Методологія наукових досліджень
Scientific research methodology

Шановні колеги! У рубриці „Методологія наукових досліджень” редакція продовжує публікацію матеріалів, що пов’язані з найважливішими аспектами наукової і навчальної діяльності: організаційно-методичним забезпеченням періодичних наукових видань, загальними принципами біостатистичного та математичного супроводження досліджень, а також оригінальними методичними підходами вітчизняних і зарубіжних морфологів.

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LEGENDS OF DNA MORPHOLOGY

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Pivovarenko Y.V. Legends of DNA morphology.
ABSTRACT. Background. It is believed that modern views on the structure of DNA molecules are based on the results of experiments that have an impeccable interpretation. This is not entirely correct, because the results of these experiments have more than one interpretation. **Objective.** Discussion of the correctness of the interpretation of the experimental results, on the basis of which the model of double-stranded DNA was proposed. **Results.** It is shown that modern views on the structure of DNA molecules are mainly determined by the ability of sodium salts to form fibers, as well as the ability of a curved glass to form fibers on its surface. In addition, an analysis of existing ideas on DNA melting and intercalation of aromatic dyes in its structure has been analyzed. **Conclusion.** The results of experiments on which modern ideas about the structure and properties of DNA molecules are based were poorly interpreted. For this reason, the models of double DNA spirals, which were the result of these interpretations, "function poorly". Thus, these DNA models do not allow satisfactory explanation of a number of properties of real DNA, including their melting. For this reason, the results of the experiments, on the basis of which the double-chain model of the DNA molecule is proposed, should receive a new interpretation that will make it possible to change the perception of the morphology of DNA molecules.

Keywords: DNA, DNA structure, melting, intercalation.

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Introduction

Modern views on the structure of DNA molecules are based on the results of x-ray studies of fibers formed mainly by sodium and, partially, lithium salts of DNA placed in glass capillaries [1, 2]. After a brief discussion, it was suggested that the parameters of these fibers are identical to those of native molecules of deoxyribonucleic acids. In other words, it was supposed to think that the structural parameters of the fibers formed by these salts in glass capillaries are similar to the structural parameters of individual DNA molecules, which, as their name suggests, are in the form of acids [1-3].

In addition, based on the results of these X-ray studies, a model of two spiral DNA molecules was proposed, which creates many problems in attempts to use it [2]. In particular, on the basis of this structure, it is impossible to explain both the melting of aqueous DNA and hypo- and hyperchromic effects in their UV absorption spectra. Moreover, this DNA model has formed a certain style of thinking, according to which DNA molecules exist and interact in high vacuum, both *in vivo* and *in vitro*.

Since all this is of fundamental importance, the sources of the appearance of the model of two spiral DNA molecules are analyzed here. In addition, we analyze known problems that arise when using such a model [2].

Aim

The purpose of this work is to analyze the causes of the appearance of models of two spiral DNA molecules, as well as an analysis of known problems arising from the use of these models.

Materials and methods

Sodium salt of DNA was purchased from Fluka (Switzerland); other reactive were purchased from Ukrreachim (Ukraine).

The potassium salt of DNA was obtained from the sodium salt of DNA by dissolving it in 0.1 M KCl, followed by precipitation. Precipitation was carried out in open vials stored in the dark. Under such conditions, the solution acquires a negative potential, which reduces the solubility of DNA and promotes its precipitation [4-6].

Results and discussion

Some features of the formation of salt crystals

The formation of salt crystals under various conditions has been studied in detail. In particular, it was shown that the shape of the crystals is very sensitive to the conditions in which they form [4-8]. It was also shown that the shapes of the crystals are highly dependent on the properties of the surfaces on which crystallization occurs. Thus, it was established that the crystal-forming properties of curved and smooth glass surfaces differ markedly (fig. 1,2).

Fig. 1. These are crystals formed on flat and curved glass surfaces during the drying of the Na_2SO_4 solution. On curved glass surfaces, the salt formed plant-like crystals.

Thus, taking into account the results obtained (fig. 1,2), it is necessary to recognize the special crystal-forming properties of curved glass surfaces. It can also be concluded that the legendary DNA fibers were obtained due to the special crystal-forming properties of curved surfaces of glass capillaries. Therefore, it can be supposed that modern views on the structure of DNA are partially determined by the ability of curved glass surfaces to form fibers.

