résultats superposables à ceux obtenus au niveau des articulations périphériques dans différentes conditions qui aboutissent à l'immobilité des structures squelettiques (explantation en greffe ou en culture[14–16], extirpation de la moelle épinière[11]). Ainsi, il apparaît possible d'associer les troubles tératologiques axiaux induits par des agents neuroactifs aux perturbations fonctionnelles inhérentes à leurs propriétés pharmacologiques reconnues.

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Comparative incorporation of uridine-^{3}H into nucleolar RNA of mouse subcutaneous and skin tissues at early times after 20-methylcholanthrene administration

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Summary. A single s.c. injection of 20-methylcholanthrene (1 mg in 0.2 ml olive oil) is found to stimulate the relative uptake of uridine-^{3}H by the skin and the s.c. tissue 17- and 3fold respectively, 24 h post-administration.

The changes in the uptake of labelled precursors of nucleic acids by DNA and RNA at an early stage following the administration of a carcinogen have been demonstrated by Paul[1]; in that case, the RNA synthesis starts rising from 24 h onwards, reaching a peak value at 48 h after the administration of DMBA[2] into the mouse skin. The changes observed in the total RNA synthetic rates might reflect a change in the nucleolar function[3]. The early response of the RNA metabolism to carcinogen, promoters and even the treatment with a growth hormone, is found to involve a stimulated incorporation of the precursors into the various components of the cellular RNA[1,4,5]. The preliminary work presented in this note deals with the alteration in the uridine-^{3}H uptake by the nucleolar RNA of the skin and other tissues exposed directly or indirectly to a single carcinogenic dose of 20MC soon after administration. The functional significance of these changes in the overall carcinogenic event is yet to be established; however, the knowledge of these changes may indicate the importance of the nucleolar function in the process of carcinogenesis.

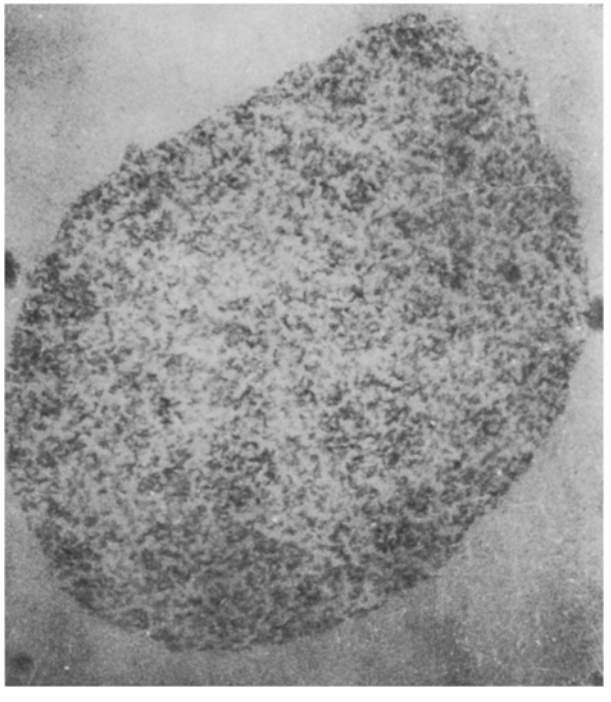

Fig. 1. Electron micrograph of a single nucleolus (× 30,000) isolated by Citric Acid-Detergent Mixture Technique[8] fixed in glutaraldehyde and osmium tetraoxide embedded in epon, stained in lead citrate and uranyl acetate. The boundry of structure is apparently free of cytoplasmic tags satisfying criteria of purity.

55 C57BL/Bcr female mice (8–9 weeks old) of about 25 g, were used in the present experiment. Animals were maintained on the standard chow diet, with water available ad libitum. Each mouse was injected with 20MC (1 mg in 0.2 ml olive oil/25 g b.wt) s.c. 15 female mice were used for the study of initial events, whereas the remaining animals were maintained to observe the development of tumors. At each point, 3 animals were used in the study and the tissues were pooled.

20MC treated mice at different hours were given an i.p. injection of uridine-^{3}H (sp. activity-2.7 Ci/mM) at a dose of 6 μCi/g b.wt and were decapitated 2 h after the injection, between 13.00 and 15.00 h to minimize the error owing to diurnal variation in the cell metabolism. Tissues were excised and chilled in 1.5% citric acid. Nuclei, isolated by citric acid procedure of Busch[6], were subjected for isolation of nucleoli (Penman[7]). The entire procedure is published elsewhere[8]. The pellet was considered to be the nucleolar fraction on the basis of evidence from electron microscopy (figure 1) and polyacrylamide gel electrophoresis[8]. The RNA from the nucleolar pellet dispersed in SDS buffer (0.1 M NaCl, 0.001 M EDTA, 0.01 M Tris/HCl at pH 7.4 and 0.5% SDS) was extracted with phenol EDTA solution at 55 °C. The optical density was determined with Backman DU-2 spectrophotometer and the activity due to tritium was counted with the liquid scin-

