ELECTRON MICROSCOPY OF CALF THYMUS NUCLEOPROTEIN

I. EFFECT OF HYDROCHLORIC ACID AND DESOXYRIBONUCLEASE*

by

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CLARK, QUAIFE AND BAYLOR[2] in 1943 prepared calf thymus nucleoprotein** by the MIRSKY AND POLLISTER[3] procedure and studied the dried salt-DNP mixtures with the electron microscope. The non-specific dendritic patterns obtained were assumed to be the result of crystal formation due to the salt present in their preparations. SCOTT[4] observed a DNA network of laterally branching and uniting fibers having a dendritic appearance. BAYLEY[5] obtained electron micrographs showing spherical bodies which were believed to be coiled globules of nucleate. ROWE, EDEN AND KAHLER[6] described the DNA molecule as a slightly kinked and slightly flexible rod with a striking tendency to spiral, twist and intertwine with neighbouring molecules. In a report on the electron microscopy of DNA by a new freeze-drying method WILLIAMS[7] described certain frequently occurring fibrillar connections (places where three fibers came together at a point) which were explained by the existence of an extremely tenuous network gel of DNA. KAHLER AND LLOYD[8] obtained fiber diameters of 15 and 25 A for DNA and DNP, respectively. The nucleate fibers were said to be intermediate in shape between a random coil and a stiff rod. "The first published picture of an extracted nucleoprotein particle that even approximates dimensions that could reasonably be expected to exist in a nucleoprotein molecule" was the description recently given an electron micrograph of DNP from sea urchin sperm by BERNSTEIN AND MAZIA[9]. The length and thickness of the fiber was 4300 A and 250–300 A, respectively. It is quite evident that estimates of the size and shape of the DNP or the DNA molecule based upon electron micrograph patterns vary considerably.

These discrepancies in the size and shape of the DNP or DNA molecule have been explained by variations in the method of preparation of the material and by variations in the techniques used in the electron microscopy (SCOTT[4] used air-drying, WILLIAMS[7] freeze-drying, and BAYLEY[5] the replica technique). The data presented below indicates that the many patterns or shapes ascribed to the DNP or DNA molecule (spherical globules, random coils, flexible rods, branched fibers, three-fiber junctions, etc.) can be obtained from a Mirsky-Pollister DNP preparation by a standard precipitation-air drying technique followed by treatment of the DNP-containing grids with hydrochloric acid and/or DNase.

* Part of this data was presented to the Division of Biological Chemistry of the American Chemical Society[1] and to the Electron Microscope Society of America.

** The following abbreviations will be used: DNP, calf thymus nucleoprotein; DNase, desoxyribonuclease; DNA, desoxyribonucleic acid or its sodium salt.

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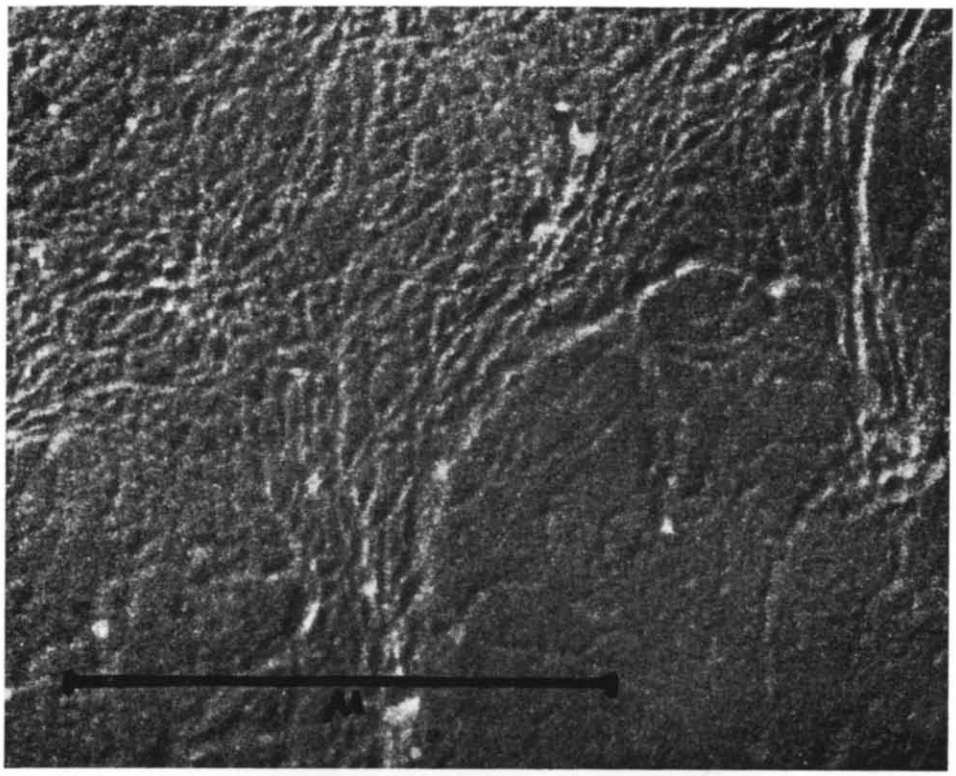

