Tumorigenicity, doubling time, saturation density and colony forming ability

Cell line	Tumorigenicity Nude	Newborn	Adult	Rates of tumor production	Latency period (days)	50% survivals (days)	Doubling time (h)	Saturation density ($\times 10^4$/cm^2)	Sizes of colonies radii (r) (arbitrary units)	Colony forming ability
MEb	–	–	–	–	–	–	96	1.2	0	0
HEL	–	–	–	–	–	–	108	2.3	0	0
Htc1-4	–	–	–	–	–	–	50	26.9	–	0.11
m 324	+	–	–	3/3***	47	106	21	38.9	0.33±0.08	0.05
m 104	+	±	–	10/13**	7	42	21	36.0	0.44±0.04	0.05
MRCB	+	±	–	7/11**	10	33	19	36.7	1.47±0.56	0.07
L	+	±	–	6/11**	9	32	22	42.0	0.62±0.08	0.52
Htd773ca	+	+	+	5/5*	82	120	–	–	–	0.07
Htd773	+	+	+	8/8*	23	52	24	45.7	0.64±0.13	0.36
F	+	+	+	4/4*	21	70	36	55.1	1.29±0.33	0.65
Fsf14MEM	+	+	+	5/5*	28	86	35	56.0	–	0.75
MC	+	+	+	4/4*	6	70	22	75.5	1.10±0.18	0.85
ME	+	±	±	3/5*	99	140	24	18.5	–	0
SY	+	+	+	11/11*	20	90	22	15.0	–	0

*** Nude, ** newborn and * adult mice.

showed no capacity to produce colonies. It is known that various factors, such as polyanionic substances in agar, collagen, serum levels, insulin, serin and other amino acids, influence the colony forming ability in a suspension culture of untransformed cells[4]. However, Sanders et al. reported that some of these factors have no relationship to the growth of transformed cells in soft agar[6]. Our SY and ME cell lines have only been maintained for less than a year after the cells had spontaneously transformed, and this is less time than other cell lines which we have been maintaining in vitro for more than 10 years. The SY and ME may not have adapted to culture conditions enough to produce colonies. We are now investigating these possibilities. The advantages of calculating colony forming ability with formulas (1) or (2) were that a) we could clearly determine the various degrees of malignancy of the cells which showed no growth in 0.3% or 0.4% soft agar, and b) we could represent the degree of malignancy as a single value, even though different values representing colony forming rates were obtained at different concentrations of agar. We could calculate the colony forming ability easily if a general function 'f(x)' could be established. Hitherto, the cells which could grow in a soft solid agar have been considered to be malignant. On the other hand, it is well-known that many cell lines, although not normal, show neither tumorigenicity nor growth in soft agar medium at these concentrations. In our method, we were able to differentiate those which were neither malignant nor normal from those which were either malginant or normal, and allocate them a position between those of normal and malignant cell lines. It could open new possibilities for the elucidation of the mechanism of the control of malignancy if cell lines having different degrees of malignancy could be compared on the same table. Finally, we would like to mention those problems which should be taken into consideration in a soft agar experiment. The colony forming ability is greatly influenced by a) the state of the cells used, b) the period of trypsin treatment, c) the temperature of the agar medium at the time when the cells were planted, and d) the lot of serum used. These 4 points should be watched with care in every experiment in which an agar medium is used. In order to elucidate the genetic control of malignancy, first of all, we must determine the phenotype of malignancy and do a quantitative study of this phenotype. In this experiment, we tried to find out whether or not growth in soft agar correlated with the degree of malignancy shown in vivo, and explore the possibility of applying this method to the quantitative study of malignancy. This experiment produced satisfactory results in this regard.

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Effect of 2-mercaptopropionylglycine (MPG) on thyroid function in sub-lethally irradiated mice[1]

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Summary. External irradiation resulted in an increase in thyroid ^{131}I uptake and plasma PB ^{131}I conversion ratio, whereas pretreatment with MPG reduced both the values significantly. Metabolic inhibition is suggested as a possible mechanism of action by the drug.

Previous studies in our laboratory have demonstrated the radioprotective effect of MPG on the different tissues which are directly responsible for the radiation death in mice[2-5]. Since thyroid plays an important role in determining the basal metabolic rate and since the metabolic state influences radiosensitivity, the present study has been carried out to determine the effect of MPG on thyroid function in mice exposed to 500 r of ^{60}Co gamma radiation.

