

ERGO RAUKAS, V. STRUCHKOV, N. STRAZHEVSKAYA

## THE MICELLAR STRUCTURE OF DNA

### Introduction

Numerous electron-microscopic investigations reveal that almost in any case the fibrillar structures of about 100 Å in diameter are present in nuclei and chromosomes (Ris, 1961, 1962). Only in a few cases and/or in small sections these fibrils seem to split into more fine structures; as a rule they have a diameter of about 50 Å. In the course of spermiogenesis these fibrils condense and yield a compact structure: no details of the inner structure of mature spermatozoa are usually visible in an electron microscope. However, it was shown that if bivalent cations are removed with ethylenediaminetetraacetate (EDTA), it is possible to extract DNP\* from sperm nuclei of nucleoprotamine type (Zubay, Wilkins, 1962). If sperm nuclei treated with EDTA are studied with an electron microscope, fibrils of about 100 Å in diameter are visible (Raukas et al., 1966). Obviously, DNP in native state is not organized into uniform two-dimensional hexagonal array (Luzzati, Nicolaieff, 1963) or even into complexes of one DNA molecule with protamine molecules (Feughelman et al., 1955), but there is an intermediate step in DNP organization in sperm nuclei, DNP micelles, connected with each other by bivalent cations. This intermediate degree of organization is usually not visible in an electron microscope, but the reflection in the 100 Å region is clearly discernible in most X-ray diffraction patterns at small angles (Raukas et al., 1966; Paykac, 1964). The most probable explanation seems to be that this periodicity in sperm nuclei is connected with the preservation of individual elementary chromosome fibrils in a compact DNP structure of sperm nuclei.

Small angle X-ray reflections are a common feature of nucleohistone diffraction patterns, too. In concentration region not far from conditions *in vivo* three reflections are observed: 110 Å, 55 Å and 37 Å (Luzzati, Nicolaieff, 1963). These reflections are attributed to aggregates of several DNA molecules accompanied by histone. Since the same reflections are observed also in case of intact nuclei (Luzzati, Nicolaieff, 1959), it may be supposed that they are derived from elementary chromosomal structures.

It is not without interest to remind here that small angle reflections in the "micellar" region are observed also in experiments with some DNA specimens (Riley, Oster, 1951). However, these observations were not confirmed by later investigators (Luzzati, 1961; Luzzati et al., 1961).

\* Throughout this investigation DNP is used in the meaning of deoxyribonucleoprotamine; the abbreviation DNH is used to mark deoxyribonucleohistone.

It was supposed, therefore, that big periods in nucleoprotein and, possibly, nucleic acid structure in all cases might be connected with special aggregation of DNA molecules within elementary fibrils.

However, the question about the number of DNA double helices per elementary chromosome fibril, whether in somatic cells (deoxyribonucleohistone) or in sperm nuclei (deoxyribonucleoprotamine), is not yet determined without ambiguity. In earlier X-ray diffraction investigations by Wilkins it is supposed that elementary chromosome fibril consists of 4 DNA double helices (Wilkins, 1956, 1957). In later investigations of DNH structure the big periods were related to sphingomyelin contamination (Wilkins, 1960). It is believed that the DNH molecule consists of one DNA double helix coated with histone. These DNH molecules are connected into pairs by histone bridges (Wilkins, 1960; Wilkins et al., 1959). This view seems to be consistent with most electron-microscopic investigations (Ris, 1961, 1962).

On the contrary, from X-ray diffraction investigations at small angles, the linear density of these 100 Å DNH fibrils is calculated to be 8 times that of DNA double helix (Luzzati, Nicolaieff, 1963). Since DNA accounts for almost 50 per cent of DNH by weight, the number of DNA double helices per 100 Å fibril has to be 4. These four DNA molecules connected with each other by protein bridges compose the core of the elementary chromosome fibril which is surrounded by a histone coat (Luzzati, Nicolaieff, 1963; Nicolaieff, 1962). If the concentration of DNH in gel is increased, these 4 double helices rearrange themselves into aggregates by two DNA molecules.

Thus at present there is no common opinion on the DNA content and structure of elementary chromosome fibril, neither there is upon the DNP and DNH structure in general.

On the basis of the mentioned above, an attempt was made to perform and reinterpret the experiments concerning the micellar structure of DNA. The X-ray diffraction and electron microscopy were used to study DNA which was extracted by soft methods from *Acipenser güldenstädti* sperm (nucleoprotamine) as well as from calf thymus and *Misgurnus fossilis* sperm (nucleohistone).

