Proceedings of the Iowa Academy of Science

Volume 72 | Annual Issue Article 77

1965

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A Quantitative Study of the DNA and RNA in the Livers of Albino Mice During the Estrus Cycle

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Abstract. Several investigators have suggested that the nucleic acid content of mouse liver varied with the estrus cycle. Nucleic acids were extracted with trichloroacetic acid and ethanol. The quantity of DNA and RNA was determined with a spectrophotometer. No significant differences were observed in the quantity of DNA or RNA throughout the estrus cycle. Diversity in the amount of nucleic acids present among the livers of non-litter mates was one-third greater that that among litter mates. In studies of the nucleic acid content of mouse liver, little is gained by controlling the estrus cycle, but using litter mates is suggested to demonstrate small fluctuations.

In the continuing attempt to understand carcinogenesis, any changes which occur during the induction of a neoplasm are of obvious interest. Hepatomas and cirrhosis of the liver have been induced by oral and intraperitoneal administration of carbon tetrachloride. Several workers (Stowell, et al., 1951; Tsuboi, et al., 1951) showed definite changes in the amount of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) present in such abnormal livers. Dudley, Coppock, and Johnson(1959) demonstrated a difference in the nucleic acid content of normal livers and that of livers of mice fed carbon tetrachloride. However, this difference was largely obscured by the variation within the experimental and control groups.

Factors such as age, enzymes, and hormones might cause a variation in the amount of DNA and RNA present in normal mice. Until these variables have been investigated, interpretation of the data pertaining to the effects of carcinogenic agents on nucleic acid content will be difficult. Spriggs (1963) found that the nucleic acid content of mouse liver varied according to age and he reported that a cyclic phenomenon, suggestive of the estrus cycle, was evident in his data.

Various effects on liver cells have been ascribed to gonadal hormones. Allan (1944) demonstrated that estrogen increased the number of binucleate liver cells in rabbits, but Bullough (1946) showed that esterone did not increase the number of mitoses in mouse liver cells. Bond (1961) described a protein found in the liver of only male rats. Bond suppressed this protein by castration and restored it by the administration of testosterone. Although Common, Chapman, and Maw (1951)

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reported that gonadal hormones increased the DNA and RNA content in fowl liver, no such effect is described for mammalian liver. The purpose of this investigation was to determine the amount of nucleic acids present in the livers of albino mice during the various stages of the estrus cycle.

Materials and Methods

The albino mice used in this investigation were obtained from a mixed stock maintained by the biology department at Drake University. The 32 experimental female mice were placed in specially constructed cages to prevent abnormal estrus cycles (Whitten, 1956). Each cage contained two to four female mice and one male separated from the females by hardware cloth. Constant light was maintained throughout the investigation.

Vaginal smears were used to determine the stage of estrus by use of the technique of Stockard and Papanicolaou (1917). The cells were stained with aqueous methylene blue (1/10,000) to facilitate their identification. All of the cells present in three randomly chosen areas were counted. The stage of the estrus cycle was determined as follows:

Proestrus	60-90%	5-30%	5-30%
Estrus	96-100	0-5	0-2
Metestrus	50-90	0-5	5-50
Diestrus	2-15	5-10	80-90

Experimental mice were sacrificed at the age of 9 weeks by cervical dislocation. The entire liver was removed, weighed, and placed in a homogenizing tube immersed in crushed ice. Distilled water was added to the liver to make a 20% homogenate by weight.

The nucleic acids of the mouse liver were extracted by modification of Schneider's technique (1945). All extractions were done in triplicate. Two and one-half ml of cold 10% trichloracetic acid (TCA) were added to 1 ml of liver homogenate. The mixture centrifuged for 5 min and the supernatant fluid was discarded. Repeating the same procedure completed the removal of the acid-soluble phosphate fraction.

The phospholipid fraction was extracted with ethanol. Five ml of 76% ethanol were added to the residue which remained after the removeal of the acid-soluble phosphate fraction. The mixture was centrifuged for 5 min and the supernatant was discarded. The residue from this extraction was treated similarly with 5 ml of 95% ethanol.

The DNA and RNA fraction was separated from the phosphoprotein fraction by extraction with 5% TCA. Two and one-half ml of 5% TCA were added to the residue which remained after the removal of the phospholipid fraction. The mixture was centrifuged for 5 min and the supernatant was placed in a 10 ml volumetric flask. Five ml of 5% TCA were added to the residue and the mixture was heated in a water bath at 90° C for 20 min. The mixture was cooled and centrifuged for 5 min. The supernatant was added to the volumetric flask. Two ml of 5% TCA were added to the residue and the mixture was centrifuged for 15 min. The supernatant was added to the volumetric flask and the contents were diluted to volume with 5% TCA.

