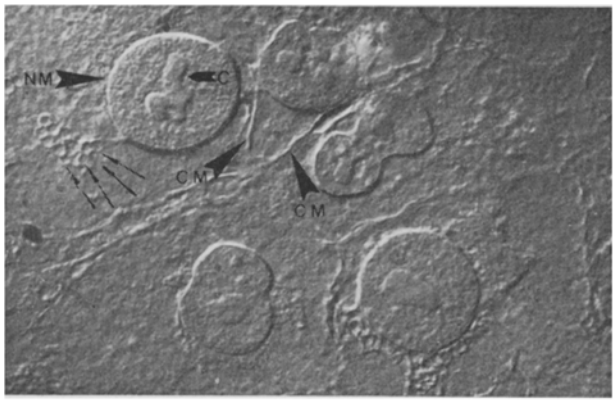

Fig. 2. HEp-2 cell culture after 60 min of contact with *Staphylococcus* α-toxin. The initial breakdown of cell membrane (CM) and numerous small depressions on the cell surface (arrows) are visible. NM = Nuclear membrane; C = chromatin. ×1000.

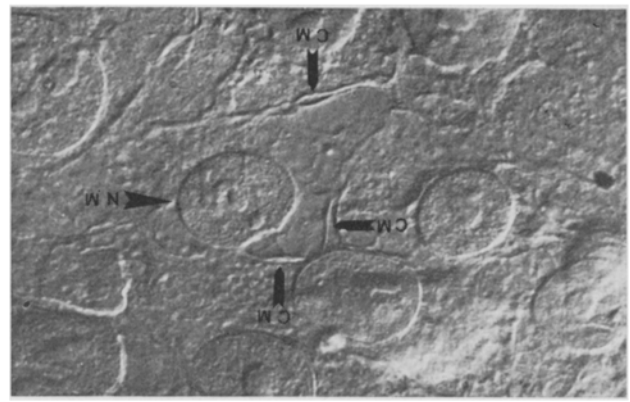

Fig. 3. HEp-2 cell culture after 180 min of contact with *Staphylococcus* α-toxin. A remarkable breakdown of cell membrane (CM) is present. The nuclear membrane (NM) and the nucleoli are barely visible. ×1000.

energetic metabolism, with a drop in the intracellular levels of ATP. This would call attention to what takes place in hemolytic anaemia associated with a congenital defect of glucose-6-phosphate-dehydrogenase activity, in which the fragility of erythrocyte membranes is caused, in part, by a decrease of the cellular content in ATP[16]. The changes of the morphology of the cell membrane described in the present paper take place with the same concentration of toxin and within the same times of incubation which cause the rapid decrease of intracellular levels of ATP[8]. On the other hand, the alterations of the membranes appear in stages notably different in different cells of the same culture. This behaviour may be due to the sensitive biochemical differences that can be checked among cellular populations[17–19]. The different stages of appearence of the alterations of the cell membranes induced by α-toxin in cells of the same culture could, therefore, make us believe that such alterations are caused by a lesion of the energetic metabolism rather than due to a direct action of the α-toxin on the membranes themselves.

Riassunto. Gli Autori hanno preso in esame, mediante il microscopio a contrasto di fase interferenziale, le modificazioni della superficie cellulare indotte dalla tossina alfa-stafilococcica in colture di cellule HEp-2. La tossina causa rapidamente collasso della membrana, seguito da rottura della membrana stessa. Vengono discussi i rapporti fra questi aspetti morfologici e le lesioni biochimiche indotte dalla tossina.

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Nucleolar Ultrastructure in the Quiescent Embryonic Cells of the Dry Seed of *Allium cepa* L.

Continuity of the nucleolus during the cell cycle is maintained by the nucleolar organizing region of the chromosomes. The recent clarification of the nucleolar chromatin, as the nucleolar organizer and the ribosomal cistron[1,2], has indeed emphasized the importance of the nucleolus in the area of protein synthesis. Nucleolar activity and its ultrastructural morphology may vary to a great extent depending on the physiology and specialization of cells[3]. Such changes also occur due to actions of various exogenously administered inhibitors[4] or promoters[5] of macromolecular synthesis. Even at the optical microscope level, reflections of physiological events on the nucleolar morphology are easily detected[6,7].

