

## Preparation of Oriented DNA by Wet Spinning\*

ALLAN RUPPRECHT

*Bacteriological Bioengineering, Department of Bacteriology, Karolinska Institutet, Stockholm 60, Sweden*

A method for the preparation of films of oriented DNA is described. It involves as a first stage the wet spinning of DNA and as a second stage the drying of the spun DNA deposit to an apparently homogeneous film. The method is convenient for the preparation of large samples which are suitable for physico-chemical investigations of oriented DNA. Birefringence, dichroism and X-ray diffraction pattern all indicate that the molecular orientation of the films is very good.

For many physico-chemical investigations on macromolecules it is a great advantage to utilize samples with a high degree of molecular orientation. In the case of deoxyribonucleic acid, or DNA, which in the capacity of the carrier of genetic information attracts a great deal of interest, two kinds of solid samples with good orientation have been prepared.

Thin films of oriented DNA, made by shearing a gel between a coverslip and slide, or by stroking the gel with a spatula, have been used for birefringence and dichroism studies<sup>1-3</sup> which among other facts have given information regarding structural changes of DNA as a function of the relative humidity.

Fibres of oriented DNA, 50–100  $\mu$  in diameter, made by slowly withdrawing a pointed glass rod from a sticky DNA gel,<sup>4</sup> have been used for X-ray diffraction studies which have played a decisive role in the determination of the DNA structure.<sup>5</sup>

An attempt to prepare oriented DNA in larger amounts was made by Wyckoff<sup>6</sup> who improved the fibre drawing method by laying the fibre tangentially on a rotating four prong spindle, which continued to draw fibres for 2–7 seconds before breakage. In one hour a circular bundle of parallel fibres weighing about one milligram was obtained. This method, which could be characterized as dry spinning of DNA, was also successfully employed by Marvin.<sup>7</sup>

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In this laboratory a method has been developed, which gives oriented DNA in large amounts and with a macroscopically uniform orientation. The films prepared are convenient for physico-chemical investigations. As is outlined in a preliminary note,<sup>8</sup> the method involves the wet spinning of DNA as a first stage, whereby the DNA fibres formed are wound on a cylinder, which is given a slow axial motion back and forth between desired limits. As a second stage the DNA deposit is dried on the cylinder giving a film of oriented DNA. After the publication of this note the method has been developed and a more complete description is given below.

#### FIBRE SPINNING AS A MEANS OF ACHIEVING MOLECULAR ORIENTATION

Fibre spinning is of great technical importance and an increasing number of chain molecules are utilized. In the "wet spinning" process a fibre-forming chain molecule solution is continuously extruded through a spinneret orifice into a precipitating liquid. During the precipitation, the chain molecule solution thread is stretched as it flows down and is thereby transformed into a solid fibre, which is wound up by a rotating take-up device. The same scheme is in principle applicable to "dry spinning" where the fibre solidification process is caused by the evaporation of the volatile solvent of the chain molecule solution, and to "melt spinning" where the fibre solidification process is caused by the cooling of a polymer melt. Some technical spinning processes have been investigated for the influence of the spinning variables on the properties of the fibres. Among other facts it has been found that a molecular orientation is always achieved during fibre spinning.<sup>9-12,16</sup>

Many theoretical considerations on fibre spinning have been published during recent years, especially by Ziabicki and coworkers,<sup>13-16</sup> and these are in good agreement with experimental results. Ziabicki<sup>14</sup> has derived that the velocity field shown in Fig. 1 should be approximately valid for all the three types of spinning processes. According to this theory, the fibre-forming molecules are subjected to two kinds of orientation. In the spinneret channel,

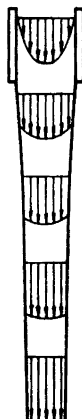


Fig. 1. Profiles of stream velocity in fibre spinning according to Ziabicki.<sup>14</sup>

orientation occurs in a velocity field with perpendicular gradient, a process which involves shearing. The orientation achieved in this region is, however, destroyed immediately below the outlet of the channel and does not contribute to the molecular orientation in the spun fibre. The second kind of orientation occurs in the velocity field with parallel gradient, which prevails during the fibre formation. This is the true fibre spinning orientation process. Some theoretical considerations have been made recently by Takserman-Krozer and Ziabicki<sup>17,18</sup> to shed light on its nature.