#### Materials and methods

"Superpolymer" DNA (Stručkov, 1964) was prepared by phenol method from sperm of *Acipenser güldenstädti* and *Misgurnus fossilis* as well as from calf thymus, as described by some of us earlier (Стручков, 1962). When extraction was being performed, special care was taken to avoid any mechanical damage of DNA molecules or aggregates of DNA molecules. In these conditions DNA solutions of a very high specific viscosity  $\eta_{sp}/c$  were obtained (about 600 dl/g) in dilute solutions (0.002 per cent DNA). The protein content of these preparations was very high, too (up to 5 per cent).

Specimens for X-ray diffraction were prepared as follows. DNA was precipitated from dilute stock solutions of DNA in 0.14M NaCl with ethyl or isopropyl alcohol, collected on a glass rod, stretched into fibres and dried over  $P_2O_5$ . The dry fibres were placed into glass capillaries with thin walls 0.8–1.0 mm in diameter and brought into contact with distilled water. After the capillary was filled with water and DNA swelled, the surplus water was removed with filter paper and the ends of the capillaries closed. To reach equilibrium, the specimens were usually kept one week at 2–4°C before exposition. In some cases ordinary cuvettes with mica foils were used instead of glass capillaries. Concentration was determined after exposition by drying DNA to constant weight at 105–110°C.

When a hydrogen-filled camera was used, stretched fibres themselves were mounted to holder and placed into X-ray beam.

X-ray techniques. Kratky-type camera with specimen-to-film distance of 300 mm

together with semi-microfocus X-ray set (Лемажихин, Лебедев, 1960) was used. CuKa radiation, usually with Ni filter, exposition 40–100 hours, RT-1 and Agfa-Laue films.

Some experiments were carried out in hydrogen-filled camera similar to that used for investigation of DNA (Langridge et al., 1960). Two 120  $\mu$  pin-holes in 1 mm lead plates, 50 mm apart from each other serve as a collimator. Small angle diffraction from the edge of pin-hole was cut off by means of 300  $\mu$  diaphragm, placed at 30 mm from the second pin-hole. Specimen-to-film distance 46 mm, exposition 50 hours, RT-1 film.

## Results and discussion

Small angle diffraction patterns of "superpolymer" DNA are usually relatively diffuse, but in most cases two reflections are clearly visible (fig. 1) in contrast to most small-angle X-ray diffraction investigations of DNA, in which the presence of only one reflection is detected (Luzzati, Nicolaieff, 1963; Luzzati, 1961; Luzzati et al., 1961; Nicolaieff, 1962; Luzzati, 1963b). Bragg spacings of these reflections are dependent upon concentration of DNA. Both spacings move synchronously with concentration, and the mean ratio of their spacings is always a little more than 2. The experiments were carried out in the concentration region from 11 to 50 per cent dry weight. In these conditions the Bragg spacings from 23 Å to 59 Å for the first reflection and from 49 Å to 136 Å for the second one were observed.

If experimental results are plotted as  $s^{-2}$  versus reciprocal of weight concentration  $c^{-1}$  (Luzzati et al., 1961; Luzzati, 1963b), two linear plots are obtained (fig. 2). According to formula for hexagonally arranged cylindrical molecules

$$s^{-2} \times 10^{-16} = \frac{\sqrt{3}}{2} \cdot \frac{m}{l} \cdot \frac{1}{\delta} [c^{-1} - 1 + \delta \bar{v}] \quad (1)$$

the linear density of 359 g cm<sup>-1</sup> is calculated for the first reflection in reasonably good agreement with the Watson-Crick model for DNA helix. In this formula  $s^{-1}$  is the Bragg spacing, m/l the mass per unit length,  $\delta$  is the density of solvent and  $\bar{v}$  is the partial specific volume of DNA in cm<sup>3</sup> g<sup>-1</sup>. Plot 2 has a slope of 4.45 times that of the plot 1. Relatively large experimental errors are caused by the difficulties in concentration determination of small specimens.

The value of the mass per unit length is determined by the tangent of the plot  $s^{-2}$  versus  $c^{-1}$ . The fact that this tangent for the second reflection is about four times bigger than the first one enables to assume that every elementary unit in the solution consists of four aggregated DNA molecules.

The character of the dependence of the Bragg spacings on concentration is in good coincidence with the one in the case of hexagonal packing of such quarterly molecules.

It is well known fact that the structural units of chromosomes are ordered in a hierarchy of pairs (Steffensen, 1959). Hence it follows that the most probable number of DNA molecules per micelle is four. The difference between the experimental and theoretical value may be caused by the deviation from the hexagonal type of packing, or by other reasons.

