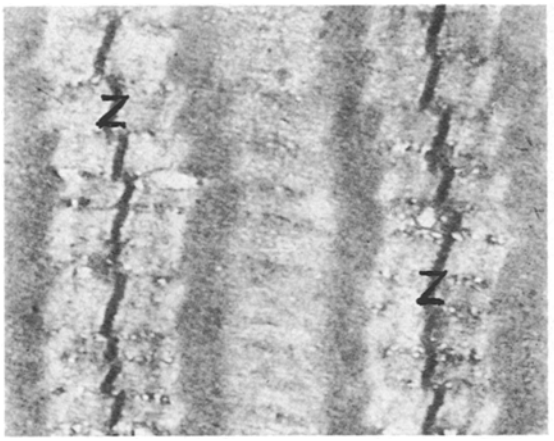

Fig. 3. Extraction of rabbit psoas with Weber-Edsall solution (48 h). Z-bands (Z) are easily distinguishable. × 25,000.

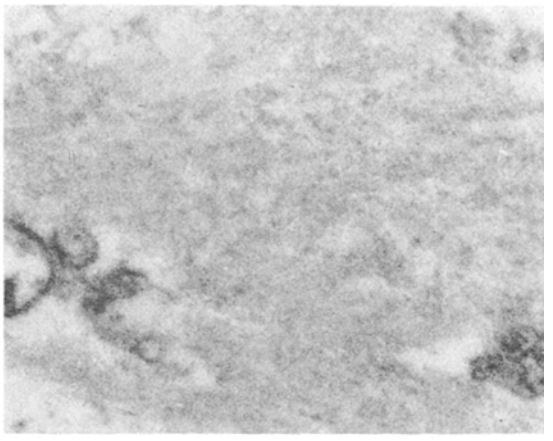

Fig. 4. Extraction of uterine smooth muscle with Weber-Edsall solution (48 h). Dense bodies were completely removed. × 25,000.

After 48 h extraction with Weber-Edsall solution, the Z-bands are present in the striated fibres (Figure 3), whereas in the smooth ones the dense bodies have completely disappeared (Figure 4).

Discussion. The entirely dissimilar behaviour of the dense bodies and Z-bands, not only after the urea extraction but also after the extraction of actomyosin, provides sufficient evidence to conclude that the dense bodies differ significantly from the Z-band material. The lack of similarity between dense bodies and Z-bands is also supported by other observations: the dense bodies number varies with the functional state of smooth muscle fibres[6–10] and myosin is localized at the level of the dense bodies[11]. Therefore, the dense bodies in vertebrate smooth muscle fibres and Z-bands in striated fibres cannot be regarded as equivalent structures.

Zusammenfassung. Die Extraktion von glatten und quergestreiften Muskelfasern mit 3 *M* Urea und Weber-Edsall-Lösung zeigt, dass die Reaktion der sogenannten «dichten Körper» und Z-Streifen eine ganz verschiedene ist. Deshalb kann die Struktur der «dichten Körper» und diejenige der Z-Streifen nicht als identische betrachtet werden.

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Occurrence of a Characteristic Nucleolar Structure in Pachytene Cells

The light microscope had already revealed to us the morphological heterogeneity of the nucleolus. A large number of authors mention the existence of empty spaces or vacuoles inside the nucleoli of plants and animals[1–11]. The use of a particular staining technique[9,12] (fixing in 10% formol and staining with 0.2% basic fuchsin) enabled us to observe a characteristic nucleolar structure in pachytene meiocytes from the anthers of *Allium cepa*. This structure was observed in all the nucleoli from the meiocytes studied during the stage in question and it consists of a small ring or button-like formation between 0.5 and 1.5 μm in diameter, which stains intensely with the basic fuchsin (Figures 1–2). Thick sections (about 0.5–1 μm) of pachytene anthers fixed in glutaraldehyde and osmium tetroxide, also show this circular structure (Figure 3). We have not been able to observe this structure at other stages of meiosis.