An important factor governing the degree of orientation in the fibre is, according to Ziabicki, a radial viscosity gradient accompanying the solidification process; in melt spinning caused by a temperature gradient, in dry spinning by a concentration gradient and in wet spinning by heterogeneous diffusion processes. This radial viscosity gradient does not lead to the appearance of a radial velocity gradient, as we have seen, but to the corresponding stress distribution. The result of this probably appears in the final fibre as a non-uniform distribution of the degree of orientation.

The results reproduced here show that fibre spinning is suitable for the purpose of achieving molecular orientation of chain molecules.

#### WET SPINNING OF DNA

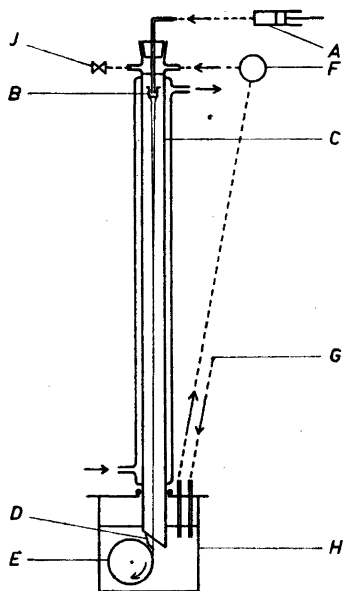
DNA consists of two interwoven helical chain molecules built up of phosphate + sugar + base repeating units.<sup>19</sup> The bases point inwards to the axis of the helix and are hydrogen-bonded to form planar pairs adenine + thymine and guanine + cytosine. DNA is soluble in water and stable around neutral pH and at temperatures below 80–100°C, the presence of electrolyte also being necessary for stability. DNA is precipitated reversibly in the form of a fibrous deposit by alcohol. This precipitation reaction has been utilized in the DNA spinning process.

The DNA solution used for the spinning was, in the case of NaDNA, prepared by dissolving thymus NaDNA (Worthington) in an aqueous solution of NaCl with the aid of a homogenizer, which consisted of a Teflon piston ( $d = 18$  mm) in a glass cylinder ( $d = 19$  mm). The DNA concentration was adjusted to 0.2–1.0 mg/ml and this was measured with a spectrophotometer. In the case of LiDNA thymus NaDNA was dissolved in 3 M LiCl, and was dialyzed against 3 M LiCl. The outer solution was renewed every eight hours, four times in total. After dialysis the LiDNA was precipitated by the addition of alcohol and was then redissolved in an aqueous solution of LiCl to give a similar concentration as in the case of NaDNA.

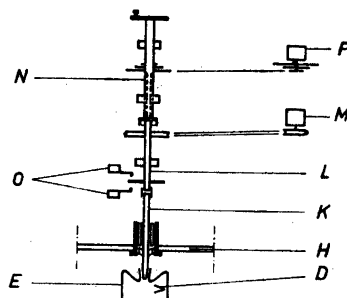
Before use the DNA solution was degassed under vacuum.

As precipitating liquid in the wet spinning of DNA, ethyl alcohol has been used at a concentration of 70–85 %. It contained NaCl in the case of NaDNA spinning and LiCl in the case of LiDNA spinning.<sup>20</sup> Before use the precipitating liquid was degassed under vacuum.

The spinning apparatus is shown in Fig. 2. A precision pump (A) continuously extrudes DNA solution through the orifices of the spinneret (B) into the precipitating liquid in the thermostatically controlled glass column (C) ( $d = 45$  mm,  $l = 1000$  mm). The resulting DNA fibres flow down the glass column and are converged by a fixed V-shaped guide (D), which also directs the bundle of DNA fibres onto the Teflon-coated surface of a rotating cylinder (E). During rotation the cylinder performs a slow axial motion back and forth between desired limits and a deposit of layers of almost parallel DNA bundles is built up. To maintain the alcohol concentration at the top of the glass column the precipitating liquid is slowly recycled by a piston pump (F) made of glass and Teflon. In spinning experiments of long duration alcohol and electrolyte are added (G) to maintain the overall alcohol and electrolyte concentration.



*Fig. 2.* Scheme of the DNA wet spinning apparatus. A, precision pump; B, spinneret; C, glass column; D, V-shaped guide; E, Teflon-coated cylinder; F, recycling pump; H, glass vessel; J, valve.



