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DNA, RNA, Lipid Phosphorus, and Acid Soluble Phosphorus in Normal A-Jax Mouse Livers[1]

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Abstract. The concentrations of these substances in wet liver tissue from randomly selected, adult mice were determined colorimetrically. DNA exhibits a modal concentration near 320 mg. per 100 gm. of wet tissue. Cytoplasmic constituents do not exhibit modal concentrations. Preliminary measurements performed upon mice fed carbon tetrachloride in olive oil indicate lower concentrations of RNA, acid soluble phosphates, and lipid phosphates in experimental mice.

A-Jax mice fed carbon tetrachloride in olive oil three times each week on alternate days develop hepatic nodules soon after the twentieth feeding. These nodules develop differently, and some show characteristics not unlike tumors of a cancerous nature. Mitochondria and cytoplasmic inclusions in A-Jax mice vary considerably. These observations coincide with those of Dalton and Edwards (1942). The number and form of mitochondria within specific hepatic cells differ greatly as feeding of carbon tetrachloride continues.

Mitochondria are sites of a variety of enzymes and are known to contain large amounts of ribonucleic acid. Phosphates are integral components of ribonucleic acid. A study of liver phosphates may give clues to the metabolism of mitochondria and to factors influencing changes associated with tumor formation.

Richter (1958) demonstrated that changes occurred in cellular phosphates of the mouse liver following one feeding of carbon tetrachloride. Preliminary studies in our laboratories indicate that one may gain valuable information from the study of DNA, RNA, acid soluble phosphates, and lipid phosphates during the period of tumor formation and subsequent tumor enlargement. Before valid comparisons can be made in such a study, it is essential that concentrations of the phosphate components of the liver cell of the normal mouse be determined. The purpose of the present paper is to describe the concentrations of DNA, RNA, acid soluble phosphates, and lipid phosphates in the livers of randomly selected adult A-Jax mice.

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Procedures

1. The A-Jax mice used in this study were a strain reared in the laboratories of Drake University which were originally obtained from the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine. They were used because of their tendency to develop hepatic tumors following the administration of carbon tetrachloride in olive oil. Mature mice were selected at random for determination of phosphate constituents of the liver.

2. All glassware was cleaned with a detergent solution, and pipettes were cleaned with chromic acid cleaning solution. All glassware and pipettes were rinsed thoroughly with distilled water and dried before using.

3. Three mice were analyzed concurrently; duplicate samples were obtained from each mouse. Thus, constituents of six samples were determined at each analysis.

4. The mouse was killed by striking the back of its head. The liver was removed immediately, weighed on a Roller Smith balance to the nearest 0.2 mg., and placed in a six-inch pyrex test tube immersed in an ice bath. Distilled water was added to make a 20 percent homogenate, and the liver was finely minced by means of a ground glass homogenizer. One ml. aliquots of the homogenate were introduced into four-inch test tubes by means of serological pipettes.

5. Acid soluble phosphorus, phospholipids, and nucleic acids were extracted according to Schneider (1945, 1946).

6. Analysis for DNA. A standard DNA solution, containing 5 mg. of DNA per 100 ml., was prepared according to the following procedure. Fifty mg. of DNA (sperm), lot no. 5615, from Nutritional Biochemical Corporation, were dissolved in 3 ml. of 1 normal KOH in a six-inch pyrex test tube. This was transferred quantitatively to a 25 ml. volumetric flask and diluted to give a solution containing 2 mg. of DNA per ml. A 2.5 ml. aliquot of this concentrate was transferred by pipette to a 100 ml. volumetric flask. Two and one-half ml. of 10 percent trichloroacetic acid (TCA) were added and the flask was filled about two-thirds full with 5 percent TCA. (The 10 percent TCA was added merely to offset the 2.5 ml. of concentrate and make the final concentration of TCA more nearly 5 percent.) The flask was heated in a water bath at 90° C. for fifteen minutes and allowed to cool until the next day before diluting to volume. The preparation of this standard was started on the same day that the livers were extracted to minimize errors of deterioration. A diphenylamine indicator solution (250 ml.) was prepared, and the extract was treated according to Snell and Snell (1953). The color was then allowed to develop overnight, and the

optical density was read on a Coleman model 14 spectrophotometer at a wave length of 600 mμ using a PC-4 filter and round cuvettes. Three ml. of the standard solution were treated at the same time and in the same way as the sample; 3 ml. of 5 percent TCA were treated in the same way for use as a blank. Since the standard solution contained 5 mg. DNA per 100 ml., the total volume of extract was 10 ml., and the sample was 0.2 gm. (1 ml. of a 20 percent homogenate), the following formula was used to calculate the DNA:

$$\text{mg. DNA/100 gm. tissue} = \frac{\text{D. samp.}}{\text{D. std.}} \times \frac{5}{100} \times \frac{10}{1} \times \frac{100}{0.2}$$

7. Analysis for RNA. A standard RNA solution, containing 16 mg. of RNA per 100 ml., was prepared according to the following procedure. Fifty mg. of RNA from General Biochemicals, Inc., lot no. 8045-C, were dissolved in 3 ml. of 1 normal KOH in a six-inch pyrex test tube. This was transferred quantitatively to a 25 ml. volumetric flask and diluted to volume to give a solution containing 2 mg. RNA per ml. Eight ml. of this concentrate were transferred by pipette to a 100 ml. volumetric flask. Eight ml. of 10 percent TCA were then added for the same reason as in the preparation of the DNA standard, and the flask was filled about two-thirds full with 5 percent TCA. The flask was heated in a water bath at 90° C. for 15 minutes and was allowed to stand until the next day before diluting to volume. The preparation of this standard solution was started on the same day extractions were made in order to minimize errors due to decomposition. The orcinol indicator (250 ml.) was prepared, and the sample was treated according to Snell and Snell (1953). Into a six-inch test tube were placed 3 ml. of the sample, 3 ml. of 5 percent TCA, and 6 ml. of the orcinol solution. The test tube was heated for ten minutes in a boiling water bath, cooled in running water, and the optical density was checked on the Coleman model 14 spectrophotometer at wave length 620 mμ. Six ml. of the standard were treated with 6 ml. of orcinol reagent and heated at the same time as the sample. Six ml. of 5 percent TCA were treated with 6 ml. of orcinol reagent for use as a blank. Since the standard solution contains 16 mg. of RNA per 100 ml., the volume of extract was 10 ml., and the sample was 0.2 gm., the following formula was used to calculate the RNA.

