The sets were run twice. The method used is reported in detail in FARROW and VAN DYKE[3]. The data were tested statistically with the Fisher Exact Probability Test, HUNTSBERGER and LEAVERTON[4].

Gibberellic acid. In plant systems gibberellins reverse genetic dwarfism in corn and pea. VARNER et al.[5] suggest that the compounds affect de novo protein synthesis.

The potassium salt of gibberellic acid (GA3) was without effect on thymidine incorporation. However, this salt inhibited the incorporation of uridine down to 30 and 50% of the control with no gibberellic acid, in our leukocyte system.

Auxins. The auxins instigate plant growth by cell elongation. Evidence that RNA is directly involved is presented by KEY et al.[6] and NOODEN and THIMANN[7]. EVANS and RAY[8], and EVANS[9] present evidence that points to an effect of auxin on growth via a system other than or in addition to the effect on the nucleic acids.

Indole-acetic acid, indole-butyric acid and α-napthalene-acetic acid did not affect thymidine incorporation at all doses. All concentrations of the auxins stimulated uridine incorporation between 40 and 60% over the control in the leukocyte system.

Cytokinins. The cytokinins are purine derivatives that are required for cell division in many plant systems[10–15]. Cytokinins occur in RNA of many species[15]. ZACHAU et al.[16] showed that in the case of brewers yeast RNA the cytokinin was located adjacent to the 3′ end of the anticodon.

Dihydrozeatin is without effect on thymidine incorporation; however, it decreases uridine incorporation to about 60% of the control at all concentrations in the leukocyte system. At the highest concentration (10^{-4} *M*) zeatin inhibited thymidine incorporation down to about 20% of the control, whereas the lower concentrations were slightly stimulatory. All concentrations of zeatin employed stimulated uridine incorporation.

The synthetic cytokinin N6-benzyladenine was without effect on thymidine corporation; however, at 10^{-4} *M* and 10^{-5} *M* it inhibited uridine incorporation down to about 10% of control. At 10^{-7} *M* a 75% stimulation over the control value was observed.

The synthetic cytokinin, kinetin, inhibited thymidine incorporation at 10^{-4} *M*; whereas a lower concentration (10^{-6} *M*) stimulated thymidine incorporation. Kinetin was either without effect or stimulatory (10^{-6} *M*) to uridine incorporation. Kinetin riboside was without effect on thymidine incorporation; however at 10^{-4} *M* it inhibited uridine incorporation to 50% of the control. Lower concentrations (10^{-5}, 10^{-6} and 10^{-7} *M*) stimulated uridine incorporation.

Phenolics. The phenolics are a diverse group of plant secondary products[17]. It has been suggested that in certain cases these compounds may act as inhibitors. Interestingly enough naringenin and ferulic acid inhibited incorporation of precursors on the order of 50% of the control in certain experiments. 6-Methoxybenzoxazolinone is a breakdown product of 2,4-dihydroxy-7-methoxy-2H-1, 4-benzoxazine-3-(4)-one which occurs in etiolated *Zea mays* seedlings[18]. Inhibition of thymidine incorporation was decreased to about 34% of control while uridine incorporation was decreased to about 60% of the control at the 10^{-4} *M*. All other doses also produced an inhibition of incorporation of the nucleoside precursor in the leukocyte system.

The compounds studied may affect nucleic acid metabolism in plants. No specific mechanisms have been established. Probably, in order to inhibit or stimulate the incorporation of thymidine-methyl-^{3}H or uridine-5′-^{3}H into DNA or RNA of the leukocyte, the compound must have acted at some point in pathway leading to synthesis of the nucleic acids. The possibility of nonspecific inhibition of the uptake of nucleosides as reported in HILL and WINGO[19] seems unlikely, because both stimulation and inhibition were observed[20, 21].

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Structural Changes of Deoxyribonucleoprotein Fibres Following γ-Irradiation under Aerobic and Hypoxic Conditions

Y. H. NAKANISHI, M. KUWABARA, G. YOSHII and T. NEI[1]

Department of Experimental Radiobiology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo (Japan), 17 July 1975.

Summary. The DNP fibres γ-irradiated under aerobic condition showed a reduction of their diameter, while no remarkable changes were observed in the DNP fibres irradiated under hypoxic condition by scanning electron microscopy.

In general, the radiosensitivity of macromolecules and biological systems irradiated in the presence of oxygen is higher than when they are irradiated in the absence of oxygen, as reviewed by DERTINGER and JUNG[2]. There are a considerable number of physicochemical and biological studies on this phenomenon. As far as the authors are aware, however, no literature of electron microscopic investigations pertaining to this subject is accessible.

The present study has been undertaken with the hope of elucidating the structural changes of deoxyribonucleo-

protein (DNP) following irradiation under aerobic and hypoxic conditions by scanning electron microscopy, maintaining water-contained state of the DNP with the aid of the new equipments designed by NEI et al.[3].