Results obtained with oriented fibres in the atmosphere of 95 per cent relative humidity confirm these experiments (fig. 3). If "superpolymer" DNA solutions are handled with care, two central equatorial reflections are present on fibre diagrams: 21.4 Å and 41 Å. Unfortunately, we have not obtained diagrams with good orientation, and this is the reason why

spots are not identified. The inner reflection is present only in case of high polymer DNA specimens. If DNA is degraded by passing through a syringe with a hypodermic needle, a diffuse central scattering is observed instead of this reflection. Only ordinary, "molecular" DNA preparations give diffraction patterns without scattering in the central part of the diagram.

Additional (although indirect) support for the aggregation of high molecular weight DNA is obtained when  $B \rightarrow A$  transition is followed.

The transition of "superpolymer" DNA from B to A form seems to be somewhat hindered in comparison with that of the ordinary, molecular DNA. On the contrary, when "superpolymer" DNA is degraded by forcing through metal hypodermic needle, the A-type pattern is observed even at relatively high humidities (95 per cent) at the same time with the B-type pattern. Since transition of DNA from the B-form to A-form includes a rotational deformation of double helix (Wilkins, 1961), it may be supposed that in "superpolymer" DNA preparations molecules of DNA are linked together to form aggregates or have linear dimensions of molecules in such an order that the free rotation of molecules is impossible. Attempts to find aggregates of DNA molecules in such a way (Wilkins, 1961) possibly failed because of nonappropriate methods of the extraction of DNA.

If "superpolymer" DNA is used to obtain complexes with protamine or basic polypeptides, a second weak, diffuse reflection is sometimes observed in the micelle region. Obviously, micelles of DNA, present in "superpolymer" DNA, do not disintegrate when a complex of DNA with protamine or polylysine is formed.

In some cases (thymus DNA) a reflection was observed in the micelle region, whose spacing was independent of concentration. On the best small angle pattern weak, but very sharp lines were visible. They have spacings of 60 and 115 Å on the diffuse background 40–115 Å. Possibly, in this case we are concerned with disputed "sphingomyelin" reflections (Wilkins, Zubay, 1963; Luzzati, 1963a) or reflections from other impurities. However, in experiments with sperm DNA these concentration-independent reflections were never observed.

In regard to our experiments it is interesting to recall the X-ray study of DNA by Riley and Oster (1951). Investigation of various DNA preparations in all cases revealed two reflections in the micelle region, the spacings and intensities of which varied with concentration. The plot  $\log s^{-1}$  versus  $\log c$  (logarithm of Bragg spacing versus logarithm of DNA volume concentration) has a slope of  $-1/2$  in all cases, which indicates that the swelling is two-dimensional and synchronous for both spacings. To calculate linear density of DNA and aggregates, the plots (fig. 4) (Riley, Oster, 1951) were transformed into coordinates  $s^{-2}$  versus  $c^{-1}$  (wt./wt.) (Luzzati et al., 1961; Luzzati, 1963b). As a result, linear plots were obtained in which the term  $(1 - \delta v)$  of equation (1) was excluded. However, this error is not a very large one and the approximate estimation of linear density values can be made, assuming that DNA molecules are arranged hexagonally, on the average. In such a way the value of  $355 \times 10^{-16} \text{ g cm}^{-1}$  for the plot "C" is obtained, in good agreement with Watson-Crick model for DNA ( $327 \times 10^{-16} \text{ g cm}^{-1}$ ) (Luzzati, Nicoliaeff, 1963). However, the second reflection is somewhat different for various specimens (plots "A" and "B"). From recalculations of plots "B" and "A" the linear density per micelle is found to be 4.8 and 11.5 that of the DNA, respectively.

Thus our observations confirm the results of Riley and Oster (1951), which were carried out some 15 years earlier and only rarely cited later.

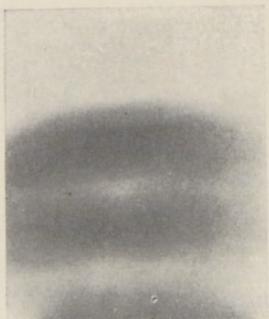


Fig. 1. Small angle X-ray diffraction pattern of *Acipenser guldentädti* sperm "superpolymer" DNA: two reflections are visible. DNA concentration 34 per cent dry weight in distilled water; Ni-filtered Cu  $\text{K}\alpha$  radiation.