Fig. 1. Calf thymus nucleoprotein deposited on the electron microscope grid from 1 *M* NaCl by the addition of distilled water, washed with water, air-dried, shadowed with Pd at 12° and photographed with an RCA electron microscope. (73,000 ×)

EXPERIMENTAL

Methods

DNP was prepared from frozen calf thymus by the MIRSKY AND POLLISTER procedure[3]. The resultant viscous solution containing approximately 0.8% DNP was diluted with 1 *M* NaCl pH 6.9 one-hundred fold before use for electron microscopy. One drop of distilled water was added to a drop of the diluted DNP solution on Formvar covered grids. This treatment reduced the salt concentration sufficiently to precipitate the DNP upon the Formvar. Excess liquid was removed by holding the grids vertically adjacent to filter paper. The grids were then swirled in Petri dishes containing 5–10 ml distilled water and allowed to remain submerged in the water for 10 minutes. This washing procedure satisfactorily removed the NaCl so that slight, if any, contamination was visible in the electron micrographs. After the DNP-containing grids were dried overnight in a desiccator containing P_2O_5 they were shadowed at 12° angle with Pd. The preparations were then viewed and photographed with an RCA electron microscope. Control micrographs were obtained by substituting a drop of 1 *M* NaCl for the DNP solution.

Effect of HCl. The above procedure to and including the water wash was repeated. The DNP-containing grid was then placed into 1 *M* HCl at 55° C for 5 minutes after which it was washed, dried, shadowed, and viewed as before. Again controls were ob-

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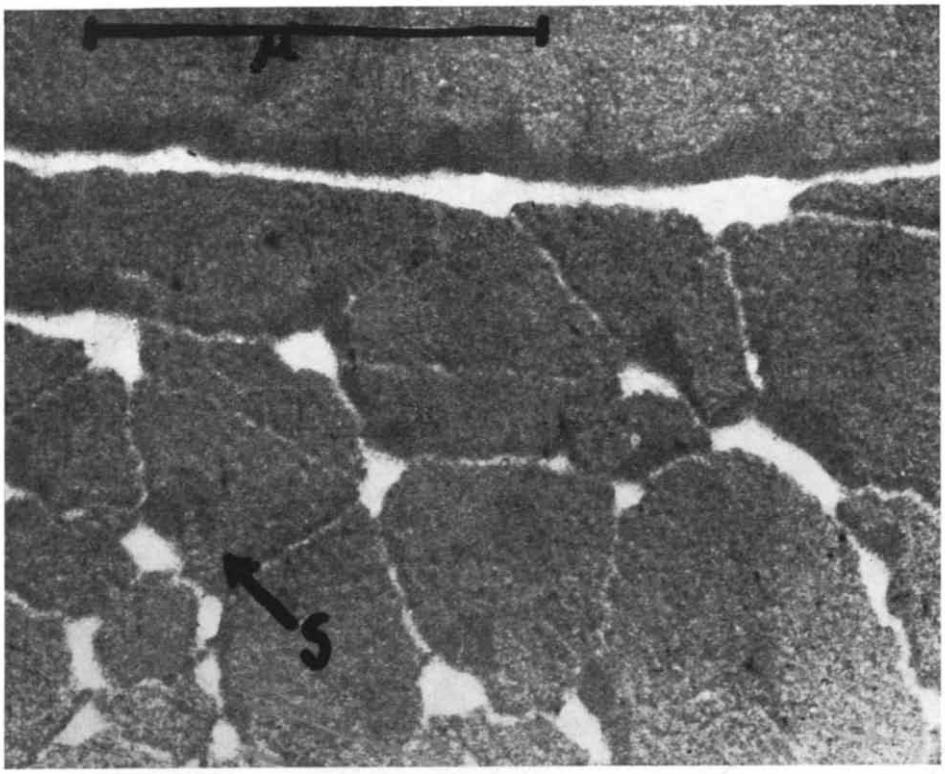