The presence of only one of these formations in each nucleolus is the most frequently observed phenomenon, but in exceptional cases as many as 3 formations of this type have been found in one nucleolus. The ring or button appears quite distinctly and clear-cut and stains homogeneously, or with an unstained area of different dimensions inside the formation and concentric with it. This

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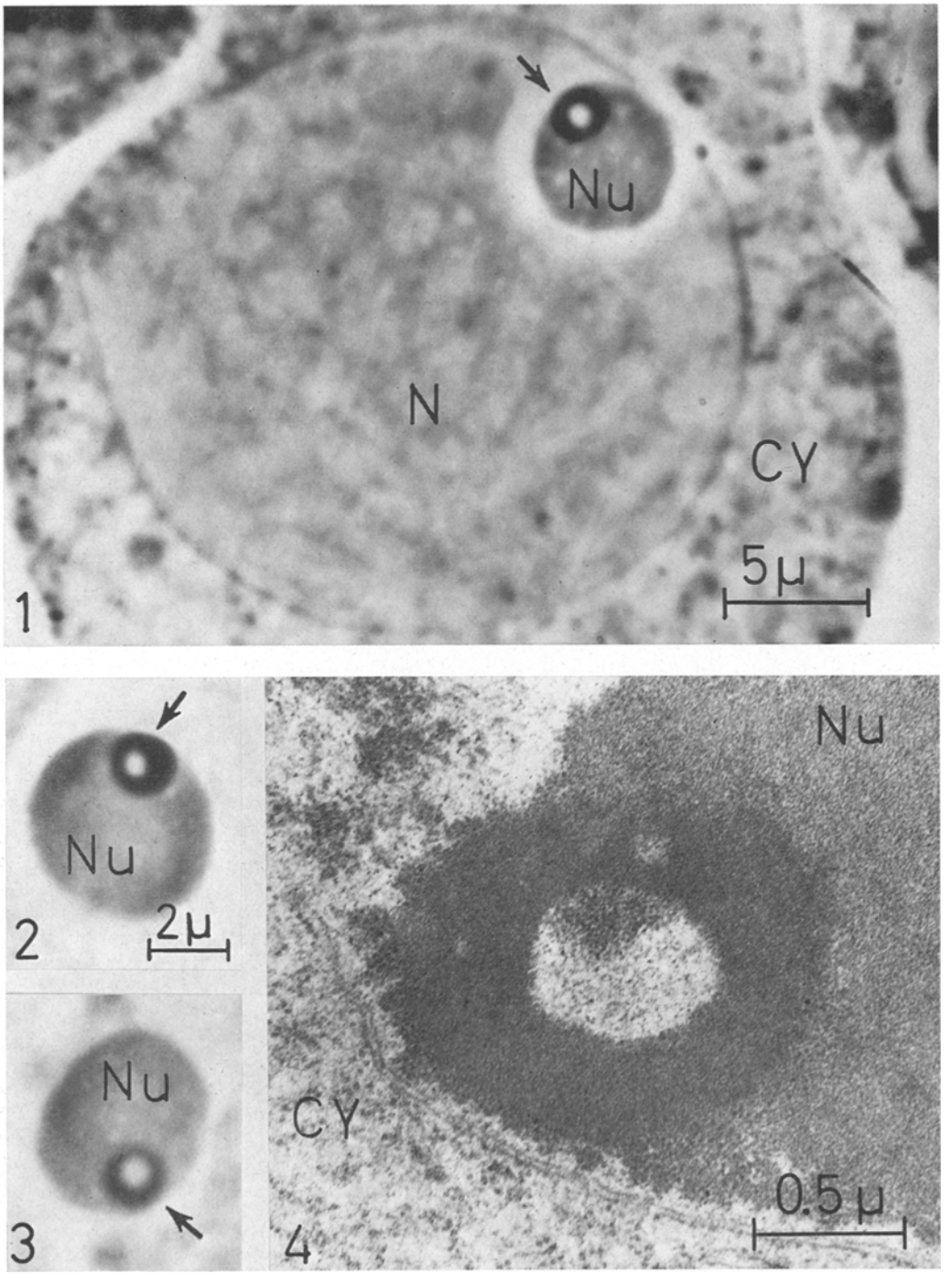
circular structure, detectable by high contrast when stained with basic fuchsin, appears for the most part peripherally and in relation to the peripheral, basophilic area of the segregated nucleoli[13].

Some authors have reported the presence of various types of structure within the nucleolus. Among such structures observed by them we find protein crystalloids[14], dense elongated cores[15], membrane systems and channels[16,17], carbohydrate inclusions[18,19], nucleolar vacuole[20], optically anisotropic nucleolar granules[21], fatty bodies[22], intranucleolar bodies[23], and 'round' and 'oblong' bodies[24]. A comparative analysis of these intranucleolar structures seems to indicate that none of them correspond to the sort of formation described above.

We are engaged upon a more thorough study of the evolution and fine structure of this nucleolar component.



