

FURTHER STUDIES ON ISOLATED CELL NUCLEI OF NORMAL RAT LIVER

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A method for preparing nuclei of the cells of normal rat liver at pH 6.0 to 6.2 already has been published (1) together with an account of six important enzymes found to be present in these nuclei in relatively high concentration. The principal objects of the present paper are twofold. The first is to compare nuclei isolated from cells of normal rat liver at pH 6.0 to 6.2 (1) with nuclei isolated at a lower pH range of 3.8 to 4.0, in regard to total lipid content and desoxyribonucleic acid content.

The second object of the paper is to show that, in isolated cell nuclei prepared by certain methods, the nucleic acid can exist either in a loosely combined, easily extractable state, or that, in nuclei prepared under different conditions, it may exist in a more firmly bound, largely unextractable state relative to mild extraction procedures. This already has been reported briefly (2). As has been stated previously, purified nucleic acid obtained by a mild extraction procedure from nuclei of rat liver cells prepared at pH 6.0 to 6.2 is in a polymerized state.

The purpose of comparing nuclei isolated at pH 6.0 to 6.2 with nuclei isolated at pH 3.8 to 4.0 is to attempt to establish whether appreciable material has been extracted from the nuclei prepared at the higher pH range during the procedure for their isolation. For reasons which will be taken up in the discussion, we believe that all of the major constituents of high molecular weight of nuclei prepared at pH 3.8 to 4.0 are retained by the nuclei during the process of isolation, although protein of high molecular weight is largely denatured. These constituents of high molecular weight comprise nucleohistone, ordinary protein, and lipid.

If ordinary protein should be extracted from nuclei prepared at pH 6.0 to 6.2, it would follow that at least some of the enzymes also would be partially extracted, resulting in a lowered enzyme activity of the nuclei. It will be shown that in all probability some protein may in fact be extracted from nuclei prepared at the higher pH range, and that therefore the measured enzyme concentrations of nuclei isolated at this pH range may be somewhat lower than the true concentrations of the enzymes as they exist in the living cell.

During the course of the work on nucleic acid, it was found desirable to

compare fractions containing nucleic acid and protein extracted from nuclei prepared at pH 6.0 to 6.2 with corresponding fractions obtained from nuclei prepared at pH 3.8 to 4.0, particularly in regard to absorption spectra. Histone fractions also were prepared from nuclei isolated at pH 6.0 to 6.2 and at pH 3.8 to 4.0, and the absorption spectrum of one of these fractions in the ultraviolet region was determined. All of the absorption spectra included in this paper, as well as many others not included, were determined by Dr. L. T. Steadman of the Department of Radiology.

In regard to extractable nucleoprotein fractions prepared from the nuclei, it should be stated that these fractions in all probability consist of nucleic acid combined loosely with histone, together with varying amounts of ordinary protein which is not combined with the nucleic acid. According to the most recent work published (3, 4), purified nucleoprotein from cell nuclei consists of nucleohistone. The nucleoprotein of nuclei thus is distinctly different in type from the firmly bound protein-ribose nucleic acid combinations which comprise some of the isolated viruses (5, 6) and the fine cytoplasmic granules of animal cells (7).

In addition to experimental work bearing on the two chief objects of the paper, some additional experiments are reported concerning a search for glycogen and a further search for cytochrome *c* in nuclei of rat liver cells.

EXPERIMENTAL

Preparation of Cell Nuclei of Rat Liver at pH 3.8 to 4.0—A mixture of roughly equal parts of crushed ice and distilled water containing 20 cc. of molar citric acid is made up to a volume of 500 cc. and is placed in a Waring blender or other suitable high speed mixer. 100 gm. of frozen rat liver are now added in small pieces as rapidly as possible without stalling the blender. The blender is run until the ice has melted, care being taken to break up at once any mass of crushed ice which forms at the top of the suspension while it is being mixed. When the ice has all melted, the mixer is stopped and the nuclei are isolated in the way already described for the preparation of cell nuclei of rat liver at pH 6.0 to 6.2 (1). There is one minor point of deviation from the procedure for nuclei isolated at pH 6.0 to 6.2, however. After the nuclei liberated at pH 3.8 to 4.0 have been washed once or twice, the pH tends to rise above 4.0 and the nuclei then agglutinate, making further washing useless. To prevent this agglutination, the pH should be maintained at 3.8 to 4.0 by the addition of a few drops of molar citric acid as needed. There will be very little trouble with loosely packed sediment, and for this reason the nuclei prepared at pH 3.8 to 4.0 may require less washing than nuclei prepared at pH 6.0 to 6.2. Fig. 1 shows an average preparation of these nuclei.