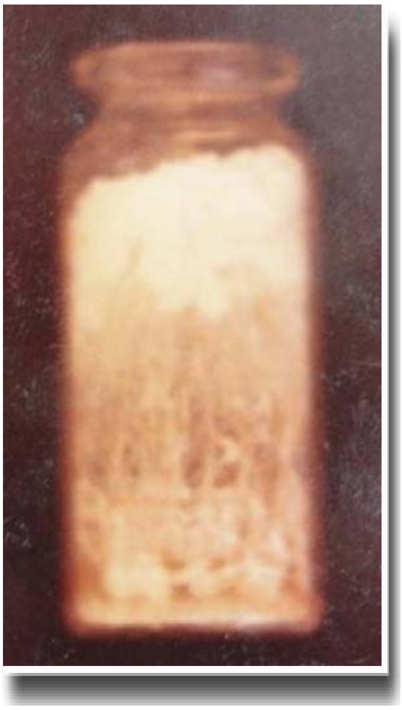

Fig. 2. Here are the serum albumin fibers formed on the surface of the curved glass. Fibers of serum albumin are not formed at the bottom of such vials, i.e. – on a flat surface.

In terms of the topic covered, it should also be noted the ability of certain substances to form spiral fibers not only on curved glass surfaces, but also near curved water surfaces (fig. 3).

Fig. 3. This is the initial stage of the formation of crystals from a solution of $CuSO_4$ near the curved water surface forming the meniscus.

As is known, potassium is the most widely represented cellular cation, and sodium cations are widely represented in cell nuclei (where their concentration is 10 times greater than in the cytoplasm [9-11]. An attempt was made to compare macrostructures of precipitation formed by sodium and potassium salts of DNA. When studying such properties of these DNA salts, it was unequivocally established that the sodium salts of DNA are able to form fibers (fig. 4, left), and potassium salts of DNA are unable to form fibers, as can be seen, they form powdered precipitates (fig. 4, right).

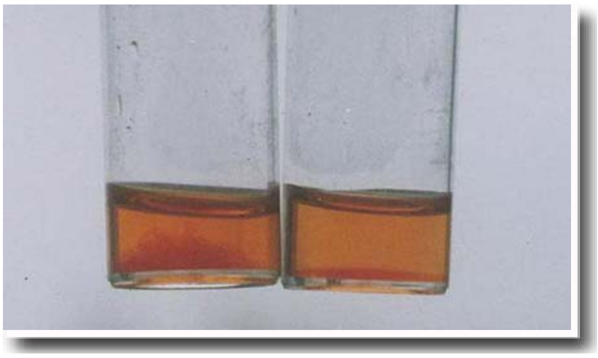

Fig. 4. This is precipitation sodium (left) and potassium (right) DNA salts. Unlike the potassium salts of DNA forming a powder, the sodium salts of DNA form fibers. Solutions contain phenazinium dye, which is added for contrast.

During additional studies it was found that sodium salts, in general, have exceptional structural properties. So, unlike potassium salts, they are able to form needle-like and fibrous crystals in various conditions (fig. 5, 6).

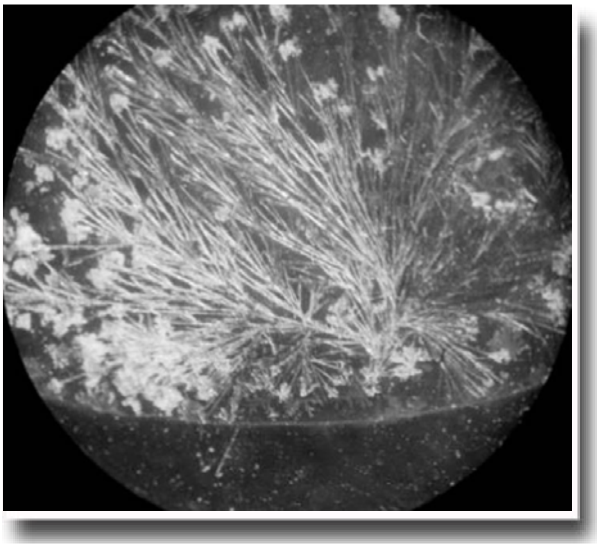

Fig. 5. The needle-shaped crystals of NaCl [4]. Potassium chloride does not form such needle-shaped crystals under the same conditions.

Thus, based on the results obtained, it is quite natural to assume that the sodium salts, in general, have the ability to form fibrous structures. For this reason, it can also be assumed that modern views on the morphology of DNA are partially determined by the morphological features of sodium salt crystals, in particular – by the ability of sodium salts to form filamentous or needle-shaped crystals.