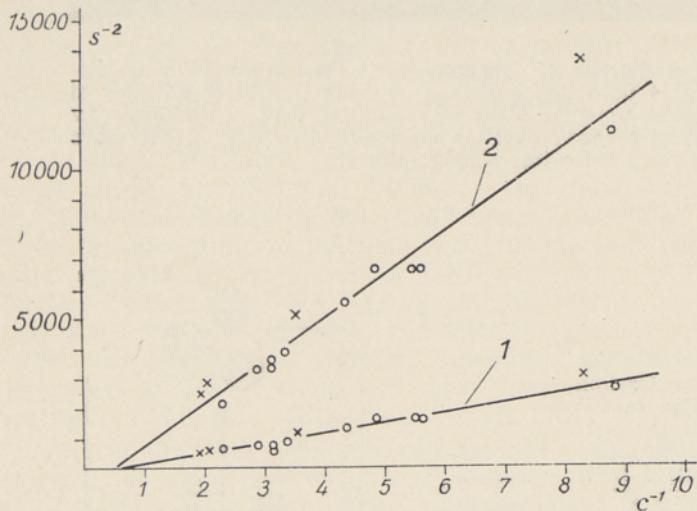


Fig. 2. Plots of the square of the Bragg spacing ( $10^{-16} \times s^{-2}$ ) versus reciprocal of weight concentration of DNA ( $c^{-1}$ ) for "superpolymer" DNA specimens:  $\times$  calf thymus DNA,  $\circ$  *Acipenser guldentädti* sperm DNA.

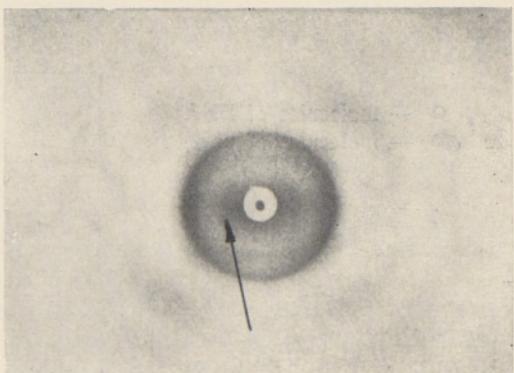


Fig. 3. Central part of X-ray diffraction pattern of "superpolymer" DNA fibre at 95 percent relative humidity. Arrow points the 41 Å reflection.

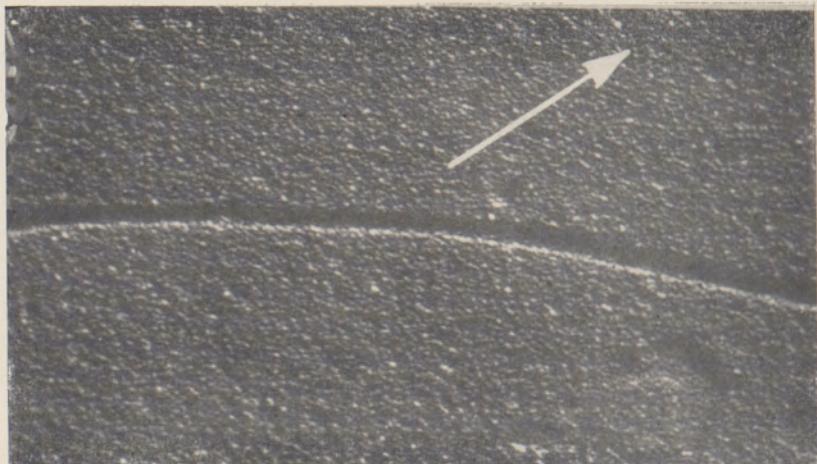
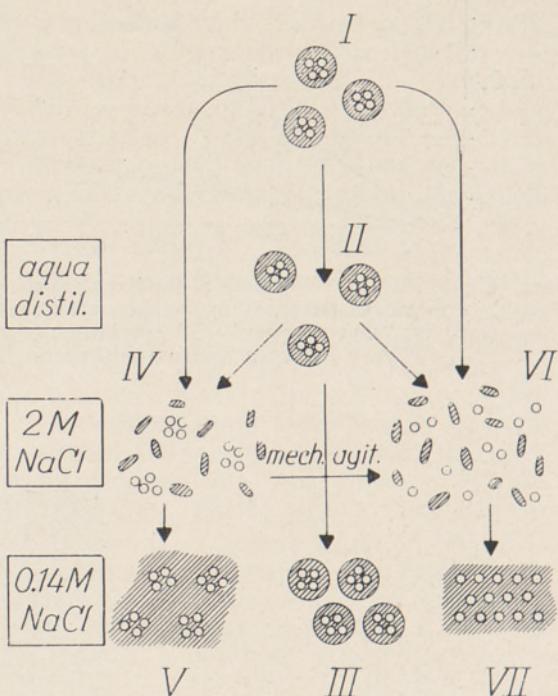


Fig. 4. Electron micrograph of "superpolymer" DNA fibre, 65 Å in diameter, consisting of 4 DNA double helices. Shadowed with Pt-Pd alloy at an angle of 8 degrees from the plane of supporting film,  $\times 100\,000$ . Arrow shows the direction of shadow. (Authors are indebted to Mrs. A. Kaftanova for this photograph.)

