THE PROPERTIES AND THE ENZYMATIC DEGRADATION OF DESOXYRIBONUCLEOPROTEIN FROM LIVER CELL NUCLEI*

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ABSTRACT

The isolation and properties of a desoxyribonucleoprotein of the rat liver cell nucleus are described. This material consists of DNA (desoxyribonucleic acid) bound to the residual chromosomal protein by what appear to be covalent linkages. Lipide is present, but can be removed by extraction in lipide solvents prior to isolation of the nucleoprotein, without much change in the physical properties of the latter. The nucleoprotein in question forms elastic, recoilable gels in molar saline at pH 7.0 or in water at pH 8.0 to 10.0 or even higher, which are similar to those that can be obtained from whole nuclei.

The effects of x-rays, heat, and enzymes on the nucleoprotein are discussed, and the composition of the protein component is investigated. The latter contains an "occult" protein that can be liberated by heating in 0.1 N HCl.

A study of the enzymatic degradation of the desoxyribonucleoprotein has been made, with the aim of attempting the isolation of small polynucleotide fragments attached to amino acids or short peptides that might be useful in characterizing the mode of attachment of the desoxyribonucleic acid to the protein in the desoxyribonucleoprotein. Evidence is presented indicating that such products can be isolated through the use of electrophoresis on paper.

This paper deals with the isolation, properties, and enzymatic degradation of a desoxyribonucleoprotein isolated from rat liver nuclei. This nucleoprotein is not desoxyribonucleohistone, but a material containing most of the DNA of the nucleus firmly bound to the residual nuclear protein remaining after the extraction of globulins and histones. This residual protein is probably identical with the residual protein of Mirsky (25), the "chromosomin" of the Steadmans (33, 34), and the protein component of the lipoprotein complex studied by Engebring and Laskowski (15) and by Wang et al. (39, 40).

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The senior author a number of years ago described the firm binding of DNA to protein in the cell nuclei (6) and later on this was related to the capability of the nuclei to form gels in alkali or strong saline solution (8, 12), but Mirsky and Ris (27) seem to have been the first to show that DNA can be firmly attached to the non-histone protein of chromosomes. Although the concept of firm binding of DNA to non-histone nuclear protein has not been accepted very readily, because of the previous firmly established belief that DNA occurs only as a salt with histone or protamine in the nucleus, there is now a fairly extensive literature indicating that DNA is bound in a variety of types of cell nuclei more firmly than could be accounted for by simple salt formation with histone or protamine (1-3, 16, 18, 19, 31, 37).

The cleavage of DNA from the residual protein of the nucleus can be brought about by heating in acid, alkali, or even in neutral solutions; by x-rays; and by certain enzymes present in mitochondria, including DNAase I and probably protease. The enzymatic breakdown of the nucleoprotein has been mentioned previously (14, 10) and has been dealt with in greater detail in a separate paper (13).

In the experimental section of this paper we shall describe a method of isolating the desoxyribonucleoprotein from rat liver cell nuclei, as well as its degradation by heating in acid to yield an apparently new "occult protein" fraction. We shall also describe the degradation of this nucleoprotein by enzymes to yield fragments containing both nucleotides and amino acids, which are thought to represent regions of the original desoxyribonucleoprotein molecules in the vicinities of points of attachment of the DNA to the protein. Some additional information concerning properties of the desoxyribonucleoprotein will be given, and in the discussion the relationship of this nucleoprotein to chromosomal structure will also be considered.

**Experimental Isolation of the Desoxyribonucleoprotein**

Nuclei were isolated at pH 4.0 in dilute citric acid (7, 9) or in 0.44 M sucrose at pH 6.0 (14). Most of the work was done with the former type of nuclei, since these can be readily isolated on a large scale (28). In either method the action of the mitochondrial enzymes in splitting the desoxyribonucleoprotein is prevented by the conditions of isolation.