The quantity of DNA present in the liver tissue was determined by comparing the nucleic acid extract from the volumetric flask to a standard DNA solution. Three ml of the contents from the volmetric flask were placed in a test tube. Three ml of standard DNA solution (.012% w/v–General Biochemicals, Inc.) were placed in another test tube. Six ml of diphenylamine indicator solution (1% w/v-Fisher Scientific Co.) were added to each of the test tubes. The test tubes were heated in a boiling water bath for 3 min and were placed in the dark overnight. The optical density of the samples was read on a spectrophotometer at 600 mμ the following morning.

The quantity of RNA present in the liver tissue was determined by comparing the nucleic acid extract to a standard RNA solution. Three ml of the contents from the volumetric flask were placed in a test tube. Three ml of standard RNA solution (.016% w/v–General Biochemicals, Inc.) were placed into another test tube. Three ml of 5% TCA were added to each of the test tubes. Six ml of orcinol indicator solution (.2% w/v–Fisher Scientific Co.) were added to each of the test tubes. The test tubes were heated in a boiling water bath for 20 min and then cooled in running water. The optical density (O. D.) of the samples was read on a spectrophotometer at 620 mμ.

The following formulae were used to calculate the quantity of nucleic acids in the liver tissue:

$$\frac{\text{mg DNA}}{\text{100 g tissue}} = \frac{\text{O. D. Sample}}{\text{O. D. Standard}} \times \frac{\text{12 mg DNA}}{\text{100 ml soln}} \times \frac{\text{10 ml soln}}{\text{.197g tissue}}$$

$$\frac{\text{mg RNA}}{\text{100 g tissue}} = \frac{\text{O. D. Sample–Corr factor}}{\text{O. D. Standard}} \times \frac{\text{16 mg RNA}}{\text{100 ml soln}} \times \frac{\text{10 ml soln}}{\text{.197 g tissue}}.$$

In these formulae 0.197 g of tissue was used because the serological pipette did not deliver 0.200 g as calculated (Spriggs, 1963). DNA gave color with orcinol so a correction factor was necessary. This factor was:

$$.00405 \times 12 \times \frac{\text{O. D. DNA Sample}}{\text{O. D. DNA Standard}}.$$

Results and Discussion

The nucleic acid content of the livers of the 32 mice sacrificed during this experiment is shown in the accompanying tables. Comparisons were made among mice at the various stages of the estrus cycle and among litter mates.

The "F" test was used to determine the significance of the differences observed. If the calculated value of "F" exceeded the critical value of "F" at the 5% level, the differences were considered significant.

The DNA content in mouse liver is shown in Table 1. Although the mean DNA content was slightly higher at proestrus and metestrus, no significant differences were found among the four stages of the estrus cycle.

Table 1. Quality of DNA in albino mouse liver during the estrus cycle expressed in milligrams of nucleic acid per 100 grams of liver tissue.

Mouse	Proestrus	Estrus	Metestrus	Diestrus
1	455	476	362	341
2	469	421	417	350
3	392	342	465	417
4	437	384	460	381
5	392	443	432	477
6	437	381	447	487
7	474	371	483	400
8	481	490	508	348
Mean	442±30*	414±46	447±39	400±50
Standard Deviation	35	53	44	57

* 95% confidence limits of the mean.

The RNA content in mouse liver is shown in Table 2. Although the RNA content increased stepwise from proestrus to diestrus, no significant differences were found among the four stages of the estrus cycle.

Table 2. Quality of RNA in albino mouse liver during the estrus cycle expressed in milligrams of nucleic acid per 100 grams of liver tissue.

Mouse	Proestrus	Estrus	Metestrus	Diestrus
1	1064	1072	974	1007
2	1104	1020	1118	1004
3	942	1053	988	1061
4	979	1067	980	1075
5	990	966	1085	1050
6	982	963	1026	1074
7	1050	1066	1036	1061
8	966	1028	1056	1031
Mean	1010±49*	1029±38	1033±45	1045±25
Standard Deviation	57	44	52	28

* 95% confidence limits of the mean.

A statistically significant difference in the DNA and RNA content of mouse liver connected with the estrus cycle was not demonstrated in this investigation. It seems evident that neither the fluctuation in the nucleic acid content of mice fed carbon tetrachloride nor the cyclic phenomenon observed by Spriggs (1963) could have been associated with the estrus cycle. Control of estrus in experimental mice is expensive, and the results of this study show that it would not reduce diversity enough to be worthwhile where DNA and RNA content of adult livers are to be measured.

The mean differences in the nucleic acid content of mouse livers among litter mates and non-litter mates are shown in Table 3. The differences among non-litter mates were one-third greater than among litter mates. In studies of small fluctuations in the nucleic acid content of mouse liver, the use of litter mates is suggested.

Table 3. Mean differences in DNA and RNA in albino mouse liver in litter mates and non-litter mates expressed in milligrams of nucleic acid per 100 grams of liver tissue.

	Litter mates at same stage of estrus cycle	Litter mates at different stages of estrus cycle	Non-litter mates at same stage of estrus cycle
DNA	37	58	53
RNA	46	45	64

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