Nuclear DNA of Mouse Hepatoma Studied by ^{3}H-Actinomycin D Binding

This study was designed to compare the distribution of DNA in normal liver and hepatoma cell nuclei, with special reference to heterochromatin. The ^{3}H-actinomycin D used in this study binds to DNA on tissue sections where it is then localized and quantitated by radioautography[1-4]. The preferential binding of this compound to heterochromatin was previously reported by SIMARD[5].

Materials and methods. The hepatoma bearing mice (Jax code BW7756) and the normal C57L/J mice which served as controls, were obtained from the Jackson Laboratory, Bar Harbor, Maine. The 3 experimental methods, differing in the sequence of fixation and ^{3}H-actinomycin D incubation, are outlined below.

Experiment 1. One mm cubes of hepatoma or normal liver were fixed for 2 h in 3% gluteraldehyde[6], post-fixed for 1 h in 1% osmium tetroxide[7], embedded in Araldite[8], and sectioned at 0.5 µm. One set of control sections was incubated in DNase, and another in RNase[9]. Slides were rinsed in 5% trichloracetic acid at 4 °C to remove hydrolyzed nucleic acid fragments[10]. All control and experimental sections were incubated for 4 h in 20 µCi/ml ^{3}H-actinomycin D (Schwarz Bioresearch Inc., Orangeburg, New York, specific activity 8.4 Ci/mM), following established methods[1-4].

Experiment 2. Tissues were incubated as unfixed 1mm cubes for 4 h in Locke solution containing 50 µCi ^{3}H-actinomycin D. They were subsequently fixed, embedded and sectioned as described above.

Experiment 3. One mm cubes of tissue already fixed in gluteraldehyde and osmium tetroxide were incubated with 50 µCi ^{3}H-actinomycin D. The embedding and sectioning procedures were those described above.

Slides from all experiments were dipped in Ilford L-4 emulsion, developed after 10–12 weeks exposure in Kodak D-19 and stained in 1% toluidine blue, following the methods of KOPRIWA[11]. Silver grains, representing ^{3}H-actinomycin D-DNA binding sites, were counted over 10 randomly selected nuclei for each of 9 hepatomas and 9 liver preparations. The liver parenchyma nuclei could be classed as diploid or tetraploid on the basis of nuclear diameter measurements made with a calibrated ocular micrometer.

Both nuclear area and grain counts per 100 µm^2 nuclear area, are presented in this table

	Liver 2 N (N = 30)	Liver 4 N (N = 30)	Hepatoma (N = 120)
Nuclear Area	28.56 µm^2	49.94 µm^2	115.47 µm^2
Expt. 1	94.33 ± 3.57	94.02 ± 1.83	200.78 ± 2.40
Expt. 2	93.04 ± 2.74	91.07 ± 2.04	202.52 ± 2.05
Expt. 3	95.21 ± 2.57	92.70 ± 1.77	204.44 ± 2.07
Expt. 1 RNase	96.13 ± 3.09	94.18 ± 2.19	204.03 ± 1.97
Expt. 1 DNase	0.75 ± 0.47	0.33 ± 0.16	0.09 ± 0.04

Silver grains represent ^{3}H-actinomycin D binding to DNA.

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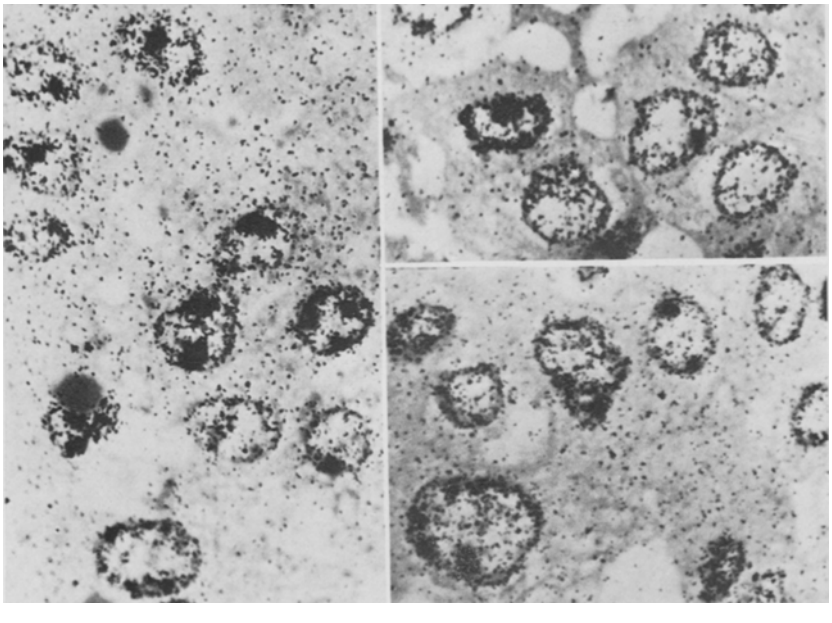

Silver grains represent ^{3}H-actinomycin D binding to DNA of hepatoma cells. 3 fields are shown. Radioactivity is particularly dense over heterochromatic regions.