The DNP was extracted from calf thymus in 0.7 m*M* phosphate buffer solution (pH 6.8) by the method of ZUBAY and DOTY[4]. The DNP solution at a concentration of about 0.1 mg per ml was kept at 0 °C and γ-irradiated from a cobalt-60 source (5,000 Ci) under aerobic or hypoxic conditions. The dosage and procedure of irradiation are described separately in respective experimental sections. About 15 min after irradiation, approximately 0.01 ml of the DNP solution, diluted with 0.7 m*M* phosphate buffer solution at a final concentration of 0.1 mg per ml, was rapidly frozen by quick immersion into Freon 22 pre-cooled with liquid nitrogen. The frozen sample was immediately transferred onto a specially designed cold stage within a pre-evacuation chamber of a JSM 50A scanning electron microscope. The specimen was sublimated to an appropriate depth at a temperature of −100 °C at 10^{-5} Torr, and subsequently coated with a thin layer of gold. The specimen kept at about −150 °C was examined under the scanning electron microscope at an accelerating voltage of 20 kV, and photographed at a magnification of

[1] Professor T. NEI's address: The Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan.

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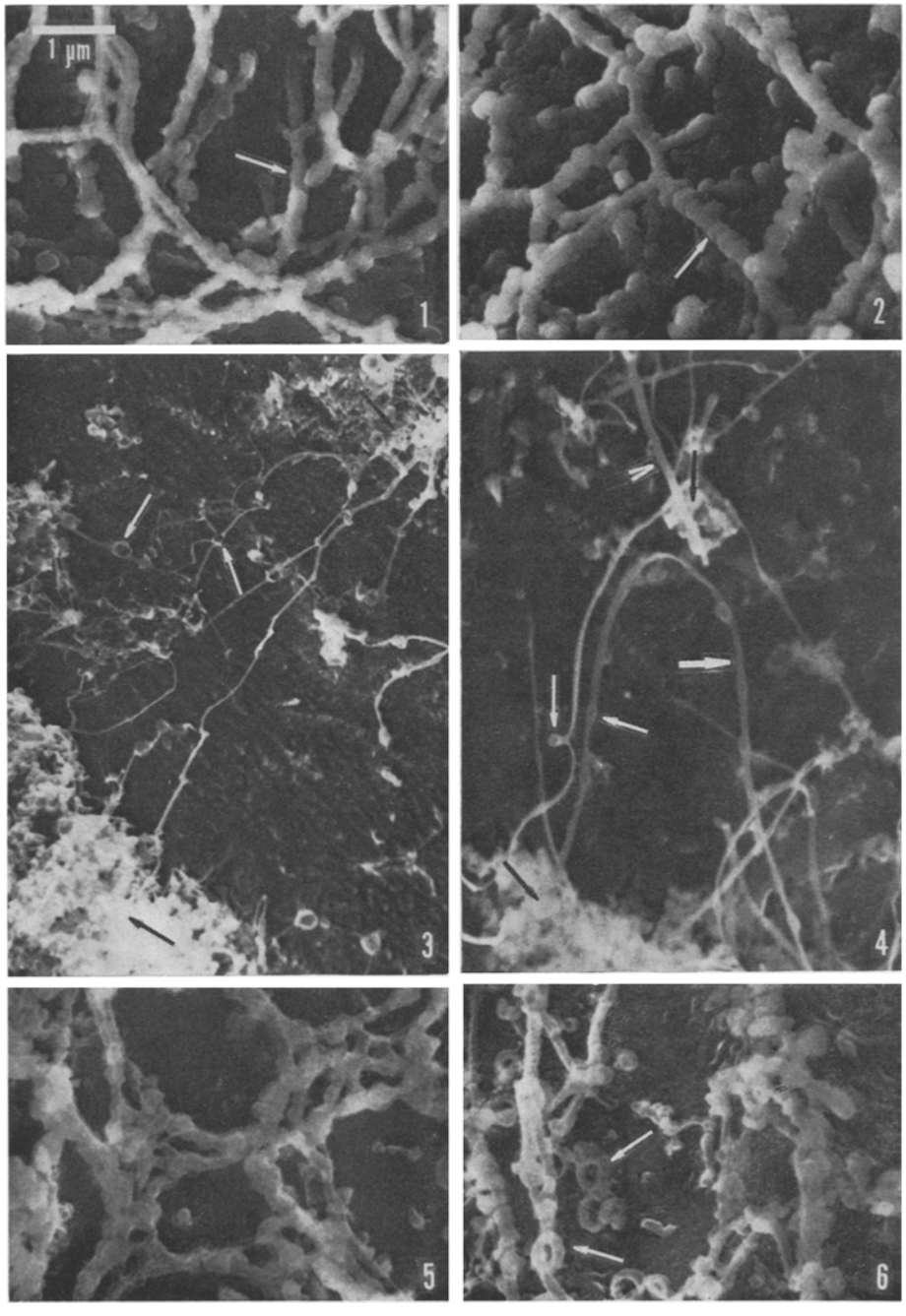

Figs. 1–6. Scanning electron micrographs of deoxyribonucleoprotein (DNP) fibres, maintaining their water-contained state with the aid of the new equipment[3,6]. The magnification of all figures is given by the scale shown in Figure 1. Figures 1 and 2. Non-irradiated DNP fibres under aerobic and hypoxic conditions, respectively. Figures 3 and 4. DNP fibres irradiated with 10 krad and 1 Mrad of γ-rays under aerobic condition, respectively. Figures 5 and 6. DNP fibres irradiated with 1 Mrad of γ-rays under hypoxic condition. For details see text.

