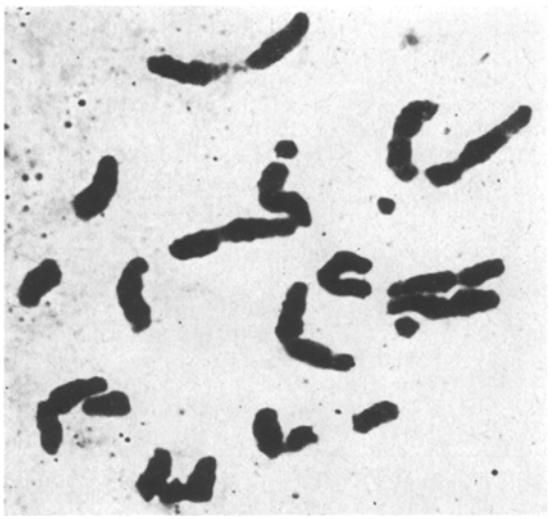
period. The cells affected could be those which were at the S-stage at the time of treatment.

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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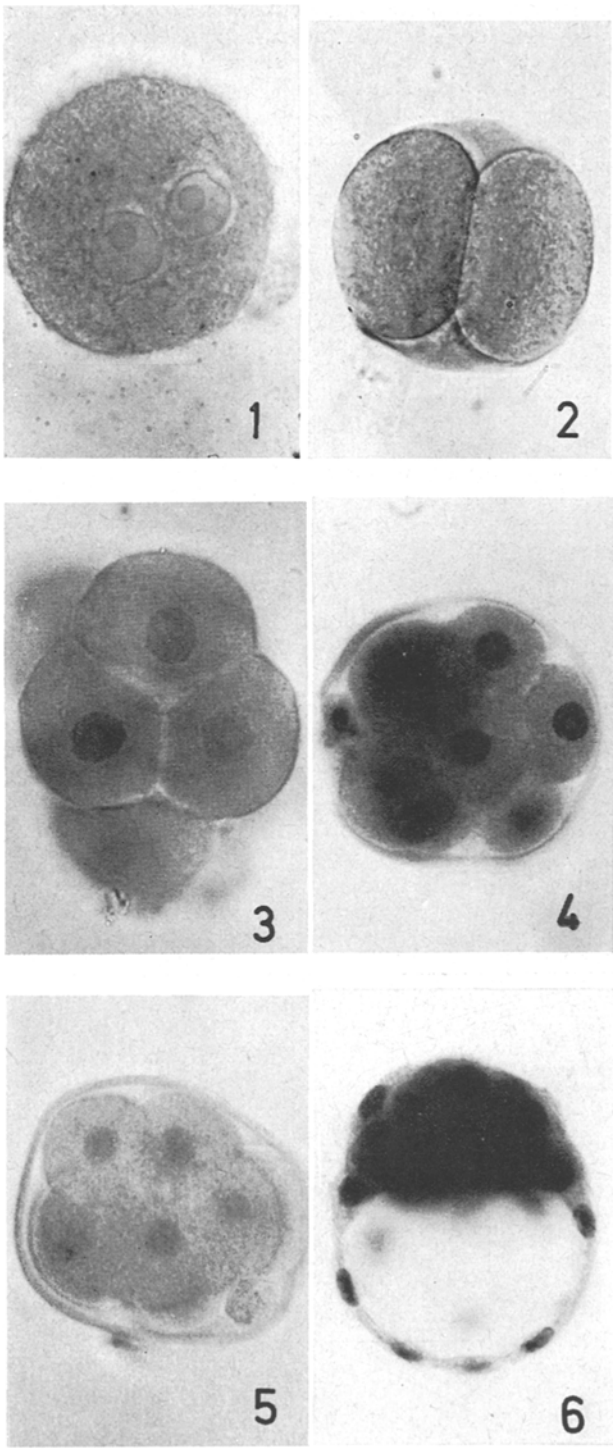
Basic Proteins of Mouse Ova and Blastocysts

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



The microphotographs do not reveal the overall staining concentration, due to the automatic correction of the exposure meter.

1. Pronuclear stage.
2. 2-cell stage.
3. 4-cell stage.
4. Early morula.
5. Early morula, acetylated.
6. Early blastocyst.

The microphotographs do not reveal the overall staining concentration, due to the automatic correction of the exposure meter. 1. Pronuclear stage. 2. 2-cell stage. 3. 4-cell stage. 4. Early morula. 5. Early morula, acetylated. 6. Early blastocyst.

blastula and later disappear. If, as in other materials, the cytoplasmic basic proteins are ribosomal[12], the diminution we observed might be related to the beginning of genetic translation.

The nuclei of 1- and 2-cell embryos bound as much stain as the cytoplasm, and from the 4-cell stage onwards, the nuclear staining increased while the cytoplasmic staining dcreased.

'Lysine-rich' and 'arginine-rich' basic proteins were differentiated by treating the ova, after TCA extraction, with acetic anhydride for 3 h in a microchamber[13] before staining with alkaline fast-green. Since acetylation inhibits the basicity due to lysine, only the 'arginine-rich' basic protein stains after this treatment[3].

Earlier than the 4-cell stage, the embryos stained similarly, whether acetylated or not; after the 4-cell stage the acetylated material stained gradually less, which means a relative increase in lysine. This suggests a regulation of genetic transcription, though the relation of histone kind to chromatin activity is not yet clear[14].

Differentiating by acetylation, we expected to witness the histone transition that reverses the process which occurs in spermatogenesis[2,15]; but the pronuclei appear equally faintly coloured, acetylated or not, as if they carried the same kind of histone, diluted in their large volume. We cannot argue for or against the masking of pronuclear histones[16] on the basis of the Sakaguchi test, since in our hands it shows that the nuclei stain stronger than the cytoplasm only for a while, during the process of fading.

In conclusion, our findings support the assumption that the changes in nuclear and cytoplasmic basic proteins associated with nucleic acids, are related to the beginning of genetic activity[17].

Zusammenfassung. Mit einer speziellen Färbetechnik auf basische Proteine wird gezeigt, dass die Intensität und damit auch die Quantität der basischen Proteine beim Übergang vom 4-Zell-Stadium zum 8-Zell-Stadium im Cytoplasma stark zunimmt. Daraus wird der Schluss gezogen, dass erst zu diesem Zeitpunkt die genetische Information für die Proteinsynthese wirksam wird.

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