*Fig. 3.* Scheme of the auxiliary equipment as seen from above. D, V-shaped guide; E, Teflon-coated cylinder; H, glass vessel; K, glass shaft; L, driving shaft; M, driving motor for shaft L; N, screwed shaft; O, micro-switches; P, driving motor for shaft N.

The guide (D) is made of a Teflon-coated platinum/iridium wire, which is bent in a V-form. It is fastened at the outlet of the glass column immediately above the rotating cylinder, as is also indicated in Fig. 3.

A glass cover on the vessel (H) prevents dust entering during the spinning operation. It is made in two halves, the anterior of which can be removed for manipulations in the vessel.

The bearing arrangement for the rotating cylinder and its auxiliary equipment is shown in Fig. 3. The cylinder (E) is attached with a ground joint to a precision ground glass shaft (K) ( $d = 10$  mm) the bearing of which, a precision ground glass socket lubricated with silicone grease, is fastened tightly in the wall of the vessel (H). A Teflon seal protects against leakage of the precipitating liquid, without preventing the rotational and axial motion of the glass shaft. A driving shaft (L) to which rotation is transmitted from a driving motor (M) by a long driving belt, is connected to the glass shaft and this shaft system can move axially. The axial motion is controlled by another screwed shaft (N) connected to the driving shaft *via* a ball bearing, an arrangement which permits independent rotational motion of the two shafts, the axial motion, however, being common to both shafts. The change of direction of the axial motion is governed by microswitches (O) activated by a round plate attached to the driving shaft. When an endpoint is reached a microswitch gives an impulse to a relay, which changes the rotational direction of the driving motor (P) for the screwed shaft, and hence the direction of the axial motion.

For convenience the spinning apparatus has been supplied with an electric counter connected to the endpoint relay controlling the axial motion of the rotating cylinder. In this way the number of axial sweeps are automatically recorded. The apparatus has

also been supplied with a stop relay controlling the whole apparatus. This relay can be activated by an electric clock, an endpoint switch for the pump (A) or the electric counter mentioned above. In this way the spinning can be stopped after a predetermined time or when the pump (A) is empty or after a predetermined number of axial sweeps. In addition a connection can be arranged between the stop relay and the endpoint relay controlling the axial motion of the rotating cylinder. The signals from the activating mechanisms mentioned earlier to the stop relay are thereby overwritten until an endpoint of the axial motion is reached, and the last axial sweep will be completed before the spinning is terminated.

When starting spinning the following procedure was used. The spinneret was mounted on its holder, a Teflon plug attached to the glass capillary tube connected to pump (A). This system was then filled with DNA solution which was filtered through a sintered glass filter contained within the Teflon plug. The outer surfaces of the spinneret were cleaned with water before mounting the holder at the top of the glass column. By opening the valve (J) which was connected to a water jet pump, the precipitating liquid from the vessel (H) was sucked up into the glass column. When the liquid surface had reached the top, the valve was closed and the precipitating liquid was thereby trapped. DNA solution was thereafter supplied at a high feed rate which caused the bundle of DNA fibres formed to move rapidly down the glass column, a glass rod being used to direct the fibres *via* the guide onto the rotating cylinder. The feed rate of the DNA solution was then diminished to the desired value to be used in the spinning.

A disturbing phenomenon was found to appear after one or two hours of spinning. Small isolated air bubbles formed in the column and gathered below the surface of the flat spinneret. This showed that air was rapidly dissolved in the precipitating liquid *via* the liquid surface in the vessel (H) and was then released in the column, where DNA solution was supplied. This phenomenon was eliminated by inserting the *degasser* shown in Fig. 4 between the vessel (H) and the pump (F). The degasser, which is made of glass, is *via* a Teflon tube connected to a side arm, which in operation dips down into the liquid in the vessel (H). Every 15 min a relay stops the circulation pump (F) and actuates a magnetic valve, which is also connected to the water jet pump, so that the degasser is evacuated through the connection (Q). Thereby precipitating liquid is sucked through the glass valve (R) and a sintered glass filter and from there passes *via* the glass valve (S) into the degasser. During filling the liquid is degassed by the action of flow and vacuum. When the liquid surface in the degasser has reached the glass bulb (T), which is connected to the valve (S), this lifts and the valve (S) is closed. After 1 min the relay starts the circulation pump (F) and simultaneously connects the degasser to atmospheric pressure by releasing the magnetic valve. Pump (F) now pumps precipitating liquid through valve (U) to the top of the column. After 14 min the degasser is nearly empty and the cycle starts again. A slow-speed electric motor with a cam on its axle controls the relay *via* a microswitch and makes the degasser work automatically as long as spinning proceeds. The degasser contains about 1 l of liquid and the time intervals used are dictated by the speed of the pump (F) and the rate of filling the degasser.