Fig. 5. A tentative scheme for deoxyribonucleoprotein extraction and small angle diffraction experiments. I — native DNH in interphase nuclei, II — water-extracted DNH, III — water-extracted DNH precipitated in 0.14M NaCl, IV — salt-extracted DNH, without mechanical rupture of DNA micelles, V — salt-extracted DNH, precipitated in 0.14M NaCl, VI — salt-extracted DNH, with mechanical rupture of DNA micelles, VII — salt-extracted DNH, precipitated in 0.14M NaCl.



The only difference between our results and those of Riley and Oster seems to be that we have never found specimens with reflections corresponding to plot "A" of Riley and Oster.

The presence of the second reflection on X-ray patterns at small angles is obviously connected with some kind of lateral aggregation of DNA. The possibility of aggregation under the influence of an elevated salt concentration is ruled out as only distilled water was used to prepare specimens for diffraction. Additional support for such an assumption is provided by X-ray diffraction experiments by Nicolaieff and Luzzati: concentrations of up to 3M NaCl were used to study gels and solutions of DNA, but in all cases only one reflection was found (Luzzati et al., 1961; Nicolaieff, 1962; Luzzati, 1963b).

The most probable explanation for the DNA micelles is their nuclear origin (Струцков, 1962). Possibly, these structures represent remains of deproteinized elementary chromosome fibrils from somatic as well as sperm nuclei and are not *de novo* formed from DNA molecules in solutions or gels. This conclusion is made very likely if the methods of DNA extraction are compared between each other. DNA specimens used in our study are extracted in conditions in which damaging of DNA molecules or their aggregates by shearing is carefully prevented (Струцков, 1962): even pipetting has been avoided when DNA samples were handled. The DNA specimens of Signer and Schwander (1949) and also of Butler and Schmidt (1950), which have been used for X-ray diffraction experiments by Riley and Oster, were also prepared in mild conditions, according to the modified Hammersten method, making use of concentrated salt solutions. If DNA solutions are handled less carefully when extracted, DNA aggregates may break up to form ordinary, "molecular" solutions of DNA, and micelles are never formed. This may be the reason why the micellar reflection (corresponding to lateral aggregates of DNA molecules) have never been observed by later investigators (Luzzati, 1961; Luzzati et al., 1961).

Similarly, the DNA specimens of Cavalieri et al. (1961) are considerably degraded: the molecular weights of these specimens are some orders of magnitude less than those of the "superpolymer" DNA. One of the most drastic treatments is obviously deproteinization by Sevag procedure, including shaking with chloroform — normal octanol at 250 linear (10 cm) strokes per minute, which leads to the emulsification of the solution. However, even in these conditions aggregation of DNA molecules into pairs is preserved if DNA is extracted from proliferating tissues (Cavalieri, Rosenberg, 1961).

These findings are in good agreement with electron-microscopic observations of "superpolymer" DNA (Струцков, 1964). The DNA fibrils prepared from rat thymus and *Misgurnus fossilis* sperm nuclei have a mean diameter of 50—70 Å, the number of laterally aggregated DNA molecules per such a filament being at least two, but the most probable value seems to be four.

Experiments with the "superpolymer" DNA from *Acipenser güldenstädti* sperm gave analogous results. Fibrils of 65 Å in diameter are a predominant feature of these "superpolymer" DNA specimens (fig. 4) in difference from the "molecular" DNA specimens with fibrils about 20 Å in diameter (Hall, Litt, 1958).

It is interesting to note that the diameter of DNA strands of Signer and Schwander specimen is also estimated to be about 50 or 60 Å, making use of electron microscope (Riley, Oster, 1951, p. 527).

It is reported that after the extensive digestion of somatic nuclei with beef pancreatic trypsin, free chromatin strands (about 50 Å in diameter) are released; the number of DNA double helices per strand is not specified (Allfrey, Mirsky, 1964). However, sometimes 4 strands having a diameter that of the Watson-Crick helix are visualized directly in the elementary chromosomal fibrils, making use of stereo-pair micrographs (Hyde, 1964).

Thus a conclusion may be drawn that the "superpolymer" DNA obtained by a mild treatment from somatic tissues as well as fish sperm (both of protamine and histone type) probably consists of 4 DNA molecules in lateral aggregation. It is not yet clear by which kind of bonds the DNA molecules are linked together (laterally, and possibly, end-to-end) in these fibrils. Experiments with the most carefully prepared DNH, "nuclear dispersion" (dispersion of nuclei in 1M NaCl) (Kuehl, 1962), i.e. a material very similar to the "superpolymer" DNA show that elevated salt concentration, Na dodecylsulphate, EDTA, urea, guanine hydrochloride, thioglycol and proteolytic enzymes do not alter the properties of nuclear dispersions; that is, electrostatic and hydrophobic interactions, bivalent cations, H-bonds, disulphide and covalent protein bridges are not essentially involved in the aggregation of DNA. Only strong shearing forces make DNA go from "nuclear dispersion" into solution. It may be supposed that 50—70 Å DNA fibrils are held together in a cable-like fashion only.