Fig. 2. DNP-containing grid washed with water, immersed in 0.6 *M* HCl at 55° C for 5 minutes, rewashed, air-dried and shadowed. Note spiral-like structure, S. (60,000 ×)

tained by substituting 1 *M* NaCl for the DNP. An additional control was obtained by replacing the 1 *M* HCl with distilled water. The pH effect was determined by substituting various concentrations of HCl for the 1 *M* reagent.

Effect of DNase. The degradation of the DNP by DNase was determined by incubating DNP-containing grids with 5 ml of a 5 mg% solution of crystalline DNase in 0.02 *M* $MgSO_4$ at 37° C. After varying periods of incubation (5–30 minutes) the DNase action was stopped by transferring the grids to a solution of Na citrate for 5 minutes. The grids were then washed, dried, shadowed, and viewed as before.

Combined effect of HCl and DNase. After the DNP-containing grids were incubated with DNase, citrated and washed, they were placed in 1 *M* HCl at 55° C for 5 minutes as before. Other grids were first treated with the HCl and then with DNase.

RESULTS

DNP. The nucleoprotein pattern of Fig. 1, typical of those obtained by the precipitation procedure described above, appears to consist of a network of fibers with many branches and junctions. Flat fibers having 15 mμ diameters can be identified; however, much larger fibers are also present. Observations of fields of less concentrated material did not reveal individual fibers but the usual branched or dendritic patterns.

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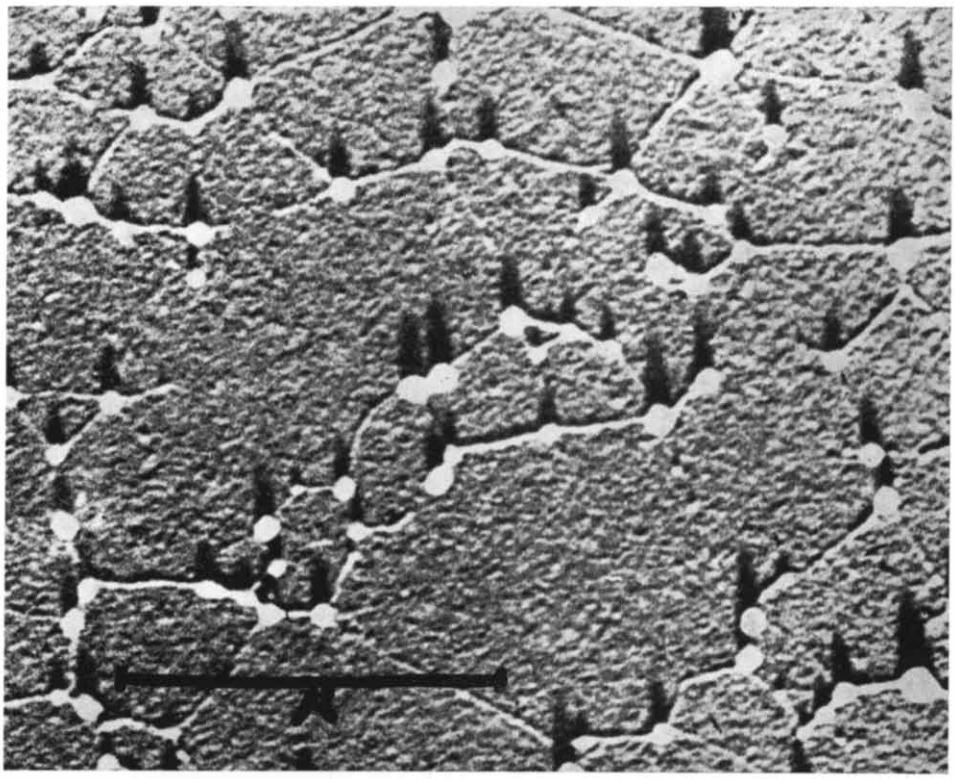