It has been found that the pump (F) can be excluded if a suitable manostatically controlled over-pressure is used in the degasser during the pumping periods. Then the liquid will be forced out through the valve (U) to the top of the column instead of being pumped.

Another phenomenon, which has always been observed during spinning, is a tendency of the fibres to bow inwards towards the center of the DNA fibre bundle immediately below the spinneret. It has been suggested<sup>21</sup> that as the bundle of DNA fibres "draws" precipitating liquid down the center of the column a compensating upward flow near

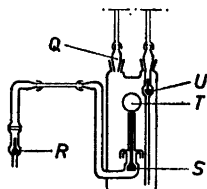


Fig. 4. Scheme of the degasser. Q, ground joint; R, non-return valve; S, valve, connected to float T; U, non-return valve.

the wall must occur. At the spinneret the upward flow turns into the central flow down the column and the peripheral fibres are thus forced to a more central position. However, the phenomenon is also observed when extruding pure solute without DNA. This indicates that the main mechanism is probably physico-chemical in nature, diffusion and surface tension playing a role. No drawback connected with this phenomenon has so far been observed.

Table 1. Values of processing variables used in the preparation of oriented LiDNA and NaDNA.

	LiDNA film	NaDNA film
DNA solution	0.6 mg LiDNA/ml in 0.4 M LiCl, (O.D. <sub>260</sub> = 13.0)	0.8 mg NaDNA/ml in 0.4 M NaCl, (O.D. <sub>260</sub> = 16.4)
Spinneret	Glass spinneret (PAN*) with 300 cylindrical channels each 100 $\mu$ diam. and 1.5 mm length	Glass spinneret (PAN*) with 720 cylindrical channels each 70 $\mu$ diam. and 1.5 mm length
Feed rate of the DNA solution	30 ml/h	45 ml/h
Mean linear velocity of the DNA solution in the spinneret orifices, $w_1$	21.3 cm/min	27.1 cm/min
Precipitating liquid	82–83 % ethyl alcohol containing 0.4 M LiCl	72–73 % ethyl alcohol containing 0.4 M NaCl
The rotating cylinder		
Diameter	96 mm	100 mm
Speed of rotation	18 rev/min	18 rev/min
Axial velocity	2.25 mm/min	2.06 mm/min
Axial displacement/rev	0.125 mm	0.115 mm
Linear take-up velocity of the DNA fibres on the cylinder, $w_2$	543 cm/min	566 cm/min
Deformation ratio, <sup>16</sup> $S = w_2/w_1$	25.5	20.9
Duration of spinning	1 h, 33 min	1 h
Number of single sweeps	84	41
Spinning temperature	+25°C	+25°C
Drying process	Dialyzation of spun de- posit for two days at +5°C in 80 % ethyl alcohol containing 0.25 M LiCl.	Dialyzation of spun de- posit for 24 h at +5°C in 73 % ethyl alcohol containing 0.1 M NaCl.

\* Paul Aschenbrenner Pan-Apparatebau GmbH, Müllheim i.B.

Table 1 continued.

	Drying for 24 h at +5°C over 0.7 g silica gel. Thereafter storage for 3 days at +25°C and 66 % r.h. before removal of the film from the cylinder.	Drying for 24 h at +5°C over 0.7 g silica gel. Thereafter storage for two days at +25°C and 75 % r.h. before removal of the film from the cylinder.
Approximate number of DNA fibres forming the film	$5.0 \times 10^5$	$7.8 \times 10^5$
Cross section dimensions of the film		
Thickness	$40 \pm 3 \mu$	$63 \pm 4 \mu$
Breadth	$2.9 \pm 0.2 \text{ mm}$	$3.7 \pm 0.2 \text{ mm}$
% r.h.	66	75
Average fibre diameter, estimated from the values above	$0.5 \mu$	$0.6 \mu$
Comment		Longitudinal stripes were observed on the NaDNA film. Use of a slightly increased axial displacement per revolution of the cylinder during spinning is therefore recommended.