Let's discuss the results. Taking into account the high sodium content of the nuclear plasma [9-11], it can be assumed that nuclear DNA exists in the form of sodium salts, rather than acids. Apparently, it is advisable to stipulate the probable presence of these cations in the DNA composition. This is all the more necessary because the structural parameters of the sodium salts can differ substantially from the structural parameters of the corresponding acids. The lack of attention to this is analogous to the assumption that the macro- and microstructures of HCl and NaCl are absolutely identical. Agree, this is a rather strange assumption, considering that under normal conditions, HCl is a gas, and NaCl is a solid. Also, for example, it would be difficult to agree that the macro- and microstructures of liquid H_2SO_4 and solid Na_2SO_4 have the same parameters.

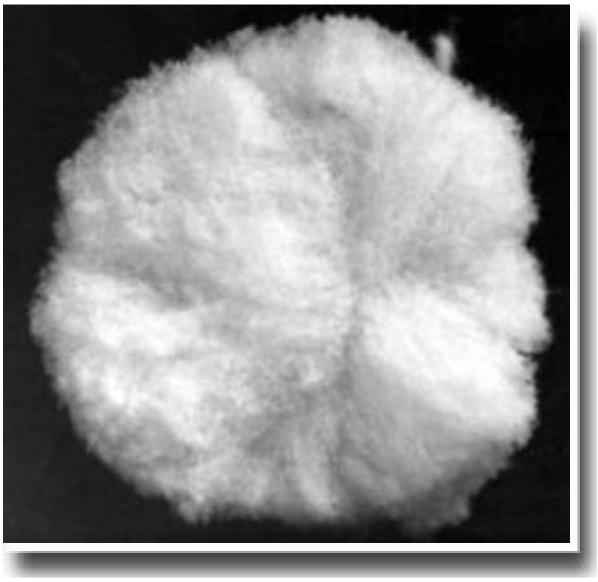

Fig. 6. The acicular crystals of NaCl formed on the surface of silica gel, pre-wetted with a water solution of NaCl; the interlacement of acicular crystals forms a kind of wool. Potassium chloride does not form such needle-shaped crystals under the same conditions [4].

Thus, based on the results obtained, two important conclusions can be drawn:

1. Non-polymeric materials, in particular sodium chloride and some sulfates, can form fibers (fig. 1,3,5,6), the most famous example being cotton candy.
2. Some polymeric materials cannot form fibers (fig. 4, right).

Let us discuss the structure and properties of DNA molecules, which were proposed on the basis of the results of X-ray studies of fibers formed by sodium and lithium salts of DNA that were placed in glass capillaries. Thus, based on these results, it was suggested that the DNA molecules have a famous double-helix structure (fig. 7) [1, 2].

As can be seen (fig. 7), DNA molecules are traditionally displayed without metallic cations. Agree, this is surprising, taking into account the composition of DNA fibers, on whose parameters, how the DNA structures of molecules are based [1,2].

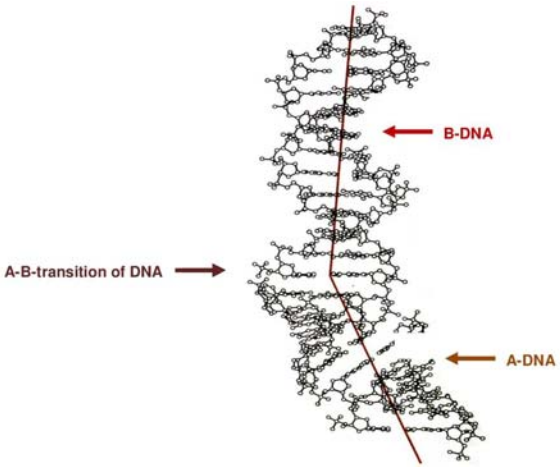

Fig. 7. This is an image of a double-stranded DNA molecule in which the A□B-conformational transition occurs [2].

Hydration of DNA

It is also surprising that images of DNA molecules are traditionally devoid of a hydrate shell. Despite this traditionally, this is not true, because, as is known, DNA molecules are highly hydrated [2]. Moreover, the relative humidity of the first DNA fibers studied was ~ 98% [1, 2], – it can even be said that the molecules of DNA salts were glued in these fibers, using structured water molecules. This is confirmed by one of the presented results (fig. 4, right) – as you can see, DNA fibers are not formed in the presence of potassium ions, which increase the mobility of water molecules and, thereby, prevent its structuring [12].

