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

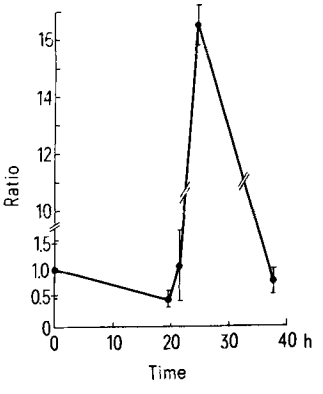

Fig. 2. The plot of relative uridine-^{3}H uptake by skin tissue injected with 20MC compared to skin distant from site of injection (T/S).

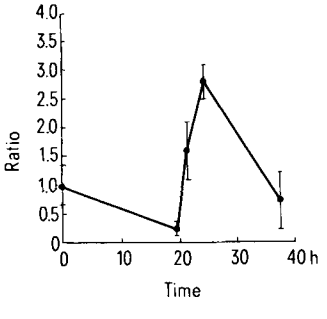

Fig. 3. The plot of relative uridine-^{3}H uptake by s.c. tissue injected with 20MC compared to s.c. tissue distant from site of injection (T/S).

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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2 The abbreviations used are 20MC for 20-methylcholanthrene; Nu-RNA for nucleolar RNA; DMBA for 7,12-dimethylbenz(a)-anthracene.
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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.

Radiation effects on protein-dye complexes

Protein and concentration	Dye and concentration	Concentration and position of peak of reference	Changes of peak on complexation (control) against refer.	Changes of peak on irradiation against control			After effects			Remarks
				3000 R	6000 R	9000 R	3000 R	6000 R	9000 R	
Histone 5 γ (rod structure)	Toluidine Blue 20 γ (65×10^{-6} M)	10 γ (33×10^{-6} M) 630 nm. Hump around 610 nm	0.6-unit increase Red shift to 640 nm. Hump around 610 nm. disappears	1-unit decrease. Stands below ref. No other change	Same as 3000 R	Same as 3000 R	–	No	–	Complexity changes in dye structure and character to give high absorbance: Absence of hump indicates dimer-monomer exchange with elimination of dimer. Irradiation causes another type of change in dye character to exhibit lower absorbance without dimer. Irradiation breaks the structure, assumed by the complex. No dose effects or after-effects occur. Effect is stable.
Histone 20 γ (Figure)	Toluidine Blue 20 γ (65×10^{-6} M)	10 γ (33×10^{-6} M) 630 nm. Hump around 610 nm	1.1-unit increase. Red shift to 640 nm. Hump around 610 nm. disappears	0.6-unit decrease Revertion to 630 nm. Stands 0.5-unit above ref. No other change	1.1-unit decrease. Revertion to 630 nm. Stands at equal height to that of ref. No other change	2-unit decrease. Revertion to 630 nm. Stands 0.9-unit below ref. No other change	–	–	–	Complexity is similar to that with low histone-content but with increased peak. Irradiation decreases and reverts the peak. Dye release occurs at 3000 R and is completed or 6000 R when radiation effects occur on released dye up to 9000 R, thus displaying dose effect without after-effect. Binding is weak.
Histone 50 γ	Toluidine Blue 20 γ (65×10^{-6} M)	10 γ (33×10^{-6} M) 630 nm. Hump around 610 nm	1.4-unit increase. Red shift to 640 nm. Hump around 610 nm. disappears	0.8-unit decrease. Stands 0.7-unit above ref. No other change	0.9-unit decrease. Stands 0.6-unit above ref. No other change	1.2-unit decrease. Stands 0.3-unit above ref. No other change	–	–	–	Complexity is similar to that with other histone contents but with more increased peak. Irradiation causes changes practically at the initial stage with dose effect above 6000 R and no after affect.
Bovine serum albumin 5γ. Axial ratio 4:1	Toluidine Blue 20 γ (65×10^{-6} M)	10 γ (33×10^{-6} M). Hump around 610 nm	No change							No complex
Bovine serum albumin 20 γ	Toluidine Blue 20 γ (65×10^{-6} M)	10 γ (33×10^{-6} M). Hump around 610 nm	No change							No complex
Bovine serum albumin 50 γ	Toluidine Blue 20 γ (65×10^{-6} M)	10 γ (33×10^{-6} M). Hump around 610 nm	0.6-unit increase. No shift. Hump around 610 nm. disappears	–	1.7-unit decrease. Stands 1-unit below ref. No other change	Same as 6000 R	–	1-unit further decrease	Same as 6000 R	Complexity changes dye character eliminating dimer. It resembles the cases with Histone but without peak shift. Irradiation releases dye at initial stages when radiation effects on released dye occur, indicating weak binding. No dose effects but slight after-effects exist.
γ-Globulin 5 γ. Axial ratio 10:1	Toluidine Blue 20 γ (65×10^{-6} M)	10 γ (33×10^{-6} M) 630 nm. Hump around 610 nm	No change							No complex