Many processing variables are involved in the wet spinning of DNA but no complete investigation of the influence of the processing variables on the degree of orientation of the DNA films prepared has as yet been made. The values of the processing variables used are the best found in non-systematic runs in the search for good spinning conditions. Among other things it has been found, that the use of a high deformation ratio<sup>16</sup> in the spinning can cause damage to the DNA molecules. Two examples of the values of the processing variables used are given in Table 1.

#### THE DRYING PROCESS FOR THE DNA DEPOSIT

The DNA deposit on the cylinder consists of a large number of minute almost parallel DNA fibres, containing water, alcohol and electrolyte. To transform the fibres into a DNA film the deposit must be dried with respect to the alcohol and the surplus of water, but first the electrolyte content must be adjusted to the desired value by "dialyzation". The process used was as follows.

The cylinder with the DNA deposit was released from the glass shaft of the spinning apparatus and transferred to a beaker containing alcohol solution of predetermined water and electrolyte concentrations. The beaker was then placed in a refrigerator at about +5°C and under slow stirring the water and electrolyte of the spun deposit were allowed to equilibrate with the concentrations used in the alcohol. After some days the cylinder was removed from the beaker, the excess of liquid was carefully dried off with a piece of adsorbant paper without touching the spun deposit and the cylinder was placed in a desiccator containing a few grams of silica gel. By absorption in silica gel, the alcohol and some of the water are slowly removed from the

DNA deposit and the individual DNA fibres thereby "fuse" together and form a homogeneous film. The shrinking of the deposit required, to avoid cracking, a movement of the hydrophilic DNA molecules across the cylinder surface. The resistance to this movement was reduced to a minimum by the hydrophobic Teflon-coating of the cylinder. The cracking tendency was also reduced by the use of slow drying and this was especially important for thick and broad preparations. By adding the silica gel in portions the rate of drying could be controlled. After drying the desiccator was brought to room temperature and later supplied with a salt solution to achieve the desired relative humidity. The film was easily released from the cylinder by cutting across the strip with a razor-blade.

Lower drying temperatures, down to  $-20^{\circ}\text{C}$ , were not suitable for broad and thick preparations because of an increased cracking tendency. The cracking tendency was also influenced by the axial displacement per revolution of the rotating cylinder, which determines the crossing angle between the bundles of DNA fibres. As should be expected the cracking tendency decreased with an increased axial displacement per revolution of the cylinder. It has also been found that NaDNA is worse in this respect than LiDNA, a higher axial displacement per revolution being required in the case of NaDNA to avoid cracking of the film during drying.

The drying procedure can be modified in many ways, for example a slow stream of dry or humid nitrogen can be used instead of silica gel. Further work is needed to clarify how the molecular orientation and crystallinity of the DNA deposit are influenced by the different procedures constituting the drying process. Two examples of the processing variables used in the drying process are given in Table I.

The drying process seems to have little effect on the DNA molecules. Oriented DNA films have thus been redissolved to undergo the spinning and drying process two or three times without appreciable decrease in spinnability. Even the transforming activity of DNA was preserved in the preparation of oriented DNA as was demonstrated for *Bacillus subtilis*-DNA in the preliminary note.<sup>8</sup>

#### ORIENTATION TESTS

Fibres obtained by spinning show *birefringence* in the visible region of the spectrum and many authors have utilized this birefringence as a relative measure of the degree of molecular orientation.<sup>10,12,16</sup> Provided that the molecular orientation of the DNA fibres is retained during the drying process, one should expect the films to be birefringent. In fact, all the preparations made showed a strong birefringence in the visible region of the spectrum.

Another optical property exhibited by oriented DNA is a *negative dichroism* in the ultraviolet region of the spectrum as has been demonstrated by others on preparations made by shearing a gel between a coverslip and slide, or by stroking a gel with a spatula.<sup>1-3</sup> By spinning directly on quartz slides attached to the surface of the rotating cylinder, thin films of oriented DNA suitable for optical investigations have been prepared. As reported previously,<sup>8</sup> when a film spun from a single fibre was made, the measurements of the absorbance



with polarized light at a wave length of 2700 Å gave at 93 % r.h. a dichroic ratio,  $R = A_{\parallel}/A_{\perp} = 0.28$ , where  $A_{\parallel}$  and  $A_{\perp}$  are the absorbancies with the electric vector of radiation polarized parallel and perpendicular, respectively, to the direction of molecular orientation.

This value of the dichroic ratio is comparable with the lowest ones found earlier and it indicated qualitatively that the molecular orientation of the film was good.