Our recent interest in quiescence[8,9] has led us to look at the nucleoli of the quiescent root meristem of *Allium cepa* L. bulb as well as the embryonic cells of its dry seeds. Differentiation observed in the nucleolar morphology are reported in this note.

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Embryos from dry *Allium cepa* L. (white pickling) seeds were dissected out and fixed in a mixture of paraformaldehyde and glutaraldehyde dissolved in phosphate buffer pH 7.2[10]. Usual procedures of post-osmication, ethanol dehydration and Epon embedding were followed. Ultrathin sections made on a Porter-Blum MT1 microtome were stained with uranyl acetate and lead citrate. A Zeiss 9S Electron Microscope was employed for ultrastructure study. Some of the embryos were fixed in acetic acid: ethanol (1:3) and embedded in paraffin for optical microscopy. The sections were stained with methyl green and pyronin for nucleolar differentiation of RNA. Parallel controls were run after ribonuclease (0.5 mg/ml in distilled water, 37°C for 3 h) digestion. Millons reaction was employed on sections for protein stains[11].

Nucleolar ultrastructure of the growing root tip cells of *Allium cepa* has been worked out extensively in recent years[12–14]. On this basis, in general terms, the interphase nucleolus may be described as consisting of the following 4 components intermingled with no definite segregation. 1. Nucleolar chromatin-dense compact fibrillar (30–40 Å fibers) patches, 2. fibrillar (30–40 Å fibers) component surrounding the nucleolar chromatin, 3. granular (150 Å granules) component and 4. vacuolar lacunae having lighter fibrillar matrix (30–40 Å fibers).

In the dry embryonic cells the nucleolus has been found to be markedly different (Figure 1), due to the lack of its granular component and vacuoles. The other nucleolar components show a clear segregation. The major bulk of the nucleolus is made of fibrillar material (30–40 Å fibers), less dense than the surrounding nuclear chromatin. The nucleolar chromatin passes through the fibrillar material and its continuity with the nuclear chromatin on both sides is clearly seen in sections. There are dense fibrillar patches embedded in a less dense fibrillar matrix in the nucleolar chromatin. In transverse sections these appear as an eccentric core.

Interestingly enough, a different organization of the nucleolus has been noted in the tip of the cotyledon (Figures 2a) and b)). Here the granular component is also missing, but the nucleolar material is vacuolated and has separated from the nucleolar chromatin, into 2 distinct spheroids. Vacuolation of nucleolar material is usually