Fig. 3. Same as Fig. 2 except that 1.0 *M* HCl was used instead of 0.6 *M*. (50,000 ×)

Effect of HCl. Hydrochloric acid treatment produced striking changes in the patterns observed. (Figs. 2 and 3). Fewer but much more distinct fibers or strands were present. The many intersections of the fibers (junctions of three or more strands) contained large round or ellipsoidal globules. Striations were apparent on some of the finer strands (Figs. 2 and 3). Some were rodlike while others appeared irregular, zig-zagged and even coiled. A spiral-like structure was also observed (Fig. 2).

Changing the temperature of the HCl to 4° or 27° C did not appreciably alter the patterns produced. The effect of HCl on the DNP was most pronounced in the concentration range 0.1 to 1.0 *M*. Micrographs of DNP treated with 0.1 *M* HCl showed many fibers or strands with much branching and crossing of the strands. However few, if any, globules were noted at these intersections. As the concentration of HCl was increased the resulting patterns had more and more globules at the intersections. A change in the tautness or stiffness of the fibers also seemed to occur. The more concentrated acid appeared to have increased the rigidity of the strands. Micrographs of DNP treated with $1 \cdot 10^{-4}$ *M* HCl were identical with micrographs of the untreated DNP. Likewise when distilled water replaced the HCl the resultant patterns were similar to the DNP patterns of Fig. 1. Varying the treatment of the DNP with HCl solutions from 1 *M* to 6 *M* produced electron micrographs showing fewer strands but more and larger globules. Dis-

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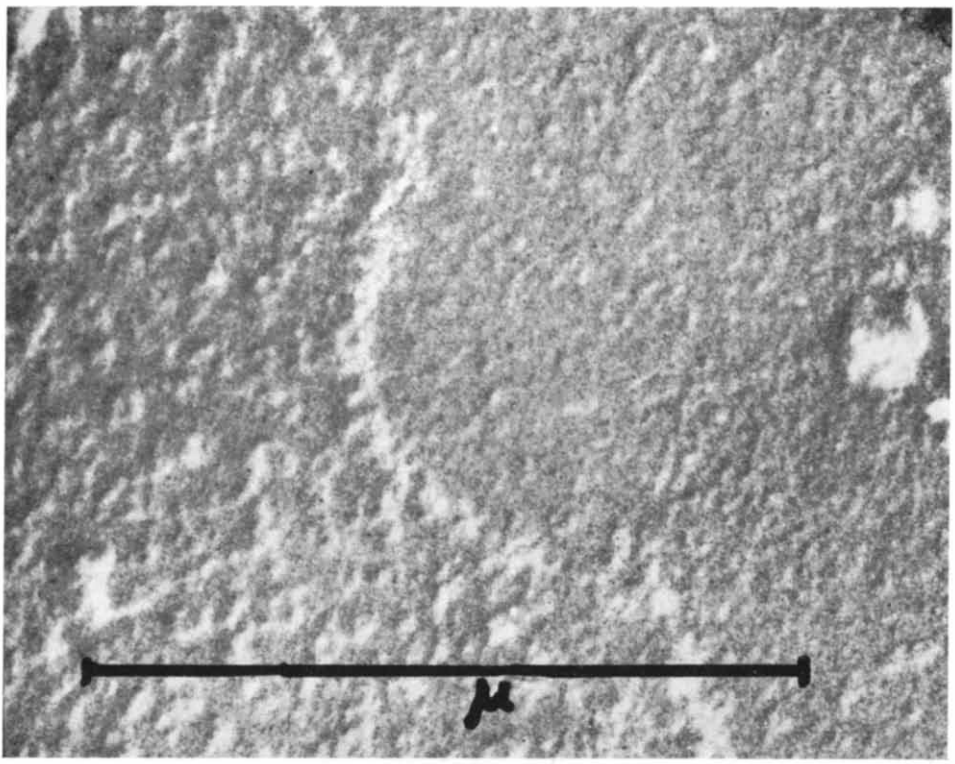