The nuclei were extracted two or three times with 0.9 per cent NaCl at 0°C. to remove globulins, with prior adjustment of the pH to 7.0 with dilute NaOH. The extracted nuclei were washed with ice cold water and then were extracted two or three times with 0.2 N HCl at 0°C. to remove extractable histones. Excess HCl was removed from the residual material by extraction with ice cold water. The remaining material is what is referred to as the desoxyribonucleoprotein. Lipide is present, but similar material can be obtained from Behrens' type nuclei (9), which do not contain appreciable lipide. The lipide does not affect the gelability of the product nor the attachment of the DNA to the protein. The desoxyribonucleoprotein prepared...
from nuclei isolated at pH 4.0 contains traces of mitochondrial degelling enzymes, and should be stored in the cold at approximately pH 4.0 to retard the action of these enzymes.

Properties of the Desoxyribonucleoprotein

The attachment of protein to DNA is recognized by the ability of the nucleoprotein to form a characteristic elastic gel in dilute alkali or strong salt solutions, a property not shared by the ionic associations of histone or protamine with DNA. This gel exhibits a pronounced recoil or unwinding tendency when subjected to a swirling motion. Free high polymer DNA, it will be remembered, has an effective limit of solubility at about 0.25 per cent (dry weight per volume) above which concentration a stiff jelly is obtained. The nucleoprotein in dilute alkali or molar saline, however, is too stiff to flow normally at concentrations in the neighborhood of only one one-hundredth of this value.

The addition of a drop of dilute (1:5) \( \text{NH}_4\text{OH} \) to a few drops of a suspension of the nucleoprotein in water below pH 7 will produce a clear yellowish gel. This gel may be greatly diluted with distilled water, and will slowly swell to fill the provided volume. The centrifugation of such very dilute solutions at 2000 r.p.m. for a few minutes (International refrigerated centrifuge) will cause the accumulation of the gel material at the bottom of the tube. Little or no DNA will remain in the supernatant. Nucleoprotein solutions formed in 1 M sodium chloride at pH 7 to 8 also can be concentrated by low speed centrifugation, and again little or no DNA remains in the supernatant.

The degree of hydration of the nucleoprotein is extremely dependent upon pH and salt concentration. At pH values below neutrality, the nucleoprotein will hydrate only poorly. In essentially salt-free environment, the nucleoprotein will imbibe water and swell gradually as the pH is raised by the addition of the hydroxides of monovalent cations. Strong swelling occurs at pH 7.5, where the gels produced tend to be opaque. At pH 8.5 clear gels form which can be diluted easily to almost any desired volume. The exact pH at which gelation begins in solutions of low ionic strength is difficult to determine because of the very low rate of hydration at pH values below 8.

The nucleoprotein appears to be completely hydrated in molar NaCl at pH values as low as 7.0, where clear gels are formed. As the salt concentration is lowered, hydration becomes correspondingly decreased. The gel is diluted only with difficulty in 0.5 M NaCl. Precipitation of the nucleoprotein occurs below 0.3 M NaCl, and redisolving cannot be effected until the salt concentration has been reduced to very low values.

The effect of divalent cations on the hydration of the nucleoprotein is worthy of note. The presence of very small amounts of such ions serves to depress the hydration of the nucleoprotein to the extent that precipitates may be formed, under any conditions except in very strong salt solutions. None of the manipula-
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Tions described above has ever been found to release either protein or DNA from the nucleoprotein preparation.

When non-gelable nuclei, such as those prepared at pH 6.0 in 1 per cent gum arabic solutions (11), are subjected to the procedure for the preparation of nucleoprotein, the material obtained has distinctly different properties. The addition of dilute alkali simply dissolves the DNA, leaving much of the protein insoluble. Sodium chloride solutions have a similar effect. After removing the DNA from such preparations, the protein largely dissolves in 1 M NaOH on standing overnight. When this solution is adjusted to pH 6.0, the protein precipitates. The same solubility properties have been reported by Wang et al. (40) for the lipoproteins from isolated liver cell nuclei.