γ-Globulin 20 γ	Toluidine Blue 20 γ (65×10^{-6} M)	10 γ (33×10^{-6} M) 630 nm. Hump around 610 nm	No change							No complex
γ-Globulin 50 γ	Toluidine Blue 20 γ (65×10^{-6} M)	10 γ (33×10^{-6} M) 630 nm. Hump around 610 nm	0.7-unit decrease. No shift. Hump around 610 nm disappears	1.7-unit decrease. Stands 2.4-unit below ref. No other change	1.4-unit decrease Stands 2.1-unit below ref. No other change	1.1-unit decrease. Stands 1.8-unit below ref. No other change	Slight farther decrease	0.75-unit further decrease	Same as 6000 R	Complexity changes dye character which are dissimilar to those with other proteins. Complexity alters the dye character to show lower absorbance, eliminating dimer. Radiation increases binding when maximum induced binding occurs initially at 3000 R; continued irradiation releases radiation induced binding of initial stage. The release is systematic with higher doses indicating dose effect. After-effect indicates recovery of the released binding.
Histone 5 γ	Methylene Blue 10 γ (27×10^{-6} M)	5 γ (14×10^{-6} M) 470 nm. Band at lower wavelength exists	1-unit increase. No other change	1.8-unit decrease. Stands 0.8-unit below ref. No other change	2.1-unit decrease. Stands 1.1-unit below ref. No other change	2.4-unit decrease. Stands 1.4-unit below ref. No other change	–	–	-	Complexity changes dye character to show higher absorbance while permitting dimer contribution. Irradiation changes dye character differently to give lower absorbance without affecting dimer contribution. Systematic dose effect without after effect is present. Effect is stable.
Histone 10 γ	Methylene Blue 10 γ (27×10^{-6} M)	5 γ (14×10^{-6} M) 470 nm. Band at lower wavelength exists	1-unit increase. No other change	1.8-unit decrease. Stands 0.8-unit below ref. No other change	2.2-unit decrease. Stands 1.2-unit below ref. No other change	2.4-unit decrease. Stands 1.4-unit below ref. No other change	–	–	–	Complexity and irradiation cause similar changes to that with low histone content. Dose effects and no after effect are present.
Histone 50 γ	Methylene Blue 10 γ (27×10^{-6} M)	5 γ (14×10^{-6} M) 470 nm. Band at lower wavelength exists	1.4-unit increase. No other change	0.5-unit decrease. Stands 0.9-unit above ref. No other change	1.4-unit decrease. Stands equal to that of ref. No other change	0.9-unit decrease. i.e. increase, compared to 6000 R. Stands 0.4-unit above ref. No other change	–	–	–	Changes on complexity are similar to that with lower histome content but with more increased peak. Irradiation causes another change in dye character unaffecting dimer contribution. Irradiation releases bound dye and completes dye release at 6000 R; continued irradiation up to 9000 R produces radiation effects on released dye showing increased absorbance. This may indicate comparatively weak binding. After effect is not present.
Bovine serum albumin 5 γ	Methylene Blue 10 γ (27×10^{-6} M)	5 γ (14×10^{-6} M) 470 nm. Band at lower wavelength exists	1-unit increase. No other change	2-unit decrease. Stands 1-unit below ref. No other change	Same as 3000 R	Same as 3000 R	–	–	Further decrease	Complexity is similar to that with 5 γ-histone. Irradiation changes dye character to show lower absorbance unhindering dimer contribution. Initial change without dose effect occurs. After effect exists.
Bovine serum albumin 10 γ	Methylene Blue 10 γ (27×10^{-6} M)	5 γ (14×10^{-6} M) 470 nm. Band at lower wavelength exists	No change							No complex

In no case precipitation takes place.

Continued next page

Radiation effects on protein-dye complexes (continuation from page 398–399)

