# THE DESOXYRIBONUCLEIC ACID CONTENT OF ISOLATED NUCLEI OF TUMOR CELLS

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In a recent communication Koller (1) has stated his belief that the nucleic acid content of chromosomes rises to abnormally high values in tumor cells. Apparently he believes that this increased content of nucleic acid is related to the cause of malignancy, as he makes the following statement. "Since the amount of nucleic acid and the rate of its production determine the frequency of division, the excess amount of nucleic acid present in the tumour must be considered as the fundamental cause of increased division rate and malignancy."

In this paper we should like to report the results of analyses for desoxyribonucleic acid in nuclei isolated from the cells of the two types of rat tumor, and to compare these results with the total desoxyribonucleic acid content of cell nuclei of normal rat liver. Also, in order to show that other types of cell nuclei may have a much higher desoxyribonucleic acid content than either the cell nuclei of normal liver or the nuclei of the two tumor cells studied, we have included analyses for the desoxyribonucleic acid content of the nuclei of fish spermatozoa and bird erythrocytes.

## EXPERIMENTAL

*Preparation of Cell Nuclei of Normal Rat Liver*—These nuclei were prepared at pH 3.8 to 4.0 according to the method given in the preceding paper from the livers of rats of the Osborne-Mendel and Wistar strains.

*Preparation of Cell Nuclei of Walker Carcinosarcoma 256*—The tumors were frozen and the necrotic areas were discarded. Then 100 gm. of frozen tumor were added rapidly to a Waring blendor containing 500 cc. of a mixture of crushed ice, distilled water, and 37.6 cc. of molar citric acid. The blendor was allowed to run for 15 minutes. The final pH of the mixture was about 3.0. Before the tumor was added, the concentration of the citric acid in the blendor was about 1.5 per cent.[1] The nuclei were then strained through cheese-cloth and were isolated by repeated differential centrifugation in an ordinary centrifuge. After the first washing, a drop or two of molar citric acid was added when needed to prevent agglutination of the nuclei because of increase in pH.

[1] Nuclei of Walker Carcinosarcoma 256 have been isolated by Haven and Levy (2) by the use of 2 per cent citric acid.

The use of pH 3.0 instead of pH 3.8 to 4.0 undoubtedly results in the removal of a certain amount of histone from the nuclei, since pH 3.0 probably is near the critical value below which the histone begins to be dissociated from the nucleic acid and since nuclei of liver cells prepared at pH 3.0 appear to lose some histone. According to the directions in Kossel's book (3) for preparing thymus histone from thymus nucleohistone, the solution must be definitely acid to Congo red in order that splitting of the histone from the nuclei may take place. This would imply a pH range of 2 to 3. It would be desirable to prepare cell nuclei of Walker tumors at pH 3.8 to 4.0, but so far this has proved to be impossible, since the cytoplasm is not well removed from the nuclei at this pH range.

A photograph of nuclei isolated from Walker Carcinosarcoma 256 at pH 3.0 is shown in Fig 1.

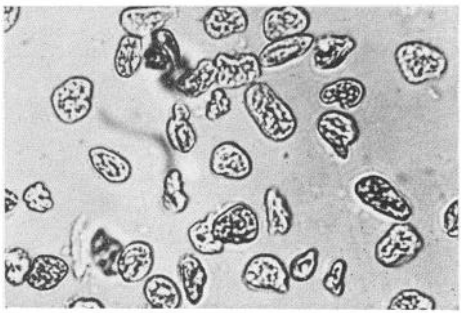

FIG. 1. Nuclei isolated at pH 3.0 from Walker rat Carcinosarcoma 256. × 720

*Preparation of Isolated Nuclei from Hepatoma 31*—It was found impossible to obtain good preparations of nuclei from Hepatoma 31 at pH values from 3.0 to 4.0. Instead it was necessary to employ concentrations of citric acid near to the concentrations employed by Stoneburg (4) and Marshak (5) in isolating the nuclei.