Fig. 4. DNP-containing grid washed with water, immersed in a DNase solution for 30 minutes at 37° C followed by Na citrate at room temperature 5 minutes and then rewashed, air-dried, and shadowed. (95,000 ×)

integration of the Formvar film by 6 *M* HCl treatment prevented the study of the effect of more concentrated HCl solutions.

Effect of DNase. The filamentous network noted in Fig. 1 for non-treated DNP was not observed after DNase incubation of the DNP. Instead a mass of ill-defined particles of varying shapes and sizes (Fig. 4) were noted.

Combined effect of HCl and DNase. When a DNP-containing grid was incubated with DNase and then treated with 1 *M* HCl as described above no strands, fibers or even threads were observed; instead many almost spherical particles of rather uniform size (from 150 to 450 A) were noted (Fig. 5). When the HCl treatment preceded the DNase the resulting patterns (Fig. 6) resembled those obtained with the HCl treatment alone, except that the shadows of the particles seen at the many intersections of the fibers were very much smaller and less pronounced. This loss of shadows is an indication that the spherical globules were changed by the DNase to flattened coin-shaped particles.

DISCUSSION

It is apparent that without any variation in either the method for the isolation of the DNP or the method for the precipitation and drying of the DNP a variety of electron micrograph patterns can be obtained. The freeze-drying technique has been favoured

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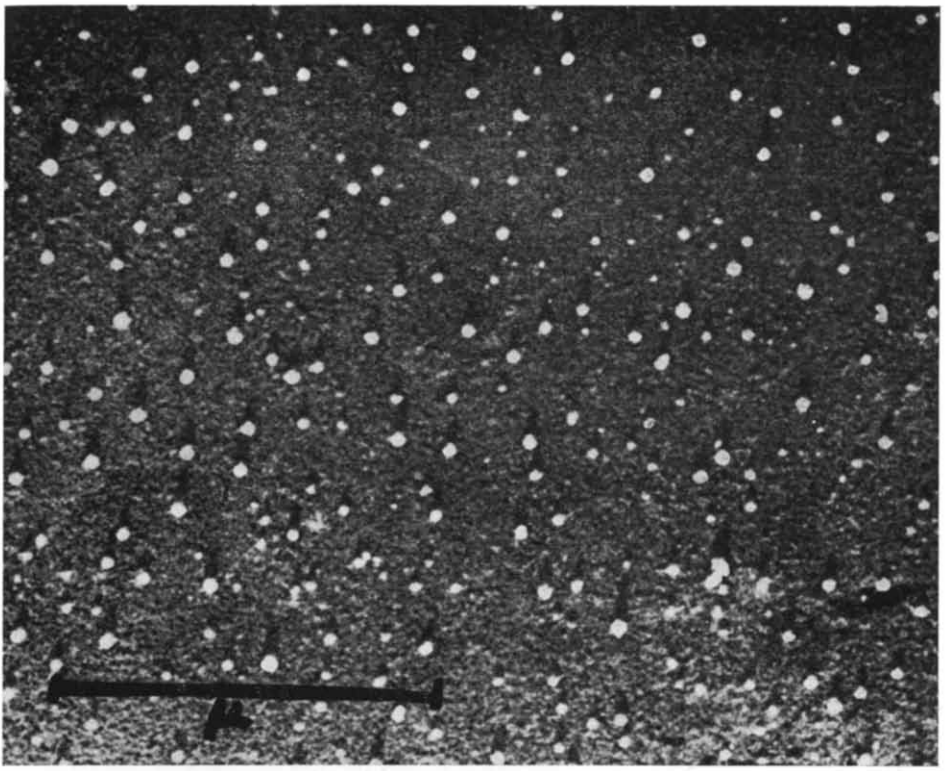