Sr. No.	Time (h)	Counts/A_{260}/min Treated (T)	Control (S)	Ratio T/S
Skin tissue				
1	0	186	186	1.00 ± 0.460
2	19.5	623	1361	0.458 ± 0.105
3	21.5	270	256	1.054 ± 0.645
4	24.5	72850	4410	16.520 ± 0.780
5	37.5	785	1000	0.785 ± 0.235
S.c. Tissue				
1	0	889	889	1.000 ± 0.3800
2	19.5	333	1370	0.243 ± 0.118
3	21.5	464	293	1.583 ± 0.658
4	24.5	25000	8890	2.812 ± 0.395
5	37.5	477	651	0.734 ± 0.506

tillation counting system (Nuclear Enterprises). The uptake of uridine has been expressed in terms of cpm per A_{260} (absorption unit) of Nu-RNA.

The incorporation of uridine-^{3}H was determined between 0 and 37 h after the administration of 20MC in the Nu-RNA extracted from (2×2 cm^2) samples, a) the skin around the site of injection, b) the skin geometrically opposite to the site of injection, c) the s.c. tissues underneath the skin coat around the site of injection, d) the s.c. tissue geometrically opposite to the site of injection. The uptake of uridine by 20MC treated skin and the s.c. tissue at different hours is expressed relative to the skin and s.c. tissue distant from the site of injection (figures 2 and 3). The choice of tissue as a control at a distance from the site of action is relevant in these experiments where circadian

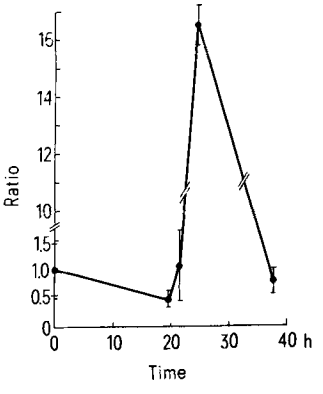

Fig. 2. The plot of relative uridine-^{3}H uptake by skin tissue injected with 20MC compared to skin distant from site of injection (T/S).

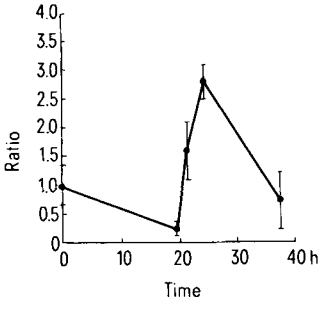

Fig. 3. The plot of relative uridine-^{3}H uptake by s.c. tissue injected with 20MC compared to s.c. tissue distant from site of injection (T/S).

rhythms are likely to affect the rate of detoxification of 20MC. It is observed that 20MC induced 17- and 3fold increase in incorporation of uridine-^{3}H at about 24 h into the Nu-RNA of the skin and s.c. tissue respectively. However, the uptake tended to be normal at 37 h after the administration of the carcinogen. The s.c. injection of 20MC was found to induce sarcoma in the skin when a palpable growth, characteristic of a tumor, was observed after a latent period of 110 $\pm$ 20 days at the site where 20MC was injected. The frequency of tumor appearance was 100% after 150 days in a group of 30 female mice maintained at 35 $\pm$ 4 °C, whereas the incidence of tumor growth in the male mice was hardly 10%.

The changes in the nucleolar functions of the skin and the s.c. cells exposed directly to 20MC reveal different rates of uridine uptake by the nucleolar RNA. The functional changes in the nucleolus become evident in the form of increased protein synthesis by the cytoplasmic ribosomes after a definite time-lag. The close agreement in the maximum alteration of the RNA synthesis by the nucleolus and the start of the increased uptake of uridine-^{3}H by the cellular RNA, which eventually reaches a peak value 48 h after the administration of the carcinogenic dose of DMBA, emphasizes the fact that the spurt in the synthetic activity of the proteins in the cytoplasm does not occur in the initiated cells until the carcinogen-induced newly synthesized ribosomes begin to accumulate in the cytoplasm. A similar result is also reported by Tata[5] for the growth-inducing and differentiation-inducing hormones. The present observation on the increased rRNA synthesis in the nucleolus by 20MC at 24 h points to the association of the nucleolus in the early stages of carcinogenesis.

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A preliminary report on γ-irradiated protein-dye complexes

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Summary. In spectral studies of γ-irradiated protein-dye complexes, influences of concentrations of the components and changes in dye character are mainly noted.

Report of radiation effects on DNA-dye[2] and DNA-cholesterol[3] complexes have appeared. Interactions of protein and organic anions[4] are being studied. Each dye produces a different conformation[5] of protein. The interaction of m-methyl red to bovine serum albumin[6] differs from that of o- or p-methyl red. Besides the nonspecific binding, rare specific binding[7] is also reported. In some proteins, new sites[8] result from conformational changes, induced by initial binding at prefered sites. The absorbance[9] of a dye, when bound to serum albumin of different species, differs. The organizations of globular proteins with different axial ratios are obscure. The amino acid sequence of histone confines the basic residues at one end. 2 terminals[10] of histone II bind DNA differently.