100 gm. of frozen tumor free of necrotic material were added rapidly to a Waring blendor containing 500 cc. of a mixture of crushed ice, distilled water, and 100 cc. of molar citric acid. (The concentration of citric acid in this mixture is about 4 per cent, and the final pH after addition of the tumor is about 2.4.) The blendor was allowed to run for 15 minutes, and then the nuclei were isolated as usual, care being taken to add a drop or so of citric acid when needed after the first washing to prevent agglutination. Agglutination is much more troublesome with nuclei prepared with strong citric acid than with nuclei prepared at pH 3.8 to 4.0.

A photograph of nuclei isolated from cells of Hepatoma 31 is shown in Fig. 2.

*Preparation of Cells of Normal Rat Liver with 4 Per Cent Citric Acid*—The same method was used which has just been described for the isolation of nuclei from Hepatoma 31. The pH is 2.4. A photograph of these nuclei is shown in Fig. 3. Some of the nuclei have been fragmented. As a rule, this does not happen.

*Preparation of Nuclei of Chicken Erythrocytes*—The method of Dounce and Lan (6) was employed, saponin being used to lake the cells in 0.9 per

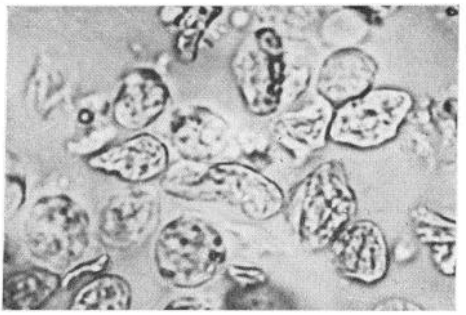

FIG. 2. Nuclei isolated at pH 2.4 from Hepatoma 31. × 1040

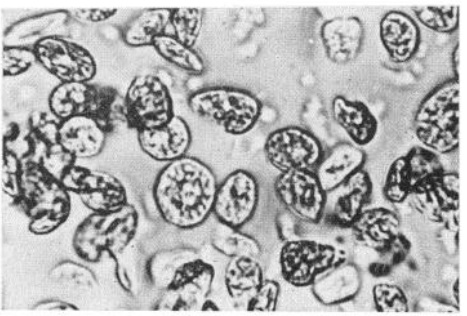

FIG. 3. Nuclei isolated from rat liver (Osborne-Mendel) at pH 2.4. × 1040

cent sodium chloride solution. A photograph of these nuclei will appear elsewhere.

*Analysis of Nuclei for Desoxyribonucleic Acid*—Desoxyribonucleic acid was determined by the method already described in the preceding paper. Samples of isolated nuclei were thoroughly suspended in distilled water and two 0.5 cc. aliquots were taken for the analysis for nucleic acid. Usually two 0.5 cc. aliquots also were dried in an oven at 100° for dry weight determination, although occasionally only one 0.5 cc. sample was dried. The values for the nucleic acid analyses are shown in Table I, and are expressed as nucleic acid, not as the sodium salt.

## DISCUSSION

The results of the analyses of various samples of isolated nuclei for desoxyribonucleic acid shown in Table I are all averages of at least two determinations on a given sample of nuclei, and different preparations of nuclei were made for each result recorded. In making a given preparation of isolated nuclei of liver cells, we used from ten to fifteen rat livers, and for nuclei from the tumor cells, parts of at least three tumors were used for every preparation of nuclei.

It should be clear from Table I that the total desoxyribonucleic acid content of the nuclei of both tumors studied is not higher than the nucleic

TABLE I

*Desoxyribonucleic Acid in Cell Nuclei, As Per Cent of Dry Weight*

Lipid was present unless otherwise stated.

| Normal rat liver, pH 3.8–4.0 | Normal rat liver, 1.28 per cent citric acid, pH 3.0 | Normal rat liver, 4 per cent citric acid, pH 2.4 | Walker tumor, 1.5 per cent citric acid, pH 3.0 | Hepatoma 31, 4 per cent citric acid, pH 2.4 | Chicken red blood cell, pH 6.8–7.0 | Fish spermatozoa |
|---|---|---|---|---|---|---|
| 22.6* | 25.3* | 38.3* | 21.0† | 21.8* | 38.1‡ | 73.0§ |
| 20.2* | | | 21.6† | 25.3* | 37.8 | |
| 23.9* | | 40.3† | | 15.2* | | |
| | | | | 21.9* | 42.0‖ | |
| 22.4† | | | | 22.4* | | |
| 19.7† | | | | | | |

* Rats of the Osborne-Mendel strain were used.