Furthermore, one can say that real DNA molecules and structured water molecules form a unique continuum, the components of which have mutual influence on each other. The existence of such a continuum should be recognized when characterizing certain properties of DNA molecules, for example, in explaining the nature of stacking interactions between adjacent DNA bases [13].

However, as it was said, the conventional images of DNA are traditionally devoid of the water component. This tradition creates misconceptions about the structure of DNA, and the effect of hydration shells on its structure and functional state. So, as is known, A□B-DNA conformational transitions cause a change in relative humidity of its fibers from 75 to 92 % or vice versa [2, 14]. But images such DNA crossings do not reflect the effect of humidity, because does not show the linked water DNA-transitions (fig. 7). Moreover, based on these figures, it can be concluded that A□B-conformational transitions of DNA occur spontaneous, without causes.

It is especially important that this tradition has formed a certain style of thinking, which in principle excludes the existence of DNA hydration shells. Moreover, this tradition had formed a representation that the DNA exist and interact in a high vacuum. It is through such representations was possible the appearance of the model of intercalation [2, 3, 15]. Causes and consequences of the emergence of this model is useful to analyze in detail.

As it is commonly believed [2, 14], DNA molecules have two hydration shells, internal and external. Also it is considered that the spaces between the bases of DNA are filled with water molecules that are part of the inner hydration shell of DNA. Thus, the spaces between any two adjacent pairs of DNA bases are not empty. For this reason, such spaces cannot be occupied “intercalators” – the proposed intercalation is possible only on models and images of dehydrated DNA (fig. 7).

Moreover, when creating such a model, it was not taken into account that the hydrated DNA envelopes are not inert structural elements, but possess properties that exclude the principal possibility of intercalation of typical intercalators. In particular, the internal hydrate shell of DNA is impermeable to cations [2]. This is an additional reason for the principle impossibility of intercalation, since typical "intercalators" (proflavine, acridine orange, ethidium bromide) are aromatic cations [2, 3, 15].

It is also worth remembering that Lerman's interpretation of intercalation arose because of the poor quality of X-ray images of fibers of the lithium salt of DNA formed from solutions containing cationic dye proflavine (fig. 8).

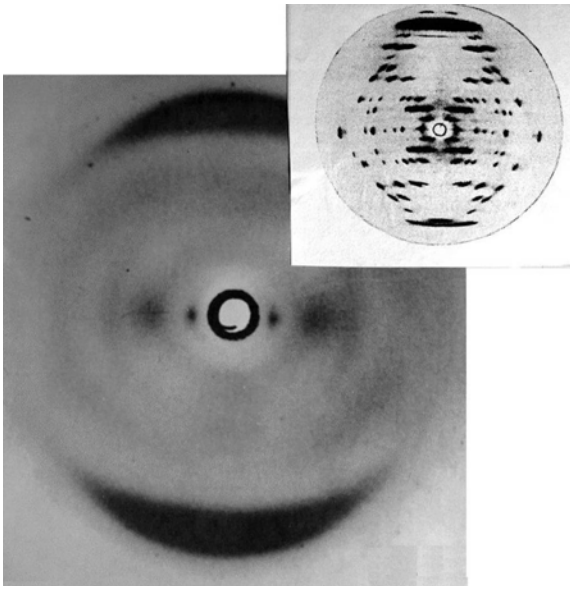

Fig. 8. This is the roentgenogram of a fiber of a lithium salt of DNA formed from a solution containing proflavine; The moisture content of this fiber was 75%. In the upper right corner is the X-ray diffraction pattern of the original fiber of the lithium DNA salt with a moisture content of 95% [15].

In explaining this deterioration in quality, Lerman ignored the fact that the original fibers of the lithium salt of DNA had 95% moisture, and the fibers modified with proflavine – 75%. Thus, Lerman drew attention to the appearance of proflavine in the studied DNA fibers, but ignored the decrease in the content of water in them [15]. Taking this into account, it can be assumed that some reflections on X-ray patterns of salt DNA fibers reflect the presence of structured water in their composition.

In addition, this deterioration in the quality of Lerman's radiographs was quite expected, since high-quality radiographs were obtained only for sodium and lithium DNA salts [2]. Replacement of lithium cations with other cations in DNA molecules (as Lerman did) will inevitably lead to a deterioration in the quality of X-ray images of fibers formed by such DNA molecules.