Obviously, this DNA complex makes up a core of 100 Å elementary chromosome fibril and is surrounded by a histone coat (Nicolaieff, 1962; Luzzati, Nicolaieff, 1963). It seems reasonable to suppose that real DNH as well as DNP elementary structures (whether or not called molecules) contain 4 DNA molecules in lateral aggregation. Proposed DNH and DNP structures, consisting of one DNA molecule, surrounded by protein (Feughelman et al., 1955; Wilkins, 1956, 1957; Zubay, Doty, 1959) are apparently inadequate if *in vivo* structures are discussed. However, such a structure may arise when protein is added to DNA solutions (especially at low DNA concentration) (Paykac, 1965) or when nucleoprotein is extracted with salt (Mirsky, Pollister, 1942; Pollister, Mirsky, 1946) or water (Zubay, Doty, 1959) methods, involving strong shearing forces.

On the basis of above-said the following scheme is suggested for further experiments and discussions (fig. 5). Nucleoprotein elementary structure in native state is identical to 100 Å elementary fibril. In interphase nuclei (I) they are free in nuclear plasm, in case of sperm nuclei of nucleoprotamine type these elementary fibrils are connected with each other by means of bivalent cations (Raukas et al., 1966). When water extraction is used, elementary chromosome fibrils obviously go into solution without any damage (II). When treated carefully, fibrils of 100—200 Å in diameter are visible in electron microscope (Ris, 1961; Struckov, 1964). The presence of more thin nucleoprotein fibrils in water-extracted DNH (Zubay, Doty, 1959) might be explained by degradation of elementary fibrils into constituent parts in hydrodynamic fields or by other reasons. DNA molecules in elementary fibril are obviously not very strongly linked together by protein and stirring and homogenization (used in preparation of DNH by Zubay and Doty) partly breaks them up into more fine structures. Other aspects of physical-chemical and structural investigation of water-extracted DNH (Zubay, Doty, 1959) are treated at length by Luzzati and Nicolaieff (1963). When salt extraction is used (Mirsky, Pollister, 1942; Pollister, Mirsky, 1946),

elementary chromosome fibrils split into DNA and histone (protamine) counterparts (IV, VI). If extraction is carried out without mechanical processing (stirring or homogenization), DNA goes into solution as 50—70 Å fibrils (IV) (Stručkov, 1964). If protein is removed with phenol, "superpolymer" DNA solution is obtained (Стручков, 1962). DNH (DNP) solutions, precipitated in 0.14M NaCl, form various DNH (DNP) structures (III, V, VII). The structure (III) must be considered as a most native one; the structure (V) is somewhat more irregular in a sense that histone (protamine) molecules might be rearranged and denatured to some extent after dissociation and precipitation. This is the material, X-ray patterns of which have characteristic reflections in micellar region. Structure (VII) is the most denatured one, micelles of DNA are mostly disrupted during the extraction (VI), and when precipitate of DNH (DNP) is examined by means of small angle X-ray diffraction, the intensity of micellar reflections are either significantly reduced or the reflections are absent altogether.

Thus we suppose that the key for interpretation of small angle diffraction patterns of DNA, DNH and DNP is not only preservation or degradation of protein structures (Zubay, Wilkins, 1964), or even presence of sphingomyelin contamination (Wilkins et al., 1959; Wilkins, 1960), but rather an integrity of DNA native, micellar structure.

### Summary

X-ray diffraction at small angles and electron microscopy is used to study "superpolymer" DNA extracted from calf thymus and fish sperm (Stručkov, 1964; Стручков, 1962). Two reflections are observed in the "micellar" region: the dependence of the Bragg spacings of these reflections upon the concentration reveals an aggregation of DNA molecules into micelles by four. This conclusion is confirmed by electron microscopic observation. It is concluded that these four DNA molecules in lateral aggregation are remnants of the core of the 100 Å elementary chromosome fibril. The possible implication of these results upon the nucleoprotein structure and methods of extraction are discussed.