Fig. 5. Same as Fig. 4 except that the citrate treatment was followed by immersion in 1 *M* HCl at 55° C for 5 minutes. (50,000 ×)

by some investigators over the air-drying method because of possible surface tension artefacts in the latter procedure. However by variation of the hydrogen ion concentration to which the DNP was subjected previous to the air-drying procedure, patterns have been obtained which are very similar to those achieved by the freeze-drying technique. The shadows observed in Fig. 3 for both the strands and the globules indicate a definite rope-like quality for the former and an almost spherical shape for the latter. It is concluded, therefore, that the ionic nature of the material present on the electron microscope grid is more important in the determination of the shape of the DNP than surface tension forces possibly resulting from the drying procedure. The above results indicate that the DNP or DNA molecule changes in shape as a function of pH and/or ionic strength. These changes appear to progress with increasing HCl concentration from long, indistinct, loose, twisted fibers to stiff, taut, rope-like structures and finally to globules. This may be interpreted as changes reflecting first the production of molecules of high net charge with the resultant high forces of repulsion between charged groups of the molecule (the stiff, taut, coiled fiber) followed by neutralization of these charges (loss of the tautness and formation of coils or globules). Recently, changes in the shape of serum albumin as a function of pH were reported by KLOTZ AND AYERS[10]. WEBER[11], using fluorescence polarization, reported similar observations on the change in shape of the enzyme, fumarase.

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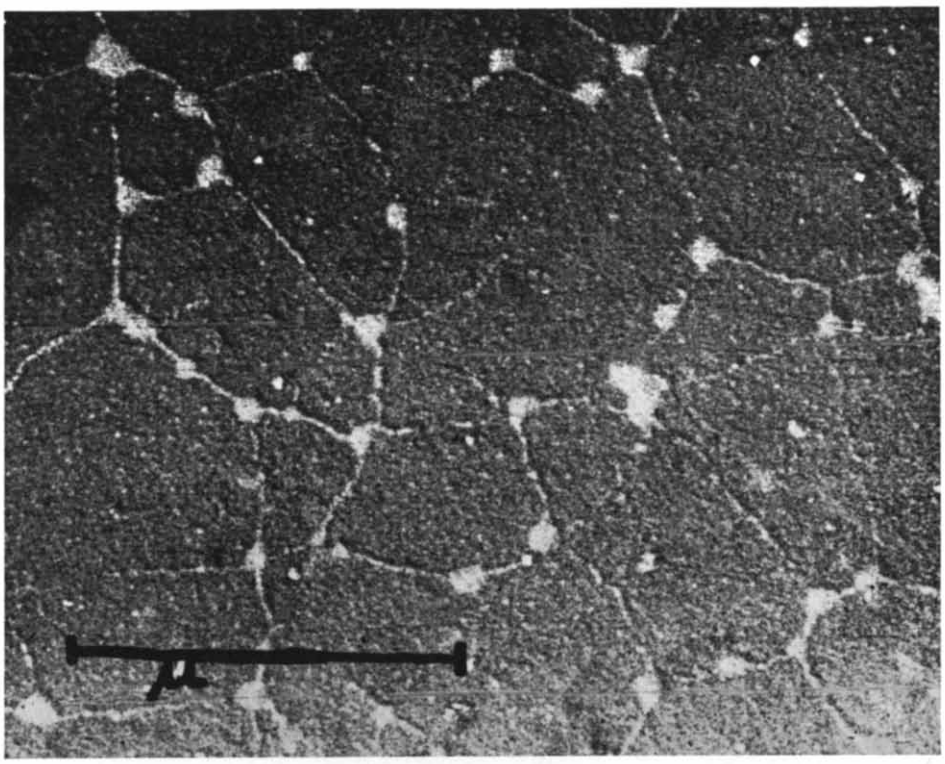