Thus, it should be recognized that the model of intercalation is a product of the imagination Lerman, who used images of dehydrated DNA (acid!), made in accordance with the existing graphic tradition. This is discouraging, since the model of intercalation still used to explain the mechanism of action of several antibiotics [16-23]. Taking this into account, it should be recognized that the notions of the real properties of the hydrated shells of DNA molecules have not only theoretical but also practical significance.

Thus, the thinking style that forms the traditional images of DNA molecules, ignoring both the salt nature of DNA, and the presence of hydrate shells in them, can create incredible ideas. Moreover, this style of thinking shapes the notion that DNA is an existence and interaction in a high vacuum. Let us analyze the significance of this thinking style for the denaturation of DNA, known as its thermal melting.

Views on DNA melting based on its usual imagines

Thus, as shown, the grounds on which the usual ideas about the structure and properties of DNA molecules are based are not well interpreted. Therefore, one should expect that these representations cannot adequately reflect the properties of real DNA. That this is not far from the truth, one can be convinced, using these ideas about the structure of DNA for the analysis of such a phenomenon as thermal melting of DNA.

It is known that the existing theory interprets the thermal melting of DNA as its thermal denaturation. It is also believed that an increase in the temperature of DNA solutions is accompanied by an acceleration of the movement of molecules of the aqueous medium and, as a consequence, by denaturation of double helices of DNA [2, 3].

Thus, according to existing views, thermal melting of DNA is largely a mechanical process (fig. 9) [2].

Let's speculate on this topic. The fundamental question immediately arises: how can the thermal chaotic motion of solvent molecules be transformed into the unwinding of the double helix of DNA? It is obvious that it is not easy to answer this question, since the process in which the thermal (chaotic) motion of molecules is transformed into their rotational motion is unknown [24, 25]. In addition, during this unknown process, solvent molecules that contact the opposite ends of the DNA molecule must rotate these ends in opposite directions – clockwise and counterclockwise. Agree, for this they must be well informed, at least. If it is additionally considered that the complementary A-T pairs melt at a lower temperature than the complementary C-G pairs [2, 3], then it is impossible to represent the melting of DNA based on its images.

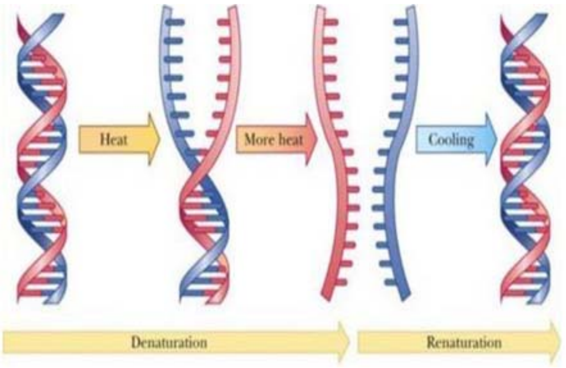

Fig. 9. This is an image of the processes of thermal denaturation (melting) of a double-stranded DNA molecule and its renaturation (upon cooling) [2].

In addition, during this unknown process, if it exists, the direction of rotation of individual DNA strands should change with decreasing temperature – obviously, this is necessary for the interweaving of separate strands of DNA into a double helix (fig. 10). (It should also be taken into account that individual strands of DNA must first meet with each other.)

It must be admitted that these representations are irrational. It is also necessary to recognize that they prevail, despite the facts that cannot be explained solely by mechanical models. As well, they cannot explain the opposite influence of sodium chloride and sodium hypochlorite on the DNA thermal stability, – as it is known, the first salt decreases and the second salt increases of them [2,3]. As you can see, DNA molecules must be well informed in order to choose different substances.

(In addition, they should also be able to select "intercalators" – as shown in Figure 8, complementary DNA strands are unraveled in their presence [2, 3], – thus, DNA molecules must be very clever, in accordance with the properties that their models possess.) So, for the productive use of conventional models of DNA molecules, one must assume that they have a mind. Since this is incredible, it is necessary to correct the traditional notions of both thermal denaturation of DNA and its thermal renaturation.

Since all these problems were not solved [26], it was suggested to replace the double-helical DNA models in the "side by side" model, which represent DNA molecules in the form of two complementary chains that do not interlace [27], – an adequate impression of this structure is given by the position of two complementary DNA strands in Figure 8 (without intercalators). Due to this substitution, thermal melting of DNA can be represented as a simple dissociation of its complementary chains, resulting from the destruction of hydrogen bonds between complementary bases [2]. Obviously, renaturation of DNA can be represented, in this case, as the association of its complementary chains (without their intertwining) [2,27]. As can be easily verified, the DNA "side by side" models are also devoid of many other drawbacks inherent in the family of double-stranded DNA [2].

