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

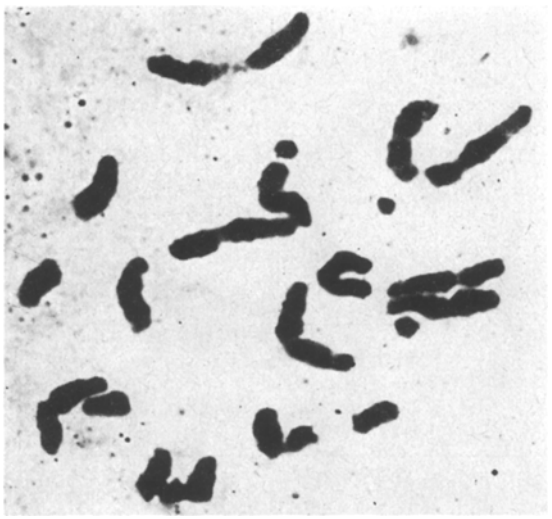
period. The cells affected could be those which were at the S-stage at the time of treatment.

The uptake of DNA precursor, H^3-thymidine, measured in terms of number of silver grain formation, is suggestive that at a comparable concentration NMU is more effective in inhibiting DNA synthesis (Table III). It is also evident that DNA synthesis when compared to normal control is significantly reduced though not completely inhibited.

Table III. Pattern of incorporation

Treatment	No. of slides	No. of cells	Mean No. of silver grains per nucleus[a]	Labelling as % of control
Control (H^3-thymidine in distilled water)	4	65	32.06	100
MNG 0.1%	2	25	12.28	38.3
MNG 0.2%	4	50	7.66	23.8
NMU 0.2%	4	70	5.60	17.4

[a] Mean calculated after deduction of background incorporation.



Metaphase showing chromosomal aberrations after 2 h of recovery.

The major portion of the chromosomal damage and the reduction in mitotic activities are present in those cells which were at synthetic stage of DNA replication at the time of treatment. DNA is considered to be the most sensitive material to alkylation within the cell and a primary site for alkylation[8,9]. Chromosomal aberrations observed in the cells belonging to G2 stage could be independent of DNA synthesis or, alternatively, DNA here is quantitatively or qualitatively different[10]. The effect of these chemicals appears to be similar to that of ionizing radiation. Their ability to induce chromosomal changes could partly be due to their capacity to alter the state and properties of DNA, but still the exact mechanism by which the aberrations are induced is not clearly understood[11].

Zusammenfassung. Mit Nitrosemethylurethan und Nitroseguanidin behandelte keimende Gerstensamen zeigten eine auffallend geringere Mitoseaktivität, chromosomale Abweichungen, nichtverzögerte Effekte und Abnahme der DNA-Synthese. Die chromosomale Schädigung scheint von der DNS-Synthese unabhängig zu sein.

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Basic Proteins of Mouse Ova and Blastocysts

We have undertaken a cytochemical study of nuclear and cytoplasmic basic proteins associated with nucleic acids on early mouse embryos, because several lines of evidence point to the precocious beginning of gene activity in this species[1,2].

Tubal and uterine embryos were obtained from random-bred, not superovulated, albino mice. The alkaline fast-green procedure[3] was standardized for whole ova by trying variations of each step. The optimal conditions are: fixation in 10% neutral formalin for 120 min; 10 min rinsing in H_2O; 30 min extraction in 5% TCA at 90 °C; rinsing in 70% ethanol for 30 min; rinsing in H_2O; staining in 0.1% fast-green FCF (National Allied) at pH 8.2 for 60 min; dehydration and mounting. The nucleic acid extraction is omitted in the controls.

This procedure revealed basic proteins, which must be linked to nucleic acids, since they do not stain when these are not extracted. The cytoplasmic staining decreased from the 1- to 4-cell embryos, and thereafter the process continued at a diminishing rate. When the nucleic acids were extracted with ribonuclease[4] (Mann Lab.), the results were somewhat inconclusive, due to the high extraction observed in the controls. But after deoxyribonuclease treatment[5] (Worthington Lab.), the cytoplasm remained as pale as in the controls, thus showing that it is with RNA that the basic cytoplasmic proteins are associated.

Our observations recall those made on the oocytes of some marine invertebrates[6-9] and in the early embryo of the sea-urchin[10,11], though, in the latter, the cytoplasmic basic proteins increase after fertilization up to the late