Protein and concentration	Dye and concentration	Concentration and position of peak of reference	Changes of peak on complexation (control) against refer.	Changes of peak on irradiation against control			After effects			Remarks
				3000 R	6000 R	9000 R	3000 R	6000 R	9000 R	
Bovine serum albumin 50 γ	Methylene Blue 10 γ (27×10^{-6} M	5 γ (14×10^{-6} M) 470 nm. Band at lower wavelength exists	0.6-unit increase. No other change	1.4-unit decrease. Stands 0.8-unit below ref. No other change	Same as 3000 R	2-unit decrease. Stands 1.4-unit below ref. No other change	–	–	Further decrease	Complexity is similar to that with 5 γ-albumin but gives lower absorbance. Irradiation produces different changes in dye character unhindering dimer contribution. No dose effects or after effect are seen at lower doses but they exist at high dose.
γ-Globulin 5 γ	Methylene Blue 10 γ (27×10^{-6} M)	5 γ (14×10^{-6} M) 470 nm. Band at lower wavelength exists	0.5-unit decrease. No other change	0.6-unit decrease. Stands 1.1-unit below ref. No other change	Same as 3000 R	Same as 3000 R	–	Slight further decrease	Slight further decrease	Complexity is different from that with other 2 proteins, as appears from decreased peak involving changes in dye character without hindering dimer contribution. Irradiation increases binding without dose effect.
γ-Globulin 10 γ	Methylene Blue 10 γ (27×10^{-6} M)	5 γ (14×10^{-6} M) 470 nm. Band at lower wavelength exists	1.2-unit decrease. No other change	0.3-unit decrease. Stands 1.5-unit below ref. No other change	1.6-unit decrease. Stands 2.7-unit below ref. No other change	Same as 6000 R	–	No	No	Complexity is similar to that of 5 γ-globulin with more decreased absorbance; Irradiation increases binding. Dose effect starts at 3000 R, attains maximum at 6000 R and then ceases up to 9000 R. No after effect exists.
γ-Globulin 50 γ	Methylene Blue 10 γ (27×10^{-6} M)	5 γ (14×10^{-6} M) 470 nm. Band at lower wavelength exists	1.2-unit decrease No other change	No change	0.8-unit decrease. Stands 1.7-unit below ref.; No other change	1.2-unit decrease. Stands 2.4-unit below ref.; No other change	No	Negligible decrease	Considerable decrease	Complexity is similar to lower Globulin content. Irradiation increases binding. No effect is seen at low dose but dose effect exists at higher dose.
Histone 5 γ	Acridine Orange 20 γ (46×10^{-6} M)	10 γ (23×10^{-6} M) 490 nm. Shoulder around 465 nm exists	0.7-unit increase. No other change	1.5-unit decrease. Stands 0.8-unit below ref. Shoul. minimized. No oth. change	Same as 3000 R	Same as 3000 R	–	No	No	Complexity changes dye character to give high absorbance permitting dimer contribution. Irradiation induces another kind of dye characteristic minimizing dimer contribution. Initial change occurs without dose effect of after effect Radiation appears to release dye immediately, when radiation effects on the released dye occurs showing decreased peak.
Histone 20 γ	Acridine Orange 20 γ (46×10^{-6} M)	10 γ (23×10^{-6} M) 490 nm. Shoulder around 465 nm exists	0.7-unit increase. No other change	1-unit decrease. Stands 0.3-unit below ref. Shoulder lessened. No other change	1.7-unit decrease. Stands 1-unit below ref. Shoulder lessened. No other change	2-unit decrease. Stands 1.3-unit below ref. Shoulder lessened. No other change	–	Further decrease	Further decrease	Complexity is similar to that with 5 γ-histone. Irradiation seems to release dye at the initial stage and radiation effects on dye occurs then. Dose effects and after effect are displayed. The radiation effect is slightly differing from that with lower histone content.

Histone 50 γ	Acridine Orange 20 γ (46×10^{-6} M)	10 γ (23×10^{-6} M) 490 nm. Shoulder around 465 nm exists	0.7-unit increase. No other change	0.7-unit decrease. Stands equal to that of ref. Shoulder minimized. No other change	Same as 3000 R	Same as 3000 R	–	No	No	Complexity is similar to that with lower histone content and radiation effects differ when only dye release occurs without dose effect or after effect.
Bovine serum albumin 5 γ	Acridine Orange 20 γ (46×10^{-6} M)	10 γ (23×10^{-6} M) 490 nm. Shoulder around 465 nm exists	No change							No complex
Bovine serum albumin 20 γ	Acridine Orange 20 γ (46×10^{-6} M)	10 γ (23×10^{-6} M) 490 nm. Shoulder around 465 nm exists	No change							No complex
Bovine serum albumin 50 γ	Acridine Orange 20 γ (46×10^{-6} M)	10 γ (23×10^{-6} M) 490 nm. Shoulder around 465 nm exists	No change							No complex
γ-Globulin 5 γ	Acridine Orange 20 γ (46×10^{-6} M)	10 γ (23×10^{-6} M) 490 nm. Shoulder around 465 nm exists	0.7-unit decrease. No other change	1-unit decrease. Stands 1.7-unit below ref. Shoulder lessened. No other change	Same as 3000 R	Same as 3000 R	–	Further decrease	Further decrease	Complexity changes dye character to show low absorbance, unherting dimer contribution. Radiation releases dye immediately when radiation effect on released dye occurs. Initial change without dose effect but with after effect occurs.
γ-Globulin 20 γ	Acridine Orange 20 γ (46×10^{-6} M)	10 γ (23×10^{-6} M) 490 nm. Shoulder around 465 nm exists	0.7-unit decrease. No other change	0.25-unit decrease. Stands 0.95-unit below ref. No other change	1.1-unit decrease. Stands 1.8-unit below ref.	–	Further decrease	Further decrease	–	Changes on complexity are similar to that with low globulin content. Radiation releases dye less drastically. At higher dose, radiation effect on released dye is exhibited. Dose effects and after effect are displayed.
γ-Globulin 50 γ	Acridine Orange 20 γ (46×10^{-6} M)	10 γ (23×10^{-6} M) 490 nm. Shoulder around 465 nm exists	0.7-unit decrease. No other change	0.8-unit decrease. Stands 1.5-unit below ref. Shoulder absent. No other change	Same as 3000 R	Same as 3000 R	–	Slight further decrease	Same as 6000 R	Complexity shows similar changes to that with lower globulin content. On irradiation dye release occurs immediately when radiation effect on released dye takes place. Initialchange without dose effect and with slight after effect occurs.