### REFERENCES

- Allfrey V. G., Mirsky A. E., 1964. Role of histone in nuclear function. The Nucleohistones, J. Bonner and P. Ts'o, editors, San-Francisco, Holden-Day, Inc. : 267—288.
- Butler J. A. V., Smith K. A., 1950. Action of ionizing radiations and of radio-mimetic substances on deoxyribonucleic acid. I. Action of some compounds of the mustard type. *J. Chem. Soc.*: 3411—3418.
- Cavalieri L. F., Deutsch J. F., Rosenberg B. H., 1961. The molecular weight and aggregation of DNA. *Biophys. J.* 1 : 301—315.
- Cavalieri L. F., Rosenberg B. H., 1961. The replication of DNA. I. Two molecular classes of DNA. II. The number of polynucleotide strands in the conserved unit of DNA. *Biophys. J.* 1 : 317—322, 323—336.
- Feughelman M., Langridge R., Seeds W. E., Stokes A. R., Wilson H. R., Hooper C. W., Wilkins M. H. F., Barclay R. K., Hamilton L. D., 1955. Molecular structure of deoxyribose nucleic acid and nucleoprotein. *Nature* 175 : 834—838.
- Hall C. E., Litt M., 1958. Morphological features of DNA macromolecules as seen with the electron microscope. *J. Biophys. Biochem. Cytol.* 4 : 1—4.
- Hyde B. B., 1964. A structural component of chromatin. The Nucleohistones, J. Bonner and P. Ts'o, editors, San-Francisco, Holden-Day, Inc. : 163—166.
- Kuehl L. R., 1962. The state of deoxyribonucleic acid in the cell nucleus. *Biochim. Biophys. Acta* 55 : 289—299.

- Langridge R., Wilson H. R., Hooper C. W., Wilkins M. H. F., Hamilton L. D., 1960. The molecular configuration of deoxyribonucleic acid. I. X-ray diffraction study of a crystalline form of the lithium salt. *J. Mol. Biol.* 2 : 19—37.
- Luzzati V., 1961. La structure de l'acide désoxyribonucléique en solution étude par diffusion des rayons X aux petits angles. *J. chim. phys. et phys.-chim. biol.* 58 : 899—902. Discussion : 902—903.
- Luzzati V., 1963a. The structure of nucleohistones and nucleoprotamines. *J. Mol. Biol.* 7 : 758—759.
- Luzzati V., 1963b. The structure of DNA as determined by X-ray scattering techniques. *Progr. Nucl. Acid. Res.* 1 : 347—368.
- Luzzati V., Nicolaieff A., 1959. Etude par la diffusion centrale des rayons X de la structure des gels aqueux de nucléoprotamines et nucléohistones et de noyaux intacts. *Compt. rend. Acad. Sci. Paris* 248 : 1426—1429.
- Luzzati V., Nicolaieff A., 1963. The structure of nucleohistones and nucleoprotamines. *J. Mol. Biol.* 7 : 142—163.
- Luzzati V., Nicolaieff A., Masson F., 1961. Structure de l'acide désoxyribonucléique en solution: étude par diffusion des rayons X aux petits angles. *J. Mol. Biol.* 3 : 185—201.
- Mirsky A. E., Pollister A. W., 1942. Nucleoproteins of cell nuclei. *Proc. Natl. Acad. Sci. U.S.* 28 : 344—352.
- Nicolaieff A., 1962. Étude par diffusion centrale des rayons X de la structure de l'acide désoxyribonucléique, des nucléoprotamines et des nucleohistones en solution.
- Pollister A. W., Mirsky A. E., 1946. The nucleoprotamine of trout sperm. *J. Gen. Physiol.* 30 : 101—113.
- Raukas É., Mityuschin V., Kafanova A., 1966. Structure of deoxyribonucleoprotein of sperm nuclei: DNP structure. (In press.)
- Riley D. P., Oster G., 1951. An X-ray diffraction investigation of aqueous systems of desoxyribonucleic acid (Na salt). *Biochim. Biophys. Acta* 7 : 526—546.
- Ris H., 1961. Ultrastructure and molecular organization of genetic systems. *Canad. J. Genetics Cytol.* 3 : 95—120.
- Ris H., 1962. Interpretation of ultrastructure in the cell nucleus. *Sympos. Intern. Soc. Cell Biol.* 1. The Interpretation of Ultrastructure. New York, Academic Press : 69—87, Discussion : 87—88.
- Signer R., Schwander H., 1949. Isolierung hochmolekularer Nucleinsäure aus Kalbthymus. *Helv. Chim. Acta* 32 : 853—859.
- Steffensen D. M., 1959. A comparative view of the chromosome. Brookhaven Sympoz. Biol. 12 : 103—118, Discussion : 118—124.
- Stručkov V. A., 1964. Die Natur der «superpolymeren» DNA und Methoden ihrer Untersuchung. Abhandl. Dtsch. Akad. Wiss. Berlin Kl. Med. (6) : 401—410.
- Wilkins M. H. F., 1956. Physical studies of the molecular structure of deoxyribose nucleic acid and nucleoprotein. Cold Spring Harbor Symp. Quant. Biol. 21 : 76—88, Discussion : 88—90.
- Wilkins M. H. F., 1957. Molecular structure of deoxyribose nucleic acid and nucleoprotein and possible implications in protein synthesis. Biochem. Soc. Sympos. (14) : 13—25, Discussion : 25—26.
- Wilkins M. H. F., 1960. Molecular structure of deoxyribonucleoproteins. Nucleoproteins. Proc. 11th Solvay Conf. Chem. 1959, New York, Interscience: 45—66, Discussion : 67—76.
- Wilkins M. H. F., 1961. The molecular structure of DNA. *J. chim. phys. et phys.-chim. biol.* 58 : 891—898.
- Wilkins M. H. F., Zubay G., 1963. X-ray diffraction study of the structure of nucleohistone and nucleoprotamines. *J. Mol. Biol.* 7 : 756—757.
- Wilkins M. H. F., Zubay G., Wilson H. R., 1959. X-ray diffraction studies of the molecular structure of nucleohistone and chromosomes. *J. Mol. Biol.* 1 : 179—185.
- Zubay G., Doty P., 1959. The isolation and properties of deoxyribonucleoprotein particles containing single nucleic acid molecules. *J. Mol. Biol.* 1 : 1—20.
- Zubay G., Wilkins M. H. F., 1962. An X-ray diffraction study of histone and protamine in isolation and in combination with DNA. *J. Mol. Biol.* 4 : 444—450.
- Zubay G., Wilkins M. H. F., 1964. A note on reversible dissociation of deoxyribonucleohistone. *J. Mol. Biol.* 9 : 246—249.
- Лемажихин Б. К., Лебедев Л. А., 1960. Конструкция микротюбусной разборной рентгеновской трубки с изменяемым фокусом. Приборы и техника эксперимента (1) : 136—138.
- Раукас Э., 1964. К структурной организации нуклеопротамина в ядрах сперматозоидов. Биофизика 9 : 653—656.