Factors Causing the Destruction of the Nucleoprotein

Three types of factors are known to result in the destruction of the desoxyribonucleoprotein. The first, namely the mitochondrial degelling enzymes, has been mentioned previously (12, 14, 10, 29), and has been considered in more detail in a separate paper (13).

The second factor capable of destroying the gel-forming capacity of the nucleoprotein, namely low doses of x-ray, has also been mentioned previously (12).

The third factor in question that can destroy the gel-forming capacity of the nucleoprotein is heat. Exposure to 100°C. for 7 minutes in the presence of dilute acid or alkali (0.1 N HCl or NaOH), or even at neutral pH, will completely destroy the ability of the nucleoprotein to form gels. Heating in alkali reduces the gel to a viscous yellow solution. If the nucleoprotein is heated in 0.1 N HCl, a precipitate forms which contains both DNA and protein. After removing the acid from this precipitate by washing with water, the addition of dilute ammonia causes the DNA to go into solution, but leaves nearly all the protein undissolved. When the nucleoprotein was heated at neutrality, only a slight amount of recollibility was observed after stirring if the solution was subsequently made alkaline, and considerable amounts of insoluble material remained suspended in the sample.

The material rendered insoluble in dilute alkali by heat treatment at any pH, was found to contain only traces of DNA. It seems reasonable to conclude that the heat treatments had destroyed the bonds attaching the DNA to protein.

The failure to obtain a protein precipitate by heating in alkali is probably due to solubilization of the protein through degradation. The acidification of these alkaline solutions after heating causes the release of hydrogen sulfide, suggesting that hot alkali may have induced appreciable decomposition of the protein moiety of the nucleoprotein.
Experiments Concerned with the Protein Components of Desoxyribonucleoprotein

When desoxyribonucleoprotein was treated with crystalline pancreatic desoxyribonuclease at pH 7.0 in 1 M NaCl containing 0.03 M MgSO₄, a precipitate of proteinaceous material was formed. This material was almost completely soluble in 1 M NaOH, and could be subsequently reprecipitated by lowering the pH of the solutions to 6.

The residual material remaining after the removal of globulins and histones from non-gelable nuclei isolated at pH 6.0 was found to consist of essentially free DNA and a protein fraction displaying solubility properties similar to those just described for the protein released from nucleoprotein by the action of DNAase I. These two protein preparations were found to have apparently identical amino acid compositions, as nearly as could be ascertained by examination of their hydrolysates (6 N HCl, 110°C, for 8 hours in sealed tubes) by two dimensional paper chromatography. Both preparations, and the nucleoprotein as well, have a pronounced rancid odor which comes from the fact that lipid is present, as already stated.

When nucleoprotein was heated at 100°C. in 0.1 N HCl, a precipitate was obtained which contained protein and DNA. This precipitate was no longer capable of gel formation. The acid supernatant fluid above the precipitate was found to contain a second protein fraction which is soluble from pH 1 to 10, but which can be precipitated from alkaline solution by the addition of 3 volumes of 95 per cent ethanol. The acid-soluble protein thus obtained from the nucleoprotein has been referred to as the occult protein.

That a limited and reasonably constant amount of occult protein could be obtained from nucleoprotein preparation was established as follows: A number of equal aliquots of a nucleoprotein suspension in 0.1 N HCl were heated for varying periods of time at 100°C. The individual samples were quickly cooled and centrifuged. The clear supernatants from each sample were decanted and the sample washed once with a small volume of 0.1 N HCl. To the combined extracts of each sample were added one-tenth volume of concentrated NH₄OH and 3 volumes of 95 per cent ethanol. The mixtures were allowed to stand at 3°C. overnight, following which the flocculent precipitates were collected by centrifugation, and dried at 105°C. for 8 hours. Fig. 1 displays the relationship between the amount of occult protein obtained and the duration of the heat treatment. The data indicated that hydrolysis for 9 to 10 minutes provided for the complete release of the occult protein. A similar experiment indicated that only about half as much occult protein could be recovered by hydrolysis of the nucleoprotein in 0.1 N NaOH for 10 minutes.