Preparation of Cell Nuclei of Rat Liver at pH 6.0 to 6.2—The method

employed has already been described, and a photograph of these nuclei has been published (1).

Preparation of Nuclei of Chicken Erythrocytes—The method of Dounce and Lan (8) was employed, saponin being used to lysis the cells in 0.85 per cent sodium chloride solution. A photograph of these nuclei will appear elsewhere.

Analysis of Isolated Nuclei for Total Desoxyribonucleic Acid—The nuclei were well suspended in water and an aliquot of 0.5 or 1.0 cc. of the suspension was dried in the oven at 100° for the determination of dry weight. For the determination of nucleic acid, the Dische reaction (9) as employed by Seibert (10) was used. The reagent is prepared by dissolving 2.75 gm. of concentrated sulfuric acid and 1.0 gm. of diphenylamine in 100 cc. of glacial acetic acid. The blank reagent is made by dissolving 2.75 gm. of

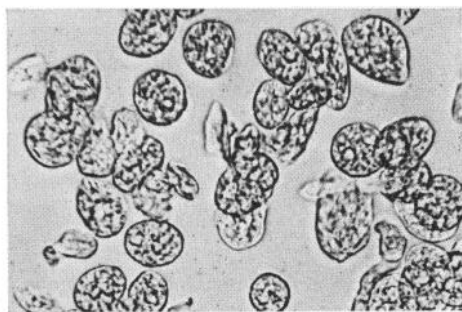


FIG. 1. Nuclei isolated from rat liver cells (Wistar) at pH 3.8 to 4.0. $\times 720$

sulfuric acid in 100 cc. of glacial acetic acid, but the diphenylamine is omitted.

0.5 cc. of the suspension of nuclei was added to each of two test-tubes. To one of the test-tubes 1.0 cc. of the Dische reagent was then added and to the other 1.0 cc. of the blank reagent was added. The tubes were immediately immersed in boiling water and were left there for 10 minutes. They were then cooled under tap water and to the tube containing the Dische reagent 8.5 cc. of the blank reagent were added. To the tube containing the blank reagent were added 1.0 cc. of the Dische reagent and 7.5 cc. of the blank reagent, so that the total volume of liquid in both tubes was now 10 cc. The contents of both test-tubes were transferred to centrifuge tubes and were centrifuged for 10 minutes at high speed to remove a precipitate which formed upon dilution with the blank reagent. After centrifuging, the supernatant solutions were read in the photoelectric colorimeter at once, and the blank was subtracted from the unknown. The total length of time elapsing between the time the tubes are placed

in the centrifuge and the time at which the colorimeter readings are taken should be kept constant and as short as possible, since the color increases on standing.

Fig. 2 shows a curve made by plotting the concentration of sodium desoxyribonucleate prepared according to Hammarsten (11) against the corresponding colorimeter readings from which the blank readings have been subtracted. The concentration of nucleic acid may be obtained by multiplying the concentration of the sodium salt by 0.944.

After the concentration of an unknown has been determined by means of this curve, if one wishes to improve the accuracy, the determination may be repeated, this time running together with the unknown a standard solution of the same strength as has been calculated for the strength of the

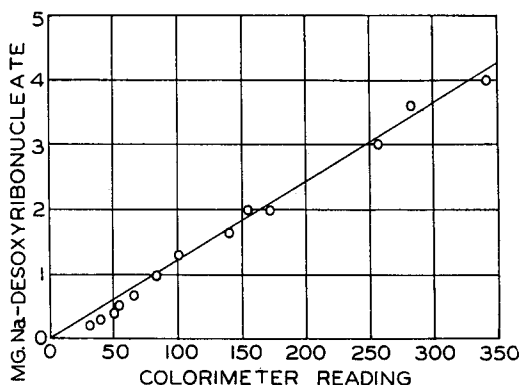


FIG. 2. Curves showing the mg. of sodium desoxyribonucleate (ordinate) plotted against the colorimeter reading (abscissa), the blank being subtracted. Klett-Summerson photoelectric colorimeter, No. 54 filter. The sodium desoxyribonucleate used in plotting the curve was about 86 per cent pure. The curve is not corrected for this impurity.

unknown by use of the curve. Since exactly the same conditions are applied to both unknown and standard, any error caused by slight differences in conditions should be compensated. The concentration of the unknown is now calculated again, the following equation being used.

$$\text{Concentration of unknown} = \frac{\text{concentration of standard}}{\text{reading for standard}} \times \text{reading for unknown}$$

Usually this value is not far removed from the first value obtained by using the standard curve.

