Experiments are described, showing that DNA solutions with lithium chloride present a hypochromicity, which increases with the molarity of the salt. Different absorbancy versus temperature curves are obtained for different solutions, and it is found that the melting point ($T_m$) increases with the lithium chloride concentration. When cooling the samples, the absorbancy decreases in a symmetric way.

In accordance with this model, a theory of DNA unwinding is proposed, assuming that before the unwinding, it is necessary a stretching of the molecule which eases this process. An extension of these ideas are applied to the transcription DNA-RNA operation.

We have communicated previously that the U.V. absorbancy (at 260 m\(\mu\)) of polyadenylic acid in water solutions, shows hyperchromism after the addition of sodium potassium or lithium chloride at high molarities. This hyperchromism, for instance, is of the order of 18\% for 0.5 – 1 M solutions. Control solutions of adenylic acid (2', 3' phosphate) do not change its absorbancy in the presence of sodium chloride of lithium chloride at high molarities. On the other hand, water solutions of DNA with high molarities of salts (e.g. 0.36 – 3 M. lithium chloride) shows hypochromism. All these facts suggest, that the polynucleotides may have a molecule capable of undergoing big structural changes, and it is therefore that we believe that a treatment of DNA unwinding, should not start by considering this polynucleotide a rigid molecule. According to this we propose a dynamic model of the unwinding, in which we first have a necessary elongation of the molecule with loosing of secondary and tertiary structure and then the proper unwinding takes place.

This previous elongation appears to be necessary so that the whole process might happen in a very short time (1 – 2 minutes), this means small energy requirements.

As an experimental basis for our hypothesis we measured the melting points of pure DNA solutions and DNA solutions with LiCl added, what means measurement of $T_m$, in a molecule with small and large secondary and tertiary structure.

We also studied the DNA unwinding after cooling.

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Material and Methods

We used water solutions ($pH$: 6) of highly polymerized salmon sperm DNA (Mann Res. Lab.) and the experimental solutions were prepared by adding lithium chloride. The U.V. absorbancy of the DNA solutions at different temperatures were measured in a Beckman DU spectrophotometer, and a constant volume of the solutions was maintained.

Results

As can be seen in the curve shown in Fig. 1,

1) There is an initial hypochromism in the lithium chloride DNA solutions, and this hypochromism is bigger the higher is the molarity of the salt;

2) The behaviour of the DNA temperature melting curves can be analyzed into two regions. In the first one these curves are flat for the heated DNA water solution and have positive slope for the heated DNA-lithium chloride solutions, the slope being the steeper the bigger is the lithium chloride molarity of the solutions. The second region shows different melting points ($T_m$) for these solutions, being higher in the DNA solutions with a bigger lithium chloride molarity. The steeper in this region is also obtained for the concentrated salt solutions of DNA;

3) When cooling the samples (during a period of three hours) we observe (Fig. 2) that DNA solutions in higher lithium molarities have first a steeper (negative) slope than the water DNA solutions and then these slopes decrease, thereby showing a symmetry between the melting DNA curves and those obtained when cooling the DNA.
Discussion

These facts can be interpreted, as if the molecule of the DNA in lithium chloride solution, with a big secondary and tertiary structure (initial hypochromism) has to be stretched during the first part of heating, before the starting of the unwinding of the two strands (2nd part). When cooling, the inverse process should happen, first the rewinding and after that, the recovery of the molecular secondary and tertiary structure. Similar molecular DNA change could occur in vivo. Before DNA unwinding, ionic variations can induce the ionization of the nucleotide phosphates and a repulsion of the adjacent nucleotides, because of the electronegativity of these radicals; and by this way, the DNA molecule could be stretched. As the repulsive force acts, on each one of the two DNA strands (not always simultaneously, being the chains antiparallel), the repulsion could have different direction and magnitude, on both sides of the molecule, inducing an elongation weakness and breakage of the bases hydrogen bonds (l.c. 3–5). This stretched DNA, with less molecular water included, has a smaller radius than the molecule at rest and is in very favourable conditions, for the unwinding process, because it needs less energy for it \[E = 16 \pi^2 n^2 \eta p^2 \lambda / T\], that, in accordance with the Watson-Crick model and the calculations