Analyses of several nucleoprotein preparations in this manner indicated that the occult protein constituted an average of 14.9 per cent of the dry weight of the nucleoprotein, with individual values varying from 12.2 to 17.0 per cent. Since the desoxyribonucleoprotein constitutes about 62 per cent of the dry weight of rat liver cell nuclei isolated at pH 4.0, the occult protein represents about 9 per cent of these nuclei.

It was found that occult protein could also be prepared from the residual material of non-gelable nuclei isolated by the gum arabic procedure. Heating in acid was performed as described above. The occult protein appears to comprise 12 to 13 per
cent of the residual material of these nuclei, and approximately 5 per cent of the whole nuclei on a dry weight basis. It is possible that the occult protein might represent a bound histone fraction, such as the histone fraction of Mirsky and Ris (26) that is not extractable with cold acid. However, the material does not dissolve in the mercuric sulfate-sulfuric acid reagent of Mirsky and Pollister (25) at any temperature and hence cannot be unaltered histone.

It was rather surprising to find, in the case of rat liver nuclei, that two dimensional paper chromatograms of the amino acids present after the hydrolysis of the desoxyribonucleoprotein are indistinguishable from those obtained from the hydrolysis of histone precipitable by ammonia alone, histone precipitable by ammonia plus alcohol, and the occult protein.

![Graph](image)

**Fig. 1.** Release of the occult protein by hydrolysis with 0.1 N HCl.

**The Enzymatic Degradation of the Desoxyribonucleoprotein**

The characterization of the chemical bonds involved in the DNA–protein linkage is severely hampered by the physical properties of the nucleoprotein molecule and by the large number of functional groups on both nucleic acid and protein moieties. It therefore seemed desirable to undertake a systematic degradation of the nucleoprotein molecule, with the hope of obtaining reasonably small molecules in which the chemical bonds in question remained intact—in other words, nucleotide-peptide complexes of reasonable molecular weight. This achievement would amount to a "purification" of the DNA–protein bonds.

Desoxyribonucleoprotein was prepared from the nuclei isolated at pH 4.0 from 150 gm. of rat liver (7, 9). This material, after several washings with water, was suspended in about 50 ml. of distilled water and adjusted to pH 8.5 by the addition of dilute sodium hydroxide. The nucleoprotein, white in the solid state, forms a clear colorless gel under these conditions. A small sample of the nucleoprotein gel was removed at this time to act as a control. This sample was subjected to the same conditions of temperature and pH as the experimental sample, but no enzymes were added to it. At the end of the experiment, the control had retained its elastic proper-
ties. This was taken to indicate that the experimental conditions, in the absence of the enzymes, had not caused the destruction of the bonds between DNA and protein.

About 5 mg. of crystallized beef trypsin was dissolved in 1 ml. of distilled water, and added to the nucleoprotein solution. The mixture was stirred gently with a mechanical stirrer. The pH of the solution was maintained between 8.0 and 8.5 by the addition of dilute NaOH when necessary. After 2 hours at 25°C., an additional 5 mg. of trypsin was added as before. After a total of 4 hours, no further pH change was observed. The gel was observed to have relaxed considerably.

The tryptic digestion was permitted to proceed for a total of 5 hours. At the end of this time the reaction mixture was adjusted to pH 8.0, and 5 mg. of four-times crystallized chymotrypsin was added in 1 ml. of distilled water. The reaction was permitted to proceed at 25°C. for a total of 5 hours with the pH maintained between 7.5 and 8.0. A second 5 mg. of chymotrypsin was added at the end of the 3rd hour.

At the conclusion of the proteolytic digestions, the solution had acquired a yellowish cast, and was now observed to be clear and highly viscous, with none of the elastic properties of the original nucleoprotein gel.