Spectroscopic analysis of our standard sodium desoxyribonucleate prepared according to the method of Hammarsten indicated the presence of some impurity. For this reason, phosphorus analyses were made¹ and

¹ We are indebted to Dr. R. F. Riley of this department for carrying out the phosphorus determinations.

the percentage of phosphorus found was compared with the percentage of phosphorus calculated for a tetranucleotide molecule containing two purine and two pyrimidine components. The results of the analysis gave an average value of 8.0 per cent for phosphorus, while the theoretical value is 9.25 per cent. This indicated that the standard was about 86 per cent pure, and hence all figures for nucleic acid were multiplied by 0.86 to correct for the impurity of the standard.

Analysis of Nuclei for Total Lipid—The total lipid was extracted from samples of dried nuclei in a small Soxhlet extractor with a solvent mixture consisting of 25 per cent ether and 75 per cent alcohol. The solvent was evaporated and the lipid residue was determined by direct weighing, since preliminary experiments had demonstrated that it was entirely soluble in petroleum ether and therefore could not have contained appreciable non-lipid material. Further extraction of the nuclei with a chloroform-methanol mixture did not remove any more lipid.

The samples of nuclei for total lipid analysis were dried either by lyophilizing or by heating in an oven at 100°. Both methods appeared to be satisfactory. The nuclei of chicken erythrocytes were first precipitated by adding dilute acetic acid until the pH was about 3.5, and then were washed at this pH with very dilute acetic acid to remove sodium chloride before being dried.

Procedure for Extracting Nucleic Acid and Protein from Cell Nuclei of Rat Liver Prepared at pH 3.8 to 4.0 or at pH 6.0 to 6.2—The water suspension of nuclei is centrifuged and the nuclei are washed three or four times with Ringer's phosphate solution at pH 7.4. By this procedure little if any nucleic acid is removed, but considerable protein is extracted from nuclei prepared at pH 6.0 to 6.2. From nuclei prepared at pH 3.8 to 4.0, only a very small amount of protein is removed, probably because the protein previously has been denatured at the low pH employed in the preparation of these nuclei.

Next the nuclei are extracted two or three times with small portions of 5 per cent sodium chloride solution. The centrifuge with high speed attachment must be used to centrifuge down the residue from nuclei prepared at pH 6.0 to 6.2, at a speed of 12,000 to 15,000 R.P.M.

The combined 5 per cent sodium chloride extracts from nuclei prepared at pH 6.0 to 6.2 contain practically all of the nucleic acid, together with protein. From nuclei prepared at pH 3.8 to 4.0, only a fraction of the nucleic acid is extracted, which is in the neighborhood of 10 per cent of the total amount present.

The ultraviolet absorption spectrum of the "nucleoprotein" fraction extracted with 5 per cent sodium chloride solution from cell nuclei of rat liver prepared at pH 6.0 to 6.2 is shown in Fig. 3. Analysis of this fraction for desoxyribonucleic acid gave a value of about 17 per cent nucleic acid

nitrogen based on total nitrogen. The spectrum of the corresponding fraction obtained from the nuclei prepared at pH 3.8 to 4.0 also is shown in Fig. 3. Analysis of this fraction gave a value for desoxyribonucleic acid nitrogen of about 10 per cent based on total nitrogen. Total nitrogen was determined by the Kjeldahl procedure, and the nucleic acid nitrogen was calculated from total nucleic acid determined by the Dische reaction.