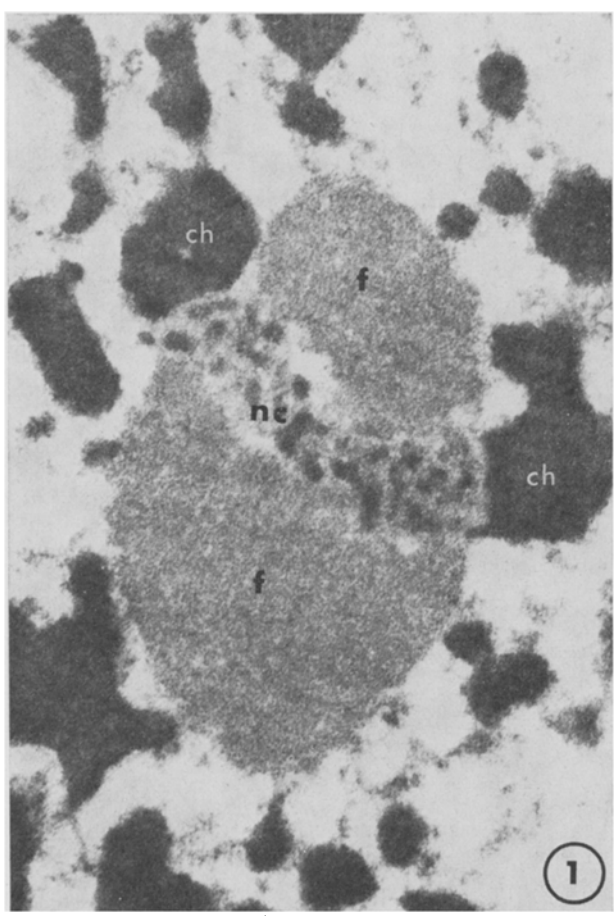

Fig. 1. Section of a nucleolus from the quiescent embryo showing the continuity of the nucleolar chromatin (nc), lying across the fibrillar matrix (f). The granular component and vacuoles are entirely lacking. Note the dense compact nature of the chromatin (ch). ×27,600.

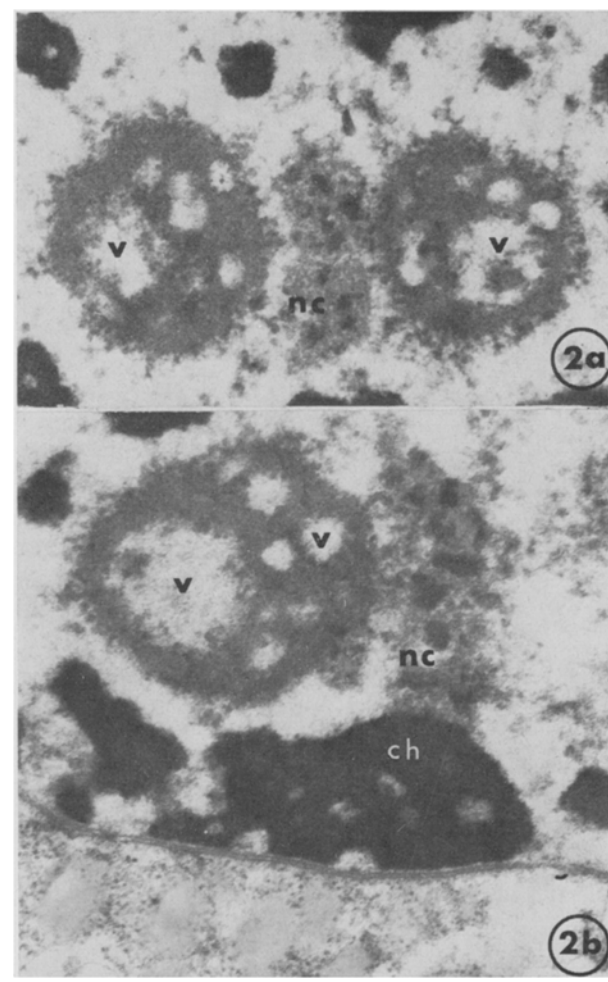

Fig. 2. a) and b). Section of nucleoli from the cotyledon tip of the quiescent embryo. Note the lack of granular component and the separation of the fibrillar material from the nucleolar chromatin (nc) into spheroids having vacuoles (v). The nucleolar chromatin in b) reveals continuity with the nuclear chromatin. ×27,600.

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associated with synthesis[15] and transfer[16] of substances into the extranucleolar surroundings. It is unlikely that in the quiescent cotyledon such configuration should imply any activity, but could possibly represent reminescence of past events. Presence of RNA and protein can be demonstrated in both types of nucleoli by pyronin and Millons stain. In view of the quiescence of the embryonic cells this ribonucleoprotein may be considered as structural to the nucleolus and may not have any direct physiological role.

The lack of the granular component in these nucleoli are related to quiescence. Quiescent root meristem cells of the bulb show a definite segregation of the granular component, which disappears with aging[17]. Similar lack of granules has been noted in actinomycin D blocked interphase nucleoli of root meristem cells[4].

From this study it appears that essential constituents that maintain the continuity of the nucleolus in quiescent cells of the embryo include the nucleolar chromatin and the associated ribonucleoprotein fibrillar component. Further studies on the evaluation of enzymes in the protein fraction of the quiescent nucleoli are in progress.