Enough sodium chloride was then added to the solution to raise its concentration to 1 M with respect to the salt. No precipitation occurred. With the addition of 1 volume of 95 per cent ethanol, a fibrous precipitate formed which was collected by winding on a stirring rod. This fibrous material resembled a crude preparation of DNA. The precipitate was redissolved in 1 M sodium chloride and reprecipitated by the addition of alcohol. It was then washed several times with 70 per cent ethanol, ethanol-ether (1:1), chloroform-methanol (3:1), and finally with diethyl ether. These solvents removed considerable amounts of lipoidal material. After air-drying, the fibrous, slightly gray material thus obtained weighed 81 mg.

This fibrous product, resembling DNA except for its color, should have represented DNA with relatively short peptides still attached, but was undoubtedly contaminated with extraneous peptides, and perhaps proteolytic enzymes, which had been coprecipitated. It was stored in a desiccator at 3°C. until used.

30 mg. of the DNA-like material was added to 0.50 ml. of 0.03 M MgSO₄ at 25°C. The material imbibed water rapidly, forming gelatinous masses owing to the high concentration used. (Pure DNA will also form gelatinous masses in sufficiently concentrated solutions.) 13 mg. of crystalline pancreatic desoxyribonuclease (Worthington Biochemicals) was added, and the pH of the mixture was maintained at about 7 using a dye for pH estimation. Within a few minutes the nuclease caused the complete liquefaction of the jelly-like nucleoprotein derivative. The reaction was permitted to proceed for 3 hours at 25°C. At the termination of the digestion, a small amount of insoluble material was observed in the tube. Since this gave no reaction with diphenyl amine, it was discarded. The solution itself was clear and non-viscous.

For comparison, 30 mg. of calf thymus sodium desoxyribonucleate, prepared by the method of Kay et al. (20), was treated in the same manner. The behavior of this material was exactly as described above, except that no precipitate resulted from the action of the nuclease.

1 The proteolytic enzymes used in these experiments were kindly provided by Dr. M. Laskowski of Marquette University.
Electrophoresis of Degradation Products of the Desoxyribonucleoprotein.—The solutions were then subjected to electrophoresis for 3 hours at 25°C. on filter paper, using 0.05 M acetate buffer of pH 4.5 and a field strength of approximately 10 volts per cm. The apparatus used was similar to that described by Kunkel and Tiselius (21). The samples were applied as streaks across 5 inch wide strips of Whatman 3MM filter paper which had been previously equilibrated for wetness in the apparatus. At the conclusion of the electrophoretic separation, the filter paper strips were air-dried in a horizontal position. Three such strips were required to accommodate the full volume of each sample, each strip carrying one-third of the sample.

The electrophoretic patterns were cut perpendicular to the line of electrophoretic migration, into strips 1 cm. in width. The corresponding strips from the three parallel patterns were jointly eluted with water, and the eluates diluted to 10 ml. Aliquots of these solutions were used for analysis.

Spectrophotometric Measurements.—Using a Beckman model DU spectrophotometer, the optical density of each eluate was measured at 260 m~. In addition, complete spectra of several samples were obtained in the region of 230 to 300 m~.

Analysis for Amino Groups.—Primary amino groups were quantitatively estimated by the photometric ninhydrin method of Troll and Carman (38). A Klett colorimeter with a No. 56 filter was used for the colorimetric measurements. Chromatographically pure glycine served as a standard for these determinations. Aliquots of the eluates of the chromatographic patterns were analyzed both before and after hydrolysis in 6 N HCl for 16 hours (at 105°C., in sealed tubes). All amino group analyses were carried out in duplicate.

Results of Analysis of Enzymatic Degradation Products of the Desoxyribonucleoprotein

At the pH used in these electrophoretic separations, most proteins and peptides would be expected to carry a zero or a net positive charge, while nucleotides and polynucleotides would carry net negative charges. The hybrid compounds of polynucleotides attached to polypeptides, representing the zones of linkage between DNA and protein, would be expected to have lower net negative charges than those of free nucleotides, and hence should migrate more slowly than the latter.

The distribution of 260 m~ absorbing material on the electrophoretic pattern is presented in Fig. 2. An examination of the data reveals that appreciable amounts of nucleotides obtained from the degradation of the nucleoprotein have mobilities lower than any obtained from pure DNA degraded in the same fashion. This phenomenon has been reproduced in five different experiments on three separate preparations of nucleoprotein.