Material and method. 40 adult male Swiss albino mice were selected from an inbred colony and divided into 2 equal groups, control (+500 r) and experimental (+500 r +MPG). The animals were treated as described earlier[3]

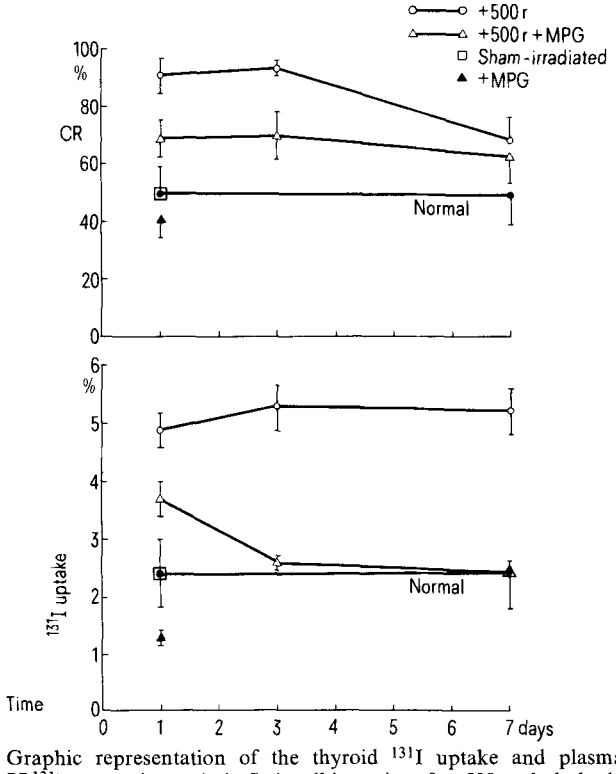
and exposed to 500 r of whole-body gamma radiation at a dose rate of 50 r/min.

Thyroid ^{131}I uptake and plasma protein bound iodine (PB ^{131}I) conversion ratio (CR) studies. The 24-h thyroid ^{131}I uptake and PB ^{131}I CR were determined at 1, 3 and 7 days after exposure in both the groups. For this, the animals of each group were further divided into 3 sub-groups. Each sub-group was injected parenterally with a tracer dose (5 μCi) of ^{131}I (supplied by BARC, Bombay, India, as sodium iodide in thiosulphate solution) in isotonic saline solution either 1 h after, 2 days after, or 6 days after irradiation. The animals were then sacrificed at 1, 3 and 7 days, respectively. The thyroids were removed, weighed separately and digested by pseudo-wet-ashing[6]. The radioactivity of the aliquot was determined over a flat crystal scintillation counter (Electronic Corporation of India, Ltd). Simultaneously the blood was withdrawn into independent heparinized tubes by cardiac puncture. The plasma was separated by centrifugation and the PB ^{131}I was precipitated by trichloroacetic acid method[6]. The radioactivity in the initial plasma and in the precipitate was determined as before. The injected radioactivity was calculated by counting the activity of 0.2-ml sample from the original solution. The values for the thyroid activity were corrected for background and decay, the uptake was calculated as cps/mg and expressed as percentage of injected activity. The plasma PB ^{131}I CR was calculated as follows:

$$\text{CR}\,\% = \frac{\text{Radioactivity in the precipitate (CPS)} \times 100}{\text{Radioactivity in the initial plasma (CPS)}}$$

Similar studies were also made in sham-irradiated animals injected with either MPG or distilled water. The experiment was repeated for confirmation.

Results. From the figure it is evident that thyroid ^{131}I uptake and CR (%) increase in both the control and experimental groups as compared to the sham-irradiated animals. However, both the values show a highly significant ($p < 0.001$) decrease in the experimental group as compared to their corresponding controls, except on day 1 in the case of ^{131}I uptake ($p < 0.05$) and on day 7 in the case of CR ($p < 0.01$), where the values are significant. But the 24-h thyroid ^{131}I uptake in the control group does not vary with the post-irradiation time, while in the experimental group the highest uptake is observed at day 1 followed by a significant decrease approaching normal value at day 3. The percent uptake on day 7 in this group shows the normal (sham-irradiated) value. The CR percentage in the control group shows a significant fall from 3 to 7 days, whereas in the experimental group the fall is considerably small, though significant as compared to the former.



Graphic representation of the thyroid ^{131}I uptake and plasma PB^{131}I conversion ratio in Swiss albino mice after 500 r whole-body gamma irradiation.

Discussion. The present finding that radiation exposure increases the thyroid ^{131}I uptake and CR in the mice is in general agreement with the earlier reports[7-11]. However, the histological structure of the thyroid in the present study indicates an inactive (hypothyroid) state[12]. Therefore, the high PB^{131}I value need not reflect a hyperfunctional condition, but as observed by Jovanovic[10] this may be due to the presence of a unique high molecular weight protein with iodine-binding capacity, not necessarily a hormone, which starts disappearing after 5 days post-irradiation. The reduction of CR in both the groups after the 5th day in the present study supports this view.

MPG pretreatment reduces the thyroid ^{131}I uptake and CR so that the values are more nearly normal in this group. The uptake falls to normal on the 7th day, when the CR also shows a similar trend, even though the normal value is not reached. The histological picture at this interval also reveals a more or less normal stucture[12]. This clearly indicates the protective effect of MPG on the thyroid which appears to be brought about by an effect on the metabolism. Metabolic inhibition by the drug is demonstrated in the normal mice, where injection of 20 mg/kg b.wt of MPG reduces the plasma PB^{131}I value significantly ($p < 0.02$). How this inhibition is brought about is not clear at present. This may be due to the disulphide formation with the proteins of the thyroid cells which ultimately blocks the activity of mitochondria, as reported by Skrede[13-15], or it may be due to peroxidyl scavenging which inhibits the formation of enzyme peroxidase, essential for the oxidation of inorganic iodine into the organic form[16-17].

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