Following the extraction with sodium chloride, a very small amount of protein and nucleic acid can be removed from the residue from nuclei prepared at either pH 3.8 to 4.0 or pH 6.0 to 6.2 by washing once or twice with 0.1 M phosphate buffer of pH 7.5 to 7.6. The residue from nucle-

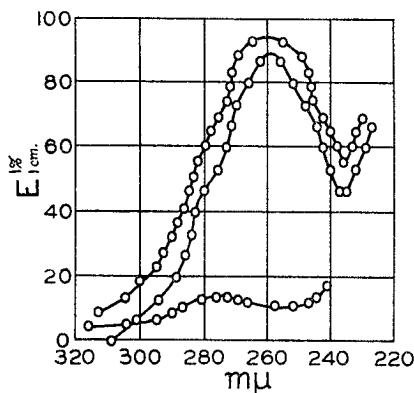


FIG. 3. The top curve represents the absorption spectrum of the nucleoprotein fraction soluble in 5 per cent sodium chloride from cell nuclei of rat liver isolated at pH 6.0 to 6.2; the middle curve, the absorption spectrum of the nucleoprotein fraction soluble in 5 per cent sodium chloride from cell nuclei of rat liver isolated at pH 3.8 to 4.0; the bottom curve, the absorption spectrum of the histone fraction at pH 4.0 prepared from cell nuclei of rat liver isolated at pH 6.0 to 6.2. $E_{1\text{ cm.}}^{\%} = (\log_{10} I_0/I) \times 1/c \times 1/d$, where c is the concentration in per cent and d is the path length in cm.

prepared at pH 6.0 to 6.2 must be centrifuged in the centrifuge with high speed attachment, as before.

The final residue obtained after washing with phosphate buffer of pH 7.4 in the case of nuclei prepared at pH 6.0 to 6.2 contains protein and lipid. Apparently it consists of nuclear membranes together with insoluble material from the interior of the nuclei.

The final residue obtained from nuclei prepared at pH 3.8 to 4.0 contains protein, lipid, and nucleic acid. If this residue is washed two or three times with distilled water, and then if distilled water is added and the pH is adjusted to 8.0 to 8.5 by the addition of ammonia, a gel is formed which persists in high dilution. A similar gel is formed by treating the original nuclei prepared at pH 3.8 to 4.0 with dilute ammonia at pH 8.0 to 8.5.

Neither the residue from nuclei prepared at pH 6.0 to 6.2 nor the nuclei themselves will form such a gel. Since the nuclei prepared at pH 3.8 to 4.0 retain much of their nucleic acid in a firmly bound condition up to the point at which the gel is formed, it appears likely that the presence of firmly bound nucleic acid is necessary for the formation of the gel. An apparently similar gel formed by adding ammonia to extracted liver tissue has been reported by Bensley and Hoerr (12).

Alternate Procedure for Removal of Protein and Soluble Nucleic Acid from Nuclei Prepared at pH 3.8 to 4.0—One adjusts the pH of a moderately concentrated suspension of cell nuclei of rat liver, prepared at pH 3.8 to 4.0, to pH 7.5 by the addition of a small amount of disodium phosphate or sodium bicarbonate solution. The nuclei swell and a certain amount of protein is dissolved. The nuclei are centrifuged and are extracted two or three times more at pH 7.5 to 7.6 with 0.5 per cent sodium chloride solution. The addition of the sodium chloride controls the swelling of the nuclei. The combined extracts are brought to pH 4.0 to 4.5 by the addition of molar acetic acid, and the precipitate, which consists of nucleic acid and protein, is centrifuged. This precipitate is entirely soluble in dilute disodium phosphate or sodium bicarbonate at pH 7.5 to 7.6, but it is only partially soluble in 5 or 10 per cent sodium chloride solution.

Most of the nucleic acid, together with some protein, can be extracted from this precipitate with 5 or 10 per cent sodium chloride solution. The material insoluble in sodium chloride contains a very small amount of nucleoprotein soluble in dilute sodium carbonate or phosphate at pH 7.5, and a fraction that is soluble at pH 2 to 3, which may be histone. The remainder of the precipitate is insoluble under mild conditions and appears to consist of denatured protein.

The residue of somewhat swollen nuclei, after being washed once or twice with distilled water, gives a gel at pH 8.0 to 8.5 in the presence of ammonia, which contains protein, lipid, and nucleic acid, and which is similar to or identical with the gel already described.

This alternate procedure for the separation of protein fractions has been applied to cell nuclei of Walker Carcinosarcoma 256, prepared at pH 3.0 with 1.5 per cent citric acid, but the results were not different from those described for nuclei of cells of normal rat liver.