Thicker films are not suitable for optical investigations because of their great optical absorbance. In this case it is convenient to use *X-ray diffraction* to test the orientation. Two films were prepared for this purpose using the values of the processing variables given in Table 1.

By folding the films back and forth in a special apparatus two "packages" of oriented DNA were prepared, consisting of 10 layers of the LiDNA film and 7 layers of the NaDNA film, respectively. After storage for two months of the concertina-like LiDNA package in an atmosphere of 66 % r.h., it was compressed and then mounted in a wide angle X-ray diffraction camera, the direction of molecular orientation being perpendicular to the central beam, and was exposed at 66 % r.h. Fig. 5a shows the wide angle diffraction pattern obtained. Similarly the NaDNA package was stored for four days in an atmosphere of 75 % r.h. and was exposed at the same relative humidity giving the diffraction pattern shown in Fig. 5b. The diffraction camera used in these experiments had a glass capillary collimation of 300  $\mu$  and a specimen to film distance of 47 mm. Nickel filtered  $\text{CuK}\alpha$  radiation was used, the exposure time being about 4 h.

A comparison with earlier X-ray diffraction studies on DNA fibres<sup>5-7,22,23</sup> shows that the values of the processing variables chosen have given the crystalline B form of DNA in the LiDNA film and the crystalline A form of DNA in the NaDNA film. It further shows that the films have a very good

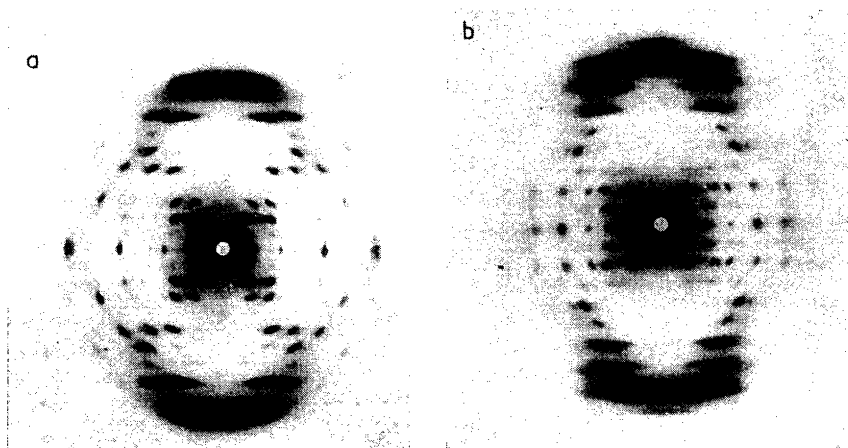


Fig. 5. Wide angle X-ray diffraction patterns of oriented LiDNA (5a) and NaDNA (5b) prepared by wet spinning.

molecular orientation. Considering the low resolution of the camera, and unavoidable departure from exact parallelity of the layers in a DNA package, it is concluded that the DNA films prepared by the wet spinning method, even at its present stage of development, have degrees of orientation comparable to and probably even higher than those of the DNA fibres used by others for X-ray diffraction studies.

It should be pointed out that dialyzation of the spun LiDNA deposit to lower LiCl concentrations gives films with the semicrystalline C form of DNA.<sup>24</sup> The C patterns have diffuse intensity distributions in the outer parts and are less suitable for the demonstration of orientation.

### DISCUSSION

It is believed that films of oriented DNA prepared by the method described above will be convenient for biochemical and physico-chemical investigations of different kinds. In cooperation with other workers studies are being prepared in electrical conductivity, X-ray diffraction, NMR, ESR, optics and mechanochemistry. The values of the processing variables now used apparently give films with a sufficiently good molecular orientation and crystallinity for most of these investigations, but they probably do not correspond to optimal conditions. It should therefore be valuable to follow up the method with an investigation of the influence of the various processing variables on the degree of molecular orientation and crystallinity of the DNA preparations. Because of the large number of variables involved much experimental work will be required. In a recent study of the influence of eleven processing variables on the physical properties of wet-spun modacrylic fibre Hersh *et al.*<sup>25</sup> have applied factorial analysis to their experiments and have obtained reliable results even with a comparatively limited number of runs. The more thorough study of the wet spinning method for DNA, which it is intended to perform, might be facilitated by using this statistical approach. This is, however, far from sure because not only the degree of orientation but also the spinnability of DNA is highly influenced by many processing variables.

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