Whatever it was, it must be acknowledged that the proposed structures of two spiral DNA molecules (fig. 7,10) are "poorly functioning". It is therefore not surprising that they cannot satisfactorily explain the causes of hypo- and hyperchromic effects of aqueous DNA solutions observed in their UV absorption spectra. Thus, these DNA models do not allow satisfactory explanation of a number of properties of real DNA, including their melting, as well as the nature of hypo- and hyperchromic effects in the UV absorption spectra of aqueous DNA solutions [28-30].

Conclusion

The results of experiments on which modern ideas about the structure and properties of DNA molecules are based were poorly interpreted. For this reason, the models of double DNA spirals, which were the result of these interpretations, "function poorly". Thus, these DNA models do not allow satisfactory explanation of a number of properties of real DNA, including their melting. For this reason, the concept of the existence of double-stranded DNA should be revised.

Feather research perspectives

Based on the results obtained, to create a DNA model that better reflects the morphological features of real DNA. So, it can be waiting that thanks to the most successfully DNA models can be explained of a number of properties of real DNA, including their melting, as well as the nature of hypo- and hyperchromic effects in the UV absorption spectra of aqueous DNA solutions [4,28-30].

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Пивоваренко Ю.В. Легенди про морфологію ДНК.

РЕФЕРАТ. Підґрунтя. Вважається, що сучасні погляди на структуру молекул ДНК ґрунтуються на результатах експериментів, які мають бездоганне тлумачення. Це не зовсім правильно, оскільки результати цих експериментів мають більш ніж одну інтерпретацію. **Мета роботи.** Обговорення правильності інтерпретації експериментальних результатів, на підставі яких була запропонована модель двохланцюгової молекули ДНК. **Результати.** Показано, що сучасні погляди на структуру молекул ДНК переважно визначаються здатністю солей натрію утворювати волокна, а також спроможністю вигнутого скла утворювати волокна на його поверхні. Крім того, проведено аналіз існуючих поглядів на плавлення ДНК та на інтеркаляцію ароматичних барвників до їх структур. **Підсумок.** Результати експериментів, на яких базуються сучасні уявлення про структуру та властивості молекул ДНК, були погано інтерпретовані. З цієї причини, моделі подвійних спіралей ДНК, які з'явилися внаслідок такої інтерпретації, "погано функціонують". Тобто, ці моделі ДНК не дають задовільного пояснення ряду властивостей реальної ДНК, включаючи їх плавлення. З цієї причини, результати експериментів, на підставі яких була запропонована двох-ланцюгова модель молекули ДНК, повинні отримати нову інтерпретацію, яка дозволить змінити уявлення про морфологію молекул ДНК та наблизити її до реальності.

Ключові слова: ДНК, структура ДНК, плавлення, інтеркаляція.

Пивоваренко Ю.В. Легенды о морфологии ДНК.

РЕФЕРАТ. Обоснование. Считается, что современные взгляды на структуру молекул ДНК основаны на результатах экспериментов, которые имеют безупречное толкования. Это не совсем правильно, поскольку результаты этих экспериментов имеют более чем одну интерпретацию. **Цель работы.** Обсуждение правильности интерпретации экспериментальных результатов, на основании которых была предложена модель двух-цепочечной молекулы ДНК. **Результаты.** Показано, что современные взгляды на структуру молекул ДНК преимущественно определяются способностью солей натрия образовывать волокна, а также способностью изогнутого стекла образовывать волокна на его поверхности. Кроме того, проведен анализ существующих представлений о плавлении ДНК и об интеркаляции ароматических красителей в их структуры. **Заключение.** Результаты экспериментов, на которых базируются современные представления о структуре и свойствах молекул ДНК, были плохо интерпретированы. По этой причине, модели двойных спиралей ДНК, которые появились вследствие такой интерпретации, "плохо функционируют". То есть, эти модели ДНК не дают удовлетворительного объяснения ряду свойств реальной ДНК, включая их плавления. По этой причине, результаты экспериментов, на основании которых была предложена двух-цепная модель молекулы ДНК, должны получить новую интерпретацию, которая позволит изменить представление о морфологии молекул ДНК и приблизить их к реальности.

Ключевые слова: ДНК, структура ДНК, плавление, интеркаляция.