In no case precipitation takes place.

From these view points, γ-irradiation effects on protein-dye complexes have been studied spectrophotometrically with varied concentrations of various proteins and dyes when the mechanisms differ from DNA-dye complexes[11,12].

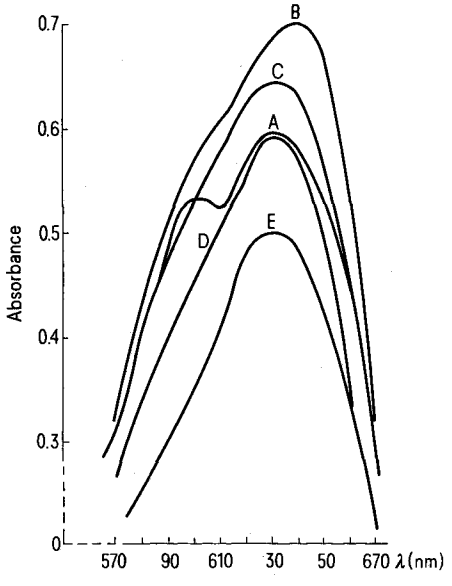
Materials and methods. Calf thymus histone, bovine serum albumin, bovine γ-globulin, Methylene Blue (Sigma), Toluidine Blue (Merck) and Acridine Orange (Schuchardt), solubilized in 0.01 M Tris-HCl buffer, pH 7.4, free from radiation effects were used[13]. Toluidine Blue required stirring[14]. Spectra of 10γ (33×10^{-6} M) Toluidine Blue, 5γ (14×10^{-6} M) Methylene Blue and 10γ (23×10^{-6} M) Acridine Orange, measured at 570–680 nm, 600–700 nm and 450–530 nm respectively, served as reference spectra. Solution of dye, in double the concentration of reference solution, was mixed with that of protein in equal volume to serve for control spectra as well as test samples, to use the concentration of reference for all measurements. Formed complexes were irradiated to doses of 3000 R, 6000 R and 9000 R at a dose rate of 245 R/min from a Cs^{137} source. Dye solutions were also irradiated. Hilger spectrophotometer was used. Effects were measured on stored samples after 24 h. Changes in complexity were estimated against reference, and that on irradiation against control spectra.

Results and discussions. The results are presented in the table and explained by remarks.

On irradiation to 6000 R, 10 γ Toluidine Blue and 10 γ Acridine Orange shows decreased absorbance without dimer, whereas 10 γ Methylene Blue shows an increased one with dimer, while none produce a peak shift. Similar irradiation on a few proteins are not significant.

The observed differences in the spectra thus appear to be due to radiation effects on the complexes. Different proteins at varied concentrations interact with different dyes differently and behave differently towards radiation. Complexity and irradiation of the complexes seem to induce different changes in dye character. In selcetive cases, radiation may increase binding. Cases of radiation release of bound dye may indicate weak binding. Concentrations of the components can influence complexity as well as behaviour towards radiation. Apparently these differences may appear to be associated with different characteristic or structure of individual components.

Results with cationic dyes and polyanions reveal that conformational changes in the polymer, shift the optical properties[15] of the bound dye. The varied behaviour of different proteins initiate complications in their different affinity and conformational consequences. The conformational change on binding may be associated with electrostatic[16,17] or hydrophobic[18] effect (at the pH used), when the latter unfolds protein by bound hydrogen. Spectral shift of Trypan Blue[19], occupying 2 identical sites in bovine serum albumin, has also been reported. Adjacently bound dyes[20] interact with each other, the 1st initiating a shift and the 2nd an absorption loss. These considerations warrent detailed studies to explain the observations correctly.



Effect of γ-rays on protein-dye complex

A 10γ Toluidine Blue, *B* 20γ histone + 20γ Toluidine Blue (50:50), *C* solution of *B* + 3000 R, *D* solution of *B* + 6000 R, *E* solution of *B* + 9000 R.

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