Figs. 1–4. Meiocytes of *Allium cepa* during the pachytene, showing nucleoli with the small rings or button-like formations (arrows). N, nucleus; Nu, nucleolus; Cy, cytoplasm. 1. and 2. Basic fuchsin stain, phase contrast. 3. Thick section of glutaraldehyde-osmium fixed material. Methylene blue borate, phase contrast. 4. Electron micrograph of the circular structure described in the text. Uranyl acetate and lead citrate staining.

In pachytene meiocytes fixed in glutaraldehyde and osmium tetroxide, some preliminary observations under the electron microscope show that the circular structure has a true existence, and it is composed of a close-packed material (Figure 4).

Resumen. Se describe el hallazgo de una estructura nucleolar característica en células paquiténicas de anteras de *Allium cepa*, fijadas en formol y teñidas con fuchsina. Esta estructura se presenta como una pequeña formación semejante a un botón o anillo en el nucleolo de todos los meiocitos observados en ese estadío.

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The Effects of MNG and NMU on Mitotic Activities and DNA Synthesis

The chromosome breaking capacity of N-nitroso-N-methyl urethane (NMU)[1] and N-methyl-N-nitro-N-nitroso-guanidine (MNG)[2] has been established. Grant and Heslot[3] indicated that NMU and NEU cause dose-dependent delay to the division of cells synthesizing DNA at the time of treatment. Savin et al.[4] reported enhancement of the mutation frequency with presoaking in barely and they correlated it with DNA synthesis peak.

In the present report an attempt has been made to compare the efficiency of NMU and MNG, the 2 potent nitroso compounds, in relation to their effectiveness on chromosome structure and DNA synthesis.

Material and methods. Germinating barley seeds of variety NP113 were treated with freshly prepared NMU and NMG solutions for 30 min at 20 ± 1 °C. The pH in aqueous solutions was in the range of 5.3–5.4. The roots were stained according to Feulgen procedure. Labelling was carried out with tritiated thymidine in conjunction with chemical solutions, keeping the final concentration to 5 μc/ml (specific activity 2.2 c/mmole). Autoradiographs were prepared with Kodak AR10 stripping film and different steps were followed as described by Darlington and LaCour[5].

Observations and discussion. In order to work out the comparable dosimetery of these 2 nitroso compounds, different concentrations were tried. The approximate range of 50% germination was reached when NMU was given at a concentration of 0.06% and MNG at 0.1%. These concentrations were used for further studies.

Looking at mitotic frequency at different recovery periods both with MNG and NMU, it seems apparent that the number of dividing cells is greatly affected even after 1 or 2 h of recovery (Table I). The normal functioning of the cells is suppressed either due to impairment of DNA synthesis or blockage at G2, the post-synthetic stage. The effect with NMU was drastic, which could be due to its immediate binding and reactivity at many sites. The sudden drop in mitotic activity particularly with NMU treatments, could be considered analogous to 'prophase poisoning'[6,7]. Maximum reduction in mitotic frequency was recorded within 12–16 h of recovery periods. These are the cells which were at synthetic stage when the treatment was given.

If we look at the percentages of abnormal metaphases, firstly at different recovery periods and secondly between the 2 treatments, an inference can be drawn that the 2 nitroso compounds affect all the stages of interphase (G2-S and G1) and even at prophase (Table II). We have observed aberrations after 2 h of recovery which is an indication of the fact that their effect is non-delayed (classifications as Kihlman[8], Figure). But the maximum damage was observed in the range of 12–16 h recovery

Table I. Mitotic frequency at different recovery periods

Recovery period	MNG 0.1% Total cells	% of dividing cells	% of control	NMU 0.06% Total cells	% of dividing cells	% of control
Control	4,675	5.4	100	4,675	5.4	100
1 h	6,189	4.6	85.1	8,289	3.13	57.9
2 h	8,336	4.5	83.3	9,869	1.16	21.4
4 h	11,586	3.4	62.9	11,077	1.66	30.7
6 h	13,231	1.8	33.3	12,006	1.64	30.3
8 h	13,345	1.7	31.4	Not recorded		
12 h	Not recorded			6,571	0.76	14.0
16 h	6,047	4.5	83.3	7,185	1.02	18,8
20 h	4,913	2.7	50.0	6,062	0.97	17.9

Table II. Percentage of abnormal metaphases

Recovery period (h)	% of metaphases with aberrations (MNG 0.1%)	Control (in distilled water) % of abnormal	% of metaphases with aberrations (NMU 0.06%)
1	7.14	0.16	40.00
2	74.28	–	90.62
4	77.7	–	92.98
6	83.33	–	95.16
8	88.88	0.70	96.77
12	90.47	–	100
16	81.35	–	Disintegration
20	64.5	–	and extreme
24	42.85	0.32	stickiness