Preparation of Histone Fraction from Isolated Cell Nuclei of Rat Liver—Nuclei isolated either at pH 6.0 to 6.2 or at pH 3.8 to 4.0 may be used in preparing this fraction. The nuclei are lyophilized and then are extracted two or three times with small volumes of 0.1 N HCl. The residue is removed by filtration or centrifugation, and the combined filtrates or supernatants are adjusted to a pH value near 5.0. Then 3 volumes of alcohol

are added and the material is placed in the ice box until the precipitated histone flocculates. The flocculated precipitate then is centrifuged down and is dissolved by adding a small amount of water and just sufficient 0.1 N HCl to cause the precipitate to pass into solution. Any trace of precipitate that remains undissolved is removed by high speed centrifugation. The histone may be reprecipitated by alcohol if desired.

The histone fraction thus prepared is soluble in dilute HCl, but if the pH is raised sufficiently by the addition of 0.1 N NaOH, it will precipitate. Near pH 8.0 it is still soluble, although slightly opalescent. The material is easily salted-out with sodium chloride.

The absorption spectrum at pH 4.0 of the histone fraction prepared from nuclei isolated at pH 6.0 to 6.2 is shown in Fig. 3.

Search for Glycogen in Isolated Nuclei—Two preparations of nuclei of cells of normal rat liver prepared at pH 3.8 to 4.0 and one preparation isolated at pH 6.0 were examined for glycogen content. The nuclei were centrifuged down from a water suspension and then were made into a paste by the addition of a small amount of distilled water. Sand was added to this paste and the mixture was ground in a mortar until microscopic examination showed that the nuclei had been reduced to fine particles. A 5 per cent trichloroacetic acid solution was then used to extract the ground nuclei, care being taken to use small amounts, so that a concentrated extract would be obtained. It was originally intended to make quantitative determinations of the glycogen precipitable by alcohol from the trichloroacetic acid extract, but, upon addition of 2 volumes of 95 per cent alcohol to the extract, no visible precipitate appeared, so that it was impossible to proceed further. All three of the preparations of nuclei gave the same negative results.

It is improbable that a material with as high a molecular weight as glycogen should be entirely extracted from the nuclei during their isolation, while much or most of the ordinary protein should remain, as it does. It might be argued that enzyme action would rapidly destroy the glycogen in nuclei isolated at pH 6.0, but this would not be likely to occur in nuclei isolated at pH 3.8 to 4.0. Furthermore, very fresh preparations of nuclei were used, and in each case glycogen was readily isolated from the original cytoplasmic fractions after the nuclei were centrifuged down.

Therefore we have concluded that glycogen is very low or absent in the cell nuclei of normal rat liver.

Further Search for Cytochrome c in Nuclei Isolated from Rat Liver Cells at pH 6.0 to 6.2—In the previous paper on isolated cell nuclei of rat liver (1), it was stated that cytochrome *c* was absent from these nuclei, since spectroscopic evidence for its presence was not found, and since it was necessary to add cytochrome *c* to the nuclei in order to obtain appreciable

cytochrome oxidase activity. Recently we have found that, if fresh preparations of cell nuclei of rat liver are extracted with a small amount of 5 per cent NaCl buffered to about pH 7.5 with phosphate, it is possible to demonstrate a small concentration of cytochrome *c* in the filtered extract. A very small quantity of solid $\text{Na}_2\text{S}_2\text{O}_4$ was added to such an extract prepared from fresh nuclei, and the solution was examined spectroscopically with a small pocket spectroscope having a scale. The small instrument was used to obtain a concentrated spectrum. The α -line of cytochrome *c* could be seen superimposed on the side of the faint hemoglobin band which is toward the violet end of the spectrum. The β -band was barely visible.

In order to identify the bands, a known sample of cytochrome *c* was diluted until the bands were approximately of the same intensity as those produced by the extract of nuclei, and then the location of both sets of bands on the scale of the instrument was observed. Corresponding bands of the known cytochrome *c* sample and of the extract of nuclei fell on exactly the same scale readings.