Zusammenfassung. Elektronenmikroskopische Untersuchungen an der Küchenzwiebel *Allium cepa* L. haben einen unterschiedlichen Aufbau der Nukleoli aus Wurzelspitzenzellen und derjenigen aus Kotyledonenspitzenzellen ergeben.

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Hemmung der cancerogenen und toxischen Wirkung von Diäthylnitrosamin auf die Rattenleber[1]

Intraperitoneale Injektionen von allogenen Geweben sensibilisierten die Transplantatabwehr gegen die nachfolgende Implantation von Impftumoren bei Mäusen und Ratten so eindrucksvoll[2], dass versucht wurde, dieses Verfahren prophylaktisch auch gegen Tumoren anzuwenden, die chemisch induziert werden. Bei Verwendung verschiedener Gewebe hat sich Thymus besonders bewährt.

Im folgenden werden Versuche beschrieben, die beweisen, dass i.p. Injektionen von homologem Thymusgewebe Ratten sowohl weitgehend gegen den toxischen Einfluss von Diäthylnitrosamin (DÄNA) auf die Leber schützen als auch die Carcinogenese durch diese Substanzen in der Leber hemmen.

Versuchsanordnung. 33 männlichen Sprague-Dawley-Ratten von etwa 150 g Körpergewicht wurden jeweils der ganze Thymus einer gleichartigen Ratte im Abstand von 1 Woche dreimal i.p. injiziert. Dazu wurde der Thymus einem frisch getöteten Tier entnommen und mit einer Tuberkulinspritze mit Hilfe von etwa $^1/_2$ ml physiologischer Kochsalzlösung durch eine Flügelnadel Nr. 14 in die freie Bauchhöhle injiziert. 1 Woche nach der letzten Thymusinjektion bekamen die so vorbehandelten Ratten gleichzeitig mit 30 gleichen, aber unbehandelten Kontrolltieren 400 mg DÄNA per kg Rattengewicht in Trinkwasser. Dazu wurde das DÄNA zu 75 mg in je 1 l Wasser gelöst. Entsprechend dem Gesamtgewicht der 63 Ratten von 9,4 kg wurden 3,76 g DÄNA in 50 l Trinkwasser verbraucht. Diese Menge wurde in 30 Tagen ausgetrunken und es entfielen auf jede Ratte von 150 g Gewicht 60 mg DÄNA. Die Trinkmenge und damit die Dosis des Carcinogens war in der unbehandelten und in der vorbehandelten Gruppe gleich. Danach bekamen alle Ratten reines Trinkwasser. Als Futter dienten Altromin-Presslinge.

Während der DÄNA-Trinkperiode blieb das Gewicht in beiden Tiergruppen konstant. Nach Absetzen des DÄNA, also nach 1 Monat, nahmen alle Tiere bei beiden Gruppen langsam an Gewicht zu. Diese Tiere wurden zu verschiedenen Zeiten danach getötet, die inneren Organe in Formalin fixiert und histologisch untersucht.

Die Untersuchung der Leber zeigte bei den 30 unbehandelten Kontrolltieren 14 Carcinome und schwere De-

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Zahl der Ratten, Geschlecht, Gewicht, Behandlung	Leberdegeneration	Anzahl der Carcinome	Tag der Tötung nach Beginn des Trinkwasserversuches mit DÄNA[a]
33, ♂, 150 g, vorbehandelt	leicht bis mässig	0	280, 285, 295, 295, 300, 300, 305, 315, 320, 330, 330, 360, 360, 365, 365, 365, 365, 365, 365, 370, 370, 370, 370, 370, 370, 380, 400, 405, 410, 410, 415, 430
30, ♂, 150 g, unbehandelt	schwer	14	270, 270, 270, 270, 270, 270, 275, 275, 275, 275, 275, 275, 280, 280, 315, 330, 335, 335, 360, 365, 365, 365, 365, 370, 370, 370, 370, 370, 375, 380

[a] Unterstrichen sind Tage, an denen die Carcinome bei den unbehandelten Kontrollen aufgefunden wurden.