† Rats of the Wistar strain were used.

‡ The value of 45 per cent reported previously (6) was based on our sodium thymonucleate standard obtained by the Hammarsten method without correction for impurity in the standard, and therefore is too high.

§ Value reported by Steudel (7), based on phosphorus analysis, lipid-free dry weight basis.

‖ Value reported by Ackerman (8), based on phosphorus analysis, lipid-free dry weight basis.

acid content of cells of normal rat liver. In the case of Hepatoma 31, it is probable that the nucleic acid content actually is much lower than the values recorded in Table I, and therefore is also much lower than the values for nuclei isolated from normal liver cells, since the use of 4 per cent citric acid undoubtedly results in the extraction of much of the histone present in the nuclei.

This is demonstrated indirectly by the analysis of nuclei of normal liver cells, prepared at pH 2.4 by the use of 4 per cent citric acid, and at pH 3.0 by the use of 1.28 per cent citric acid. The per cent of desoxyribonucleic acid in nuclei prepared at pH 2.4 is about 1.8 times the per cent of desoxy-

ribonucleic acid in nuclei prepared at pH 3.8 to 4.0, while the per cent of desoxyribonucleic acid in nuclei prepared at pH 3.0 is about 1.17 times the per cent of desoxyribonucleic acid in nuclei prepared at pH 3.8 to 4.0. These factors are calculated from average values for the per cent of desoxyribonucleic acid in the nuclei of cells of normal rat liver prepared at pH 2.4, pH 3.0, and pH 3.8 to 4.0. If we divide the average value for the per cent of desoxyribonucleic acid in nuclei of Hepatoma 31, prepared at pH 2.4, by the factor 1.8, we get a "corrected" value of about 11.8 per cent. If we divide the average value for the per cent of desoxyribonucleic acid in Walker Carcinosarcoma 256 by the factor 1.17, we get a "corrected" value of about 18.2 per cent. It is not possible to state how close these "corrected" values for the desoxyribonucleic acid content of the tumor nuclei are to the true values, since we do not know how the histone content of the tumor cell nuclei compares with the histone content of cell nuclei of normal liver.

All of the work in this paper probably should be considered as dealing with nuclei in the resting stage, since even in the tumors the percentage of cells undergoing mitosis does not appear to be very high.

While we do not wish to contradict the observations of Koller, who used different tumor cells, a different technique, and who referred mainly to chromosomes rather than to whole nuclei, we should like to point out that his conclusions may be too sweeping, particularly in regard to the consideration of an enhanced nucleic acid content of tumor cells as a cause of malignancy.

## SUMMARY

1. Nuclei isolated from Walker Carcinosarcoma 256 at pH 3.0 with 1.5 per cent citric acid, and nuclei isolated from Hepatoma 31 with 4 per cent citric acid have been analyzed for desoxyribonucleic acid, and the results have been compared with similar analyses of cell nuclei from normal rat liver, chicken erythrocytes, and fish spermatozoa.

2. Values obtained for the desoxyribonucleic acid content of nuclei of the Walker tumor cells are probably not greatly in error, but values obtained for nuclei isolated from Hepatoma 31 undoubtedly are too high because of extraction of histone from the nuclei by the strong acid.

3. The nuclei of Walker tumors in the resting stage have nearly the same concentration of desoxyribonucleic acid as the nuclei of cells of normal rat liver in the resting stage, while nuclei of Hepatoma 31 appear to have a much lower desoxyribonucleic acid content than this.

4. Nuclei of bird erythrocytes and fish spermatozoa have a much higher concentration of desoxyribonucleic acid than do nuclei of the two tumors studied, or the cell nuclei of normal rat liver.

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