- Раукас Э., 1965. Температура плавления комплексов дезоксирибонуклеиновой кислоты с протаминами, основными полипептидами и полиэтиленполиамином. Биохимия 30 : 1122—1131.
- Стручков В. А., 1962. О природе «сверхполимерной» дезоксирибонуклеиновой кислоты. Биофизика 7 : 538—550.

Academy of Sciences of the Estonian SSR,  
Institute of Experimental Biology  
Academy of Sciences of the USSR,  
Institute of Biological Physics

Received  
Dec. 1, 1965

ERGO RAUKAS, V. STRUTSKOV, N. STRAZEVSKAJA

### DNH MITSELLAARNE STRUKTUUR

#### Resümee

«Superpolümeerse» DNH (Стручков, 1962; Stručkov, 1964) uurimiseks kasutati röntgenidifraktsioonimeetodit väikestel nurkadel ja elektronmikroskoopi. Difraktsioonil ilmnes, et «mitsellaarses» piirkonnas võib selgesti eristada kaks refleksi. Refleksi kau-guse sõltuvus DNH kontsentratsioonist paneb arvama, et DNH molekulid on grupeerunud mitsellideks, igaüks neli molekuli. See järeltus leiab kinnitust elektronmikroskooplisel uurimisel. Oletatakse, et nelja DNH molekuli lateraalne aggregaat on elementaarse kromosoomse fibrilli tervikuna säilinud osa ja kajastab DNH struktuuri *in vivo*. Arut-luses on esitatud DNP ekstraktsiooni skeem.

Eesti NSV Teaduste Akadeemia  
Eksperimentaalbioloogia Instituut  
NSV Liidu Teaduste Akadeemia  
Biolooligilise Füüsika Instituut

Saabub toimetusse  
1. XII 1965

ЭРГО РАУКАС, В. СТРУЧКОВ, Н. СТРАЖЕВСКАЯ

### МИЦЕЛЛЯРНАЯ СТРУКТУРА ДНК

#### Резюме

«Сверхполимерная» ДНК (Стручков, 1962; Stručkov, 1964) была исследована при помощи рентгеновской дифракции под малыми углами и электронной микроскопии. При малоугловой дифракции наблюдается два отражения; зависимость брэгговских расстояний этих отражений от концентрации ДНК показывает, что молекулы ДНК расположены по четыре в каждой мицелле. Этот вывод подтверждается электронно-микроскопическими наблюдениями. Предполагается, что боковой агрегат четырех молекул ДНК не образуется *in vitro*, а является неразрушенной средней частью элементарной хромосомной фибрilli. Обсуждается молекулярная структура и методы экстракции ДНП.

Институт экспериментальной биологии  
Академии наук Эстонской ССР  
Институт биологической физики  
Академии наук СССР

Поступила в редакцию  
1/XII 1965