Therefore we wish to correct the previous statement that cytochrome *c* is absent from nuclei isolated from the cells of normal rat liver at pH 6.0 to 6.2, and to state instead that it is present but in low concentration. The principal reason for our failure to detect the cytochrome *c* before was that we did not add $\text{Na}_2\text{S}_2\text{O}_4$ to obtain the spectrum of reduced cytochrome *c* which is much easier to detect than that of the oxidized material. Cytochrome *c* in the isolated nuclei is in the oxidized state. The concentration of this substance in the nuclei appears to be so low that it would be difficult to decide whether it actually belongs there or is merely adsorbed during the preparation of the nuclei, as hemoglobin appears to be (1).

Attempted Fractionation of Nuclei of Bird Erythrocytes—All attempts to prepare fractions of soluble nucleoprotein from nuclei of bird erythrocytes so far have failed. The nuclei form a gel in 5 or 10 per cent sodium chloride solution, saturated sodium chloride (13), or in alkaline buffer. In distilled water, they agglutinate and form a single mass of material which gives a gel on attempts to extract it in neutral or alkaline solutions. It seems likely that anything which completely destroys the tenuous stroma, stable in 0.85 per cent sodium chloride solution at pH 7.0, will favor agglutination if the nuclei are agitated or centrifuged.

It is not likely that appreciable protein is extracted from the nuclei of chicken erythrocytes by the 0.85 per cent sodium chloride solution used in preparing them, since the second washing of the nuclei with this solution is almost protein-free. Moreover, the nuclei do not appear to shrink during treatment with 0.85 per cent saline, as do nuclei of rat liver cells when they are treated with Ringer's phosphate solution, which extracts protein from them.

DISCUSSION

It appears probable that little material of high molecular weight is lost from the nuclei isolated at pH 3.8 to 4.0. Protein of high molecular weight is undoubtedly denatured and thereby rendered insoluble; and nucleohistone must be quite insoluble at this pH range, which is very close to pH values usually reported for isoelectric points of nucleohistone (14). There is no reason why appreciable lipid should be removed from intact nuclei prepared at pH 3.8 to 4.0. Glycogen has been shown to be low or absent in nuclei of rat liver cells. Therefore it seems reasonable to use

TABLE I

Percentage of Total Lipid and Desoxyribonucleic Acid in Cell Nuclei of Rat Liver

Each figure for desoxyribonucleic acid represents an average of two or more determinations on a given preparation of nuclei, and each figure for total lipid represents a single determination on a given preparation of nuclei. From ten to fifteen rat livers were used in making a given preparation of nuclei.

	Nuclei of normal rat liver	
	pH 3.8-4.0	pH 6.0-6.2
Desoxyribonucleic acid	22.6*	12.8*
	20.2*	20.2*
	23.9*	18.8†
	22.4‡	16.2‡
	19.7‡	
Lipid	3.2*	7.5*
	3.2*	10.7†‡
	6.0†	10.8†‡
	6.0†	
	6.3†	
	7.2†	

* Rats of the Osborne-Mendel strain were used.

† Rats of the Wistar strain were used.

‡ Values previously reported (1).

the analytical values for lipid and desoxyribonucleic acid obtained for nuclei prepared at pH 3.8 to 4.0 as standard values with which to compare the corresponding values found for nuclei prepared at other pH values or by other methods.

Nuclei prepared at pH values much below 3.0 undoubtedly lose much or all of their histone content, as will be demonstrated elsewhere, and so will give high values for lipid and nucleic acid. On the other hand, as may be seen from the figures in Table I, nuclei isolated at pH 6.0 to 6.2 appear to lose some nucleic acid, and therefore give somewhat high values for total lipid content. The amount of nucleic acid lost appears to be somewhat variable, as can be seen from Table I, and preliminary observations not

included in the table indicate that the nuclei which have lost the greatest amount of nucleic acid show the highest values for total lipid. It appears probable that nuclei stirred in the Waring blender so long that they become broken are those which lose relatively large amounts of nucleic acid.

It seems unlikely that nuclei should lose nucleic acid without also losing some protein, and therefore some of their enzyme content. For this reason it is possible that some of the values reported for the concentrations of certain enzymes of the isolated nuclei (1) may actually be lower than the true concentrations of these enzymes in the nuclei as they exist in the living cell. However we do not wish to apply this reasoning to the enzymes catalase and succinic dehydrogenase, which were found to be absent or present only in traces. There seems to be no reason why these enzymes should be almost quantitatively removed from nuclei, while the other enzymes investigated should remain in relatively high concentrations. These two enzymes probably are lacking in the nuclei as they exist in the living cell.