Fig. 6. Same as Fig. 3 except that the acid treatment was followed by the DNase incubation as in Fig. 4. (50,000 ×)

On the basis of X-ray studies, helical-chain, coiled structures for DNA have been postulated by WATSON AND CRICK[12] and by PAULING AND COREY[13]. Several investigators have reported the tendency for the DNA molecule to spiral, coil or twist[5,68]. Though the spiral-like structure of Fig. 3 may be too large to be a single DNA or DNP molecule and though efforts to reproduce similar structures have been unsuccessful, it appears possible that with minor modifications of the techniques described above the postulated coiled structure for DNA may soon be verified by electron microscopy.

ACKNOWLEDGEMENTS

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SUMMARY

1. Procedures for the electron microscope study of the effects of HCl and DNase on DNP have been described. DNP is precipitated from a 1 *M* NaCl solution directly upon the electron microscope grid by the addition of a drop of distilled water. Excess salt is removed by transferring the grid into a dish of distilled water. Acid or enzyme treatment of the DNP is accomplished after the washing by transfer of the DNP-containing grid into the acid or enzyme solutions.

2. Untreated DNP resulted in an ill-defined tree-like network of material. DNase treatment produced a mass of irregularly-shaped particles without any network or connections. After the HCl treatment a very pronounced network of fibers with round masses at the intersections was observed. When HCl treatment followed the enzyme action, only small round globules were observed. When the enzyme treatment followed the acid treatment the only noticeable difference was a change in the height of the round masses at the intersections of the fibers.

RÉSUMÉ

1. Le présent mémoire décrit les procédés employés pour l'étude des effets de HCl et de la DNase sur le DNP au moyen du microscope électronique. Le DNP est précipité d'une solution 1 *M* NaCl directement sur la grille du microscope électronique, par l'addition d'une goutte d'eau distillée. L'excès de sel est enlevé en plaçant la grille dans un plat d'eau distillée. Le traitement à l'acide ou à l'enzyme du DNP s'effectue après le lavage, en plaçant la grille contenant le DNP dans la solution d'acide ou d'enzyme.

2. Le DNP non traité résulta en un réseau de matière, mal défini et en forme d'arbre. Le traitement à la DNase produisit une masse de particules de formes irregulières et sans réseau ou connexions. Par suite du traitement à l'HCl, un réseau de fibres très prononcé fut observé, contenant des masses rondes aux intersections. Lorsque le traitement à l'HCl avait suivi l'action enzymatique, de petites globules rondes furent l'unique résultat observable. Lorsque le traitement à l'enzyme avait suivi le traitement à l'acide, la seule différence perceptible consiste en un changement dans la hauteur des masses rondes aux intersections des fibres.

ZUSAMMENFASSUNG

1. Es werden Verfahren für die elektronenmikroskopische Untersuchung der Einwirkung von HCl und DNase auf DNP beschrieben. DNP wird durch Hinzufügung eines Tropfen destillierten Wassers, aus einer 1 *M* NaCl Lösung direkt auf das Gitter des Elektronmikroskop ausgefällt. Der Salzüberschuss wird durch Einbringen des Gitters in ein destill. Wasser enthaltendes Gefäss entfernt. Säure- oder Enzymbehandlung der DNP wird nach dem Waschen durch Einbringen des DNP-enthaltenden Gitters in eine Säure- oder Enzymlösung erreicht.

2. Unbehandeltes DNP ergab ein schlecht definiertes, baumartiges Netzwerk. DNase-Behandlung erzeugte eine Masse unregelmässig gestalteter Teilchen ohne Netzwerk oder Verbindungen. Nach Behandlung mit HCl wurde ein ausgesprochenes Fasernnetzwerk mit runden Massen an den Schnittpunkten beobachtet. Wenn die HCl-Behandlung auf die enzymatische Einwirkung folgte, wurden nur kleine runde Kügelchen beobachtet. Folgte die Enzymbehandlung der Säurebehandlung, dann bestand der einzige merkliche Unterschied in einer Änderung der Höhe der runden Massen an den Schnittpunkten der Fasern.

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