The analyses for amino groups were undertaken in an attempt to show a coinciding of nucleotides and peptides on the electrophoretic pattern. The distribution of amino nitrogen in the acid-hydrolyzed samples is presented in Fig. 3. No ninhydrin reaction was obtained in any zone before hydrolysis. It should be noted that acid hydrolysis under the conditions employed causes
considerable degradation of purines, rendering some of their nitrogen ninhydrin reactive. The data in Fig. 2 are not corrected for the contribution of the nucleotides, as such a correction would require the exact determination of the purines and pyrimidines present in each zone.

The ninhydrin-reactive substances which were significantly displaced cathodally from the origin are very probably peptides. Their mobility is such as would be expected of peptides, they were not ninhydrin-positive before hydrolysis, and they could not be located on the electrophoretic pattern by means of bromphenol blue staining. No significant absorption of ultraviolet light was observed in these regions.

Considerable amounts of protein were found at the origin in both patterns. This probably represents the desoxyribonuclease, and, in the case of the nucleoprotein, residual traces of the proteolytic enzymes. This material stained intensely on the paper with brom-phenol blue. It was also unreactive with ninhydrin before hydrolysis. The presence of the proteins in this location was to be predicted from electrostatic considerations (28, 30).

Approximately equal amounts of absorption at 260 m\(\mu\) were also found at the origin in patterns from the desoxyribonucleoprotein and from DNA. This fact suggests that the nuclease may be capable of binding some nucleotides to the filter paper, or else that some poorly degraded DNA remains. On the patterns obtained from both DNA and the nucleoprotein derivatives, the distribution of ninhydrin-positive material roughly parallels the distribution of nucleo-
tides. However, notice should be taken of the zone spread approximately 5 cm. anodally from the origin of the pattern obtained from the nucleoprotein derivatives. In this region the ratio of the intensity of the ninhydrin reaction to the optical density at 260 mμ is so high that the ninhydrin reaction can hardly be entirely due to the decomposition of nucleotides that are present. This ratio is about 9 to 3, whereas in the zone containing the bulk of the nucleotides from the electrophoresis of the DNA digest, the ratio is only 3.7 to 3. It is to be remembered that this zone also contains nucleotides with abnormally low mobilities. It seems very probable therefore that this zone contains nucleotides still attached to peptides by the chemical bonds which link DNA and protein together in the intact nucleoprotein. The presence of nucleotides and peptides in this zone is otherwise very difficult to account for.

There was no indication of any spontaneous splitting of DNA from protein in the control not subjected to enzymatic degradation. Unfortunately the only means at present for testing such a control is to observe the elastic gel that forms upon the addition of alkali, and this is not a quantitative assay. However, the strength of the gel did not appear to diminish during the time required for the enzymatic degradation of the nucleoprotein and the electrophoretic separation of the products.

DISCUSSION

The biochemical argument that DNA exists in nature in chemical union with protein depends primarily upon the observation that DNA cannot be separated...
from protein until some chemical change has been brought about by enzymes, heat, or irradiation. The final proof that this attachment is covalent must await the characterization of the chemical bond involved.

The data obtained from enzymatic degradation of the nucleoprotein suggest that it may be feasible to prepare substantial quantities of the nucleotide-peptide complexes for direct chemical study. The DNA-like product obtained after proteolytic digestion of the nucleoprotein appears to be stable for at least 3 weeks, and could be accumulated from a number of nucleoprotein preparations. By substituting starch plates for paper, the electrophoretic separation could be carried out on a preparative scale.