In connection with the lipid analyses, it is of interest to note that the total lipid content of nuclei of liver cells of rats of the Wistar strain appears to be higher than the total lipid of nuclei of liver cells of Osborne-Mendel rats.

In regard to the state of the nucleic acid in isolated nuclei, it has been shown that this substance can exist either in an easily extractable, loosely combined state, or that it can be to a considerable extent in a more firmly bound, non-extractable state, presumably combined with protein. Either state can exist in nuclei which have been prepared by relatively mild methods. Thus in nuclei of rat liver cells prepared at pH 6.0 to 6.2, we have the nucleic acid almost entirely in the loosely combined state, while in nuclei of rat liver cells prepared at pH 3.8 to 4.0, as well as in nuclei of chicken erythrocytes prepared at pH 6.8 to 7.0, the nucleic acid is largely in the firmly combined state. It seems obvious that the nucleic acid of nuclei must exist in the loosely combined state or be thrown into this state when tissues are subjected to procedures such as that of Hammarsten (11) for preparing thymus nucleic acid, or of Mirsky and Pollister (3, 4) for preparing nucleoproteins. Otherwise, the yields of nucleic acid or nucleoprotein would be very small. Under the same conditions, the nucleic acid of animal cell cytoplasm, which is known to exist in granules as a liponucleoprotein complex (7), must remain firmly combined, or otherwise it would contaminate the desoxyribonucleic acid extracted from the nuclei.

Denaturation of protein evidently is not responsible for the firmly bound state of the nucleic acid of isolated nuclei, since this state occurs in nuclei of chicken erythrocytes which are prepared at too high a pH for denaturation of protein, and since in general denaturation is stated to liberate firmly

bound nucleic acid from protein (5, 6, 15). It might be thought that when nuclei are prepared at pH 3.8 to 4.0 a change in the permeability of the nuclear membrane could cause the nucleic acid to become unextractable. However, this is not probable in view of the fact that a small amount of the nucleic acid actually can be extracted from these nuclei. Moreover, the nuclei of chicken erythrocytes, which have all of their nucleic acid in a firmly combined state, were not prepared at low pH. Finally, when nuclei of liver cells prepared at pH 3.8 to 4.0 are suspended in 100 cc. of distilled water and then are fragmented by prolonged stirring at high speed in the Waring blender, the nucleic acid still is firmly bound to the residue obtained by centrifugation at high speed. This was determined by extracting the residue with 5 and then 10 per cent solutions of sodium chloride and analyzing the residue for nucleic acid. The nucleic acid content was found to be 19.5 per cent, and very little precipitable nucleoprotein appeared in the extract.

It is interesting to speculate as to whether the two states of nucleic acid in nuclei are physiologically reversible, or whether one state is normal for the living cell and the second state occurs after death or disruption of the cell, but at present these questions do not seem to be answerable from biochemical evidence. Changes in the viscosity of nuclei of tumor cells have been reported (16), and possibly these changes might be connected with a change in the state of combination of the nucleic acid in the nuclei.

SUMMARY

1. A method has been given for isolating cell nuclei of rat liver at pH 3.8 to 4.0. This pH results in denaturation of much protein, but is favorable to retention of nucleic acid and protein by the nuclei.

2. A study has been made of the total lipid and desoxyribonucleic acid content of cell nuclei of rat liver prepared at pH 3.8 to 4.0 and at pH 6.0 to 6.2. From this it is concluded that some protein is probably lost from nuclei prepared at the higher pH range, and that therefore the true concentrations of several enzymes already reported to be present in the nuclei may be even higher than the reported values.

3. It has been shown that the desoxyribonucleic acid of isolated cell nuclei may exist either in an easily extractable, loosely combined state, or in a more firmly combined state, unextractable by mild procedures. Nuclei of bird erythrocytes have been included in this study.

4. Some absorption spectra have been given for the principal nucleic acid-containing fractions prepared from isolated nuclei of rat liver cells.

5. Glycogen appears to be very low or absent in nuclei of rat liver cells.

6. Cytochrome *c* appears to be present in low concentration in nuclei isolated from cells of normal rat liver, instead of being absent as was previously reported.

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