Results and discussion. The Table presents measurements of average nuclear area for both liver and hepatoma cell nuclei. The area of the tetraploid liver cell nuclei (49.94 μm^2) is nearly twice that of the diploid nuclei (28.56 μm^2), while the hepatoma cell nuclei are much larger than either (115.47 μm^2). To compensate for these differences in nuclear size, all the grain count data on the Table are presented as a standard unit measure: grains per 100 μm^2 nuclear area.

Proof that ^{3}H-actinomycin D binds specifically to DNA is provided by the grain count data obtained from the enzyme treated control sections. The Table shows that RNA extraction did not influence ^{3}H-actinomycin D binding, but when the DNA was extracted, no binding occurred.

The grain density over diploid and tetraploid liver cell nuclei was almost identical in all experiments (about 93 grains per 100 μm^2), showing that DNA distribution was not altered by the increase in ploidy. In marked contrast, the ^{3}H-actinomycin D binding to hepatoma cell nuclei (200 grains per 100 μm^2) was more than twice as dense as in the normal liver cells. In the Figure, silver grains are not only visible over the nuclear area, but over the cytoplasm as well, however, the latter are not background fog. Evidence that they represent ^{3}H-actinomycin D binding to cytoplasmic DNA has been documented in other publications by the present authors[4, 12].

The mouse hepatoma used in the present study is aneuploid and shows wide variations in the total amount of DNA per nucleus[4]. Aneuploidy and polyploidy have been reported in many types of tumors[13, 14]. However, the increased ^{3}H-actinomycin D binding reported above for hepatoma nuclei cannot be explained on the basis of increased ploidy alone.

It is of particular interest that heavy concentrations of silver grains were observed over chromatin masses near the nuclear membrane and around nucleoli. Heavy ^{3}H-actinomycin D binding in these heterochromatic regions was particularly prominent in the hepatoma nuclei, as shown in the Figure. High levels of heterochromatin are characteristic of many types of tumor cell nuclei[15, 16]. SIMARD[5] has reported that ^{3}H-actinomycin D binds exclusively to the heterochromatin in cultured hamster fibroblasts, and interprets his results in terms of either more DNA in heterochromatin or preferential binding of ^{3}H-actinomycin D to heterochromatin. A similar interpretation of preferential ^{3}H-actinomycin D binding to heterochromatin in the hepatoma cell nuclei has been adopted by the present authors to explain the results reported here.

Résumé. Le ^{3}H-Actinomycin D se fixe seulement sur l'ADN dans des coupes histologiques; les noyaux de l'hépatome en liant plus que ceux du foie normal. Ces observations sont interprétées en relation avec l'augmentation de l'hétérochromatine de l'hépatome.

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Radio-Protection of Arousing Ground Squirrels (*Citellus tridecemlineatus*) by Endogenous Catecholamines

Radio-protection by exogenous catecholamines has been known for many years[1] and has recently been studied in greater detail on the hibernator, *Mesocricetus auratus*[2]. The radio-protection is thought to be due to a vasoconstriction-hypoxia mechanism. That is, intense vasoconstriction leads to a local tissue hypoxia of the bone marrow which is then protected through the 'oxygen effect'. The quantities of catecholamines necessary to elicit a radio-protection response (1.5 mg/kg) are well above the physiological range, and until now radio-protection by endogenous catecholamines has not been demonstrated.

Naturally occurring radio-protection in hibernating ground squirrels has been reported[3]. It was thought that radio-protection could also occur during arousal from hibernation since severe vasoconstriction occurs at that time, thus indicating the release of large amounts of norepinephrine[4]. Vasoconstriction is strong enough to allow the thorax of the 5°C animal to warm quickly to 30°C before the rectal temperature begins to rise. Furthermore, bretylium β-TM 10 eliminates the differential warming rate and greatly prolongs the arousal process[4]. The possibility that vasoconstriction of such severity could cause radio-protection due to tissue hypoxia in blood forming organs seems likely. In addition, if the radio-protection were due to the release of norepinephrine, it should be blockable with an α-blocker such as phentolamine.

Methods. The radiation source used in these experiments was a U.S. Nuclear of California model Gamma 12 ^{60}Co. The description and calibration of the source has been reported previously[3]. The dose rate was 200–185 rads/min due to the decay of the ^{60}Co over the time in which the experiments were done.

The animals used for this study were 13 lined ground squirrels (*Citellus tridecemlineatus*) of either sex. They were born in our vivarium to pregnant females trapped in Kansas. The ages ranged from $1^1/_2$ to $2^1/_2$ years, and it was the first experience with hibernation for each animal.

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