It was noted that less than 2 per cent of the total nucleotides of the nucleoprotein appear in the electrophoretic zone suspected of containing the nucleotide-peptide compounds in question. Assuming that extensive destruction of the DNA-protein bonds has not occurred during the degradative procedure, it follows that there can be at most only one link to protein for every 50 to 100 nucleotide residues on the DNA chain. As it is unlikely that the nuclease leaves only mononucleotides attached to the peptide chains, the frequency of attachment points may be several fold smaller than this value. Such a small number of sites of attachment would make more intelligible the rapidity with which the intramitochondrial enzymes release DNA from the nucleoprotein and the efficiency with which low doses of ionizing radiation cause the loss of elasticity of the nucleoprotein gels.

It is of interest that there is also a biological argument that seems to indicate a necessity for a firm chemical union between DNA and chromosomal protein. If DNA is indeed genetic material or an important part of the genetic determinants, then presumably each DNA molecule must be rigidly established at a fixed locus on a particular chromosome. Contemporary experiments with genetic mapping would indicate that necessity, unless the improbable assumption is made that DNA molecules extend throughout the length of the chromosome and have different genic functions in different sections of the molecule. This location must be maintained throughout the profound changes that occur in the degree of hydration of the chromatin material during the mitotic cycle, and must be passed on with a high degree of accuracy to the progeny of the cell. A mechanistic consideration of these attributes would be greatly facilitated by the assumption that DNA was rigidly fixed to a macromolecular skeleton.

Several attributes of the nucleoprotein presently under consideration suggest that it may represent such a macromolecular structural unit of the chromosome. The fact that it can be easily concentrated from dilute solutions by the centrifugal application of only 1000 g presumably indicates an extremely high molecular weight. The broad changes in the degree of hydration which may be produced by varying the pH and the ionic composition of the medium, could be useful in explaining the cyclic changes in the appearance of the chromosomes. Also, the observed radiation susceptibility involves doses of the same order of
magnitudes as those used to induce chromosome breakage and fragmentation 
(4, 5, 24, 32, 35, 36), an effect poorly understood at the chemical level.

As yet there is very little data which have bearing on the spatial arrangement 
of these macromolecules. Studies with the electron microscope of preparations 
of nucleoprotein, freeze-dried after spraying on collodion membranes (41), 
indicated a complex fibrillar network, similar to that described by Frajola et al.
(17). The smallest fibers observed displayed a diameter of 50 to 100 A. Chemical 
analyses indicate that in the case of liver the DNA constitutes slightly less than 
30 per cent of the dry weight of the nucleoprotein, the remainder being made 
up of residual protein, including the occult protein, and lipid. The number 
of sites of binding of DNA to residual protein is probably small, perhaps only 
one or two per DNA molecule.

All the above data are compatible with either of the following two postulated 
structures for the macromolecule: it could be an alternating linear polymer of 
residues of protein and DNA, or it could be a protein backbone to which DNA 
molecules are attached as side branches. In addition to these two simple 
structures, other more intricate combinations can also be envisioned. The in-
volveinent of DNA in an alternating linear polymer structure apparently is con-
tradicted by studies of Mazia et al. (22, 23), but there is need for further investi-
gation on this point.

It is hoped that the work outlined in this paper will help to focus attention 
upon the importance of the attachment of DNA to the residual chromosomal 
protein and to encourage further studies of the mode of attachment of the DNA 
to this protein.

SUMMARY

1. The isolation and properties of a non-histone type desoxyribonucleoprotein 
of rat liver cell nuclei are described.
2. The action of heat, x-rays, and mitochondrial enzymes on the nucleoprotein 
is mentioned.
3. The protein component of this nucleoprotein is shown to be separable into 
an "occult protein" and an insoluble protein by the action of hot HCl.
4. The occult protein resembles histone and the unfractionated protein com-
ponent of the desoxyribonucleoprotein, but cannot be unaltered histone.
5. A systematic enzymatic degradation of the nucleoprotein is described 
with subsequent separation of the products by electrophoresis. Evidence is 
presented showing that complexes containing both nucleotides and amino 
acids can be isolated by this procedure, which may represent regions of the 
original desoxyribonucleoprotein molecule that include points of attachment 
of DNA to the protein component.
6. The possible relationship of the desoxyribonucleoprotein to chromosomal 
structure is discussed.
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