4 S. Lewin, Biochem. J. 95, 44 [1965].
of Levintthal and Crane; and others. On the other hand, in the elongated and rigid DNA, we can divide for simplicity, the topological circular unwinding movement, in two passive and successive displacement; a vertical and a transversal one, by which each stiff strand, comes back every time to a rest position, after beaking the hydrogen bonds; Fig. 3. As a sequence of these two movements we have the unwinding of a cross point. Even we assume that for the achievement of this process, it is necessary to take advantage, of the thermal energy of the system. In the addendum we calculate, the energy consumed in this mechanical process of unwinding and as shown, is lower than the thermal energy of the system at 300 K. The calculation was done, for a DNA of a molecular weight of $10^8$ and for one minute unwinding time. If, following the idea worked out by the Argonne Nat. Lab. Biologists (Argonne Nat. Lab. Annual Report, 1964, p. 66), on the existence in the DNA, of a “master” strand which carries the genetic information and being the other strand “messenger”, then, we can assume in this kind of model, that the principal strand could remain, during the unwinding time attached to the nucleoprotein, maintaining its structure and the secondary strand released from the nucleoprotein, stretched and then separated by a trivial way. (See Fig. 4.) The fact described by us, that in the presence of concentrated sodium chloride there is a hyperchromism in polyadenylic solutions and hypochromism in DNA solutions, suggests the existence of a similar mechanism for the DNA transcription to mRNA. In that case, the DNA should conserve its structure in the presence, for instance, of sodium chloride and the mRNA elongated by the ion effect, could be released from the DNA molecule (see Fig. 5). It is possible to discuss about the space, occupied in the cell, by this stretched DNA, but that objection which is valid also for other theories, can be solved, if we accept foldings or (and) breakages of the molecule. In agreement, with the well known phenomena of breakage and recombination of DNA, in vivo.

We have not discussed in this paper the hypothesis of DNA duplication of Fong, in which he suggests a DNA replication, with rotation of the bases of each strand and simultaneous pairing with a new complementary bases, at each side; because that model is not applicable to in vitro DNA dissociation.

Fig. 3. Perpendicular and transversal displacements of DNA stretched strands, when unwinding.

Fig. 4. Separation of the stretched “messenger” DNA, from the “master” one.

Fig. 5. Separation of an elongated mRNA, from the DNA, after transcription.

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8 W. Kuhn, Experientia [Basel] 13, 301 [1957].
10 M. Fixman, J. molecular Biol. 6, 39 [1963].
Addendum

Comparison of the energy necessary for the mechanical process of the DNA unwinding and the thermal energy of the system at 300 °K.

We shall consider a DNA of $10^8$ M.W., which has around $10^5$ nucleotides in each strand and $2 \times 10^4$ turns, with a length of $3.4 \times 10^5$ Å and a total unwinding time of one minute. For simplicity, we are considering the unwinding’s circular displacement of the strands (after the breakage of the hydrogen bonds), resolved in two movements, one in a vertical plane (with respect to a molecular axis) and the other in a transversal one. In this displacements, each one of the strands goes over a path, that, for the unwinding of the first crossing (turn), is the only some angstroms and for the last one, cannot be greater than the length of the strand. This follows from the fact, that a radian corresponds to an angle, of around $57^\circ$ and it is obvious, that the angle between the two strands at the crossing point, is always very much smaller than $114^\circ$. Then, the mean distance $\bar{s}$, travelled in the oscillation of each strand, when unwinding one turn, is less than $3.4 \times 10^5$ Å, so, the calculated kinetic energy for each strand, in the total DNA unwinding is $2 \times 10^{-16}$ ergs, and it follows that $\frac{1}{2} m v^2 \ll \frac{1}{2} kT$ (300 °K).

In this calculation we have used, relations of the kinetic theory of gases and we assume, a non random movement of each strand, because the rigidity of the model accepted when unwinding.

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