10,000×. The DNP solution contained fibrous elements, preferring to use a term of DNP fibres[5,6].

In order to prepare hypoxic DNP solution, about 3 ml of the DNP solution in a Pyrex glass tube (17 cm³) were kept at 0 °C, and bubbled with nitrogen gas (99.95%) through a fine needle at a flow rate of 100 ml per min for 15 min. The hypoxic DNP solution thus prepared revealed essentially the same configuration of the DNP fibres as that of the DNP fibres observed in the aerobic condition (Figures 1 and 2). The DNP fibres, about 2,100 Å in diameter, were formed by a coiling of subfibres with about 850 Å diameter (Figures 1 and 2, arrows), as previously reported by NAKANISHI et al.[6].

The DNP solution under aerobic condition was irradiated with 10 krad and 1 Mrad at dose rates of 800 rad and 15 krad per min, respectively. Remarkable alterations of the DNP fibres in both the irradiated samples were a reduction of their diameter, about 800 Å in diameter (Figures 3 and 4). In some places, 2 thin fibres were closely contacted, and run parallel, representing a tape-like wide structure (Figure 4, thick arrows). The evidence suggests that the DNP fibres are extended by irradiation under aerobic condition, resulting in the formation of the thin fibres. Furthermore, it was observed that the aggregates and ring-shaped loops were constructed by the thin fibres, the former being indicated by black arrows and the latter by white arrows in Figures 3 and 4, respectively.

On the other hand, when the hypoxic DNP solution was irradiated at a dose rate of 15 krad per min giving a high dose of 1 Mrad, no apparent change of the DNP fibres was demonstrated in their structure, except that the surface of the DNP fibres exhibited rough appearance (Figure 5) and that the ring-shaped loops were found to be formed (Figure 6, arrows). The image seems to be more similar to that of the DNP fibres observed in the non-irradiated aerobic and hypoxic DNP solutions than to that of the DNP fibres irradiated with 10 krad and 1 Mrad under aerobic condition.

Although a final conclusion must be left to future studies, it seems probable that the different appearances of the DNP fibres are affected by the presence or absence of oxygen during irradiation. Further detailed and quantitative studies on the fine structure of the DNP fibres under various conditions are now in progress[7].

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Organization of Myosin Molecules in the Muscle Thick Filament

C. L. DAVEY and A. E. GRAAFHUIS

Meat Industry Research Institute of New Zealand Inc., P.O. Box 617, Hamilton (New Zealand), 8 September 1975.

Summary. Tryptic treatment of muscle thick filaments reveals the underlying backbone of aggregated L-meromyosin as a coil of 3 secondary filaments (helical repeat ~130 nm) each in turn a coil of 3 finer ones.

The cyclical interaction of the myosin heads of thick filaments with the g-actins of the thin filaments is at the basis of muscular contraction[1]. This interaction appears to involve subtle steric orientations of the heads and as such, the precise organization of myosin in the thick filaments is an important aspect of the contractile mechanism[1,2].

The reflections from low angle X-ray diffraction of pre-rigor vertebrate muscle are interpreted as showing that the thick filaments have their myosin-head projections arranged at successive levels of about 14.5 nm along the filament backbone[3]. The heads are in a helical array described severally as two-stranded 6/1 of pitch 86.4 nm[3]; four-stranded 6/1 of pitch 86.4 nm[4]; three-stranded 9/1 of pitch 129.6 nm[5]. The values 6 or 9 refer to the number of projections in each helical turn of each strand.

Further advance in resolving the molecular organization is likely to come from electron-microscopic identification of key details not shown by X-rays. We report pertinent new structural features revealed by controlled tryptic digestion of thick filaments to remove the H-meromyosin moiety, and to expose the underlying aggregated backbone of L-meromyosin.

Hen pectoral muscle was used to prepare natural thick filaments[6] and the myosin for forming synthetic filaments[7]. Digestion was carried out in two ways. Trypsin, 10 mg/ml in 0.1 *M tris*-HCl, pH 7.6[8] was added in equal volume to dilute suspensions of the filaments. After digestion, droplets of the suspensions were applied to carbon-coated grids. Alternatively the trypsin (5 mg/ml) in the same buffer was added as droplets to grids on which the filaments had already been lodged. In both cases digestion at 20 °C was continued for 40 min. Head removal was incomplete at lower trypsin concentrations or shorter digestion times. Undigested and digested filaments on the grids were then fixed for 5 min in 1% glutaraldehyde, 2 m*M* imidazole-HCl, pH 7.0. Negative staining was carried out with 1% uranyl acetate[9].

Although both methods revealed the same structures, the Figure illustrates those obtained by digestion in the suspensions. The untreated natural (a) and synthetic (b) filaments, as expected, were covered in projections except for relatively smooth central regions. The roughly oblong

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