$$\frac{\text{mg.RNA}}{\text{100 gm. tissue}} = \frac{(\text{D.samp.})\ (2) - (.0156)\,(\text{mg.DNA/100 ml.})}{\text{D. std.}} \times \frac{16}{100} \times \frac{10}{1} \times \frac{100}{.2}$$

$$\text{mg.DNA/100 ml.} = 5 \times \frac{\text{density of DNA sample}}{\text{density of DNA standard}}$$

A factor of 2 times the density of the samples was necessary because the sample was diluted 1:1 before being treated with the orcinol reagent. The factor (mg. DNA/100 ml.) subtracted from the den-

sity was necessary because DNA also gives a color with orcinol. It was found that five mg. of DNA per 100 ml. of solution gave an optical density of .078. This was .0156 per mg. per 100 ml.

8. Analysis of the phospholipid fraction. The analysis was done according to Fister (1950). The present analysis was modified during the digestion with sulfuric acid and hydrogen peroxide. It was necessary to repeat the addition of H_2O_2 two or three times because of the large amount of organic material present. The standard phosphate solution was made according to Hawk (1954) and one ml. of this solution was treated in the same manner as the sample. One ml. of ethanol was treated as a blank.

9. Analysis of acid soluble phosphate fractions. One ml. aliquots of the acid soluble extract were treated in the same manner as the phospholipid extract. The only difference was that for a reagent blank one ml. of 10 percent TCA was treated, instead of one ml. of ethanol.

Data

The results of DNA, RNA, acid soluble phosphates, and lipid phosphates determinations and the ratio RNA:DNA are shown in Table 1. The arithmetic mean determination is listed in the bottom line of the table. DNA concentrations show a mode near the arithmetic mean. Concentrations of RNA, acid soluble phosphates, and lipid phosphates show no mode but are distributed from the lowest to the highest concentration. The RNA:DNA ratio varies between 3.08 and 3.64 and possesses a mode near 3.5.

Table 2 contains data from four mice considered to be other than normal. Data listed after number one were obtained from a mouse possessing a spontaneous tumor on the hind leg. Data listed after numbers 2, 3, and 4 were obtained from mice fed carbon tetra-

Table 1

Liver Constituents Determined in Normal Mice, Expressed As Mg. Per 100 Gms. of Wet Tissue

Mouse Number	DNA	RNA	Acid Soluble Phosphates	Lipid Phosphates	RNA / DNA
1	320	1090	89.5	152	3.40
2	330	1157	88.0	167	3.53
3	355	1247	98.0	169	3.51
4	322	1173	96.7	158	3.64
5	342	1052	93.4	144	3.08
6	324	1049	93.2	147	3.28
7	302	1065	97.0	141	3.53
8	318	1100	97.6	142	3.46
9	318	1024	95.2	130	3.22
10	286	1040	91.2	156	3.64
11	327	1150	98.6	175	3.52
12	293	1034	92.3	178	3.53
Mean	319	1098	94.2	155	3.45

Table 2

Liver Constituents Determined in Abnormal Mice, Expressed as Mg. Per 100 Gms. of Wet Tissue

Mouse Number	DNA	RNA	Acid Soluble Phosphates	Lipid Phosphates	RNA / DNA
1 spontaneous tumor on hind leg	370	969	72.0	127	2.62
2 28 feedings of carbon tetrachloride	331	767	84.1	112	2.32
3 28 feedings of carbon tetrachloride	303	686	89.5	107	2.27
4 34 feedings of carbon tetrachloride	359	800	84.8	108	2.23

chloride 28 or 34 feedings. It will be noted that concentrations of the constituents varied within broad limits. The cytoplasmic contents of the abnormal mice were considerably less concentrated than in the normal mice, and also the RNA:DNA ratio was lower.

Discussion

The nuclear concentrations of DNA of both normal and experimental animals were relatively stable and within similar limits. The concentrations of RNA, acid soluble phosphates, and lipid phosphates differed significantly in that they were lower in experimental mice than in normal mice. Likewise, there was a large difference in the RNA:DNA ratio between the normal and experimental mice. It is interesting to note that the liver of the mouse with a spontaneous tumor on the hind leg showed many of the same characteristics as those of mice fed carbon tetrachloride. This mouse had not been fed carbon tetrachloride. The possible influence of a foreign tumor upon liver structure and function should receive further attention.

Mice fed carbon tetrachloride for 28 or 34 times possessed lower concentrations of RNA, acid soluble phosphates, and lipid phosphates, and a lower RNA:DNA ratio than the normal animals. Although concentrations of cytoplasmic phosphate compounds varied greatly and no modal point was suggested from the study of 12 mice, it appears that limits of concentrations of these compounds in normal mice can be defined. These concentrations differed radically from homologous contents in experimental mice. The study of litter-mates under normal and experimental conditions is indicated. It would be expected that gross limits were determined by random selection of mice in the present report. A study of litter-mates may give more refined data and make possible validating experimental studies while using fewer animals.

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