

Cytotoxic and radiomimetic activity  
of irradiated culture medium on human  
leukocytes

P.C. Kesavan and M.S. Swaminathan,  
Indian Agricultural Research Institute  
New Delhi.

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Although as early as 1947, Stone, Wyss and Hass<sup>1</sup> reported the incidence of mutations in Staphylococcus aureus cultured on ultra-violet irradiated substrate, interest in the study of the indirect effects of radiation as measured by the effects observed in unirradiated test organisms grown on irradiated media, has been negligible <sup>until recently</sup>. During 1955-65, Swaminathan and co-workers carried out a series of experiments <sup>2-6</sup> 2-8 which indicated that in a wide range of material such as potatoes, fruit juices, and different types of culture media, cytotoxic and radio-mimetic compounds are generated following their exposure to dosages of gamma rays of the order used in the pasteurization and sterilization of food. The precise biochemical nature of these compounds has not yet been identified, although data suggesting that they may not be short lived were obtained<sup>6</sup>. Recently, Holsten, Sugii and Steward<sup>7,9</sup> have confirmed these observations and have further provided evidence for the formation of some new chemical substances in irradiated culture media containing sugar. The present paper relates to observations made during mitosis in human leukocytes ~~when they were~~ cultured on irradiated medium.

M. Idnani  
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Using a slight modification, of the technique of Moorhead et al.,<sup>8</sup> mitosis was studied in leukocyte cultures from four different healthy human males, ranging from 20 to 30 years in age. The composition of the medium was as follows:

- a) T.C. 199 7 ml.
- b) AB human serum 1.5 ml.
- c) Bacto-Phytohaemo-glutinin M  $\frac{1}{2}$  drops.

In one set of cultures, normal T.C.199 was used and in the other, T.C. 199 exposed in intact vials to different dosages of gamma rays in a 2000-curie  $\text{Co}^{60}$  source was added.

The prominent effects observed in cultures in which irradiated T.C. 199 was used, were (a) a considerable inhibition of mitosis, (b) pyknosis, chromosome fragmentation and polyploidy and (c) micronuclei formation at telophase (Table 1). Thus, the effects on mitosis, as observed in leukocytes, are similar to those earlier reported in plant meristems. When the T.C. 199 medium was irradiated, stored in a refrigerator for <sup>95</sup>15 days and then used, the cytotoxic properties ~~increased~~ <sup>persisted</sup>.

We have pointed out in several of our earlier papers that the radiomimetic effects produced by the irradiation of culture media are ~~small~~ small (about 1% in relation to the effect of direct irradiation) and ~~can~~ can be demonstrated in most cases only in relation to controls of comparable size. In recent literature there are reports both supporting our findings and contradicting them, <sup>9-17</sup>

There have been extreme views on the implications of the results obtained in such studies. While on the one hand Holsten et al.<sup>7</sup> hold that the work has obvious bearing on the radiation-sterilization of food, especially in cases in which the irradiated material is relatively rich in sugar, Goldblith<sup>16</sup> feels that the data already available indicate that irradiated food 'can be consumed with impunity'. Radiation sterilization of food is a very important technological development and could find even more application in the developing countries, than in the developed ones. It is hence ~~important~~<sup>desirable</sup> that the products and pathways of the indirect effects of radiations are studied critically. Golblith<sup>18</sup> believes that even if cyto-toxic products are produced in irradiated food, they may be of no consequence since animal systems have "mechanisms for modification, alteration and digestion of the foods by the alimentary tract and the detoxification and excretion mechanisms of the liver and kidney". The extensive data now available on the wholesomeness of irradiated food unfortunately all relate to somatic effects and no serious study has been made to detect possible genetic harm.<sup>19</sup> Elegant genetic tests such as those used by Russel in mice are available for obtaining critical data within a short time which would help ~~to reach~~<sup>in drawing</sup> scientifically satisfactory conclusions. In view of the great economic attractiveness of radiation food technology, support for such research would be extremely prudent.

At the level of genetics, there is a remarkable unity in the biological response to mutagenic agents.

Table 1.

## Observations during mitosis in human leukocyte cultures

Case No.	Medium and dose.	No. of hrs/day of storage of medium.	No. of leukocytes analysed.	Mitotic index.	No. of cells studied at mitosis	Percentage of cells with abnormalities				
						Euploid cells.	Gaps & breaks	Fragmentation.	Micronuclei (Scored at interphase)	Total No. of cells with abnormalities during division
I a)	Control	24 hrs	6401	2.99	20	0	0	0	0	0
b)	Irradiated (0.10 mega rads).	24 hrs	2294	0.21	9	0	22.22	66.66	11.00	88.88
II a)	Control	7 days	4779	7.95	26	0	3.84	0	0	3.84
b)	Irradiated (0.10 mega rads)	7 days	2635	0.30	8	0	-	62.50	16.50	62.50
III a)	Control	9 days	9199	3.42	34	0	5.88	2.94	0	8.82
b)	Irradiated (0.50 mega rads)	9 days	2194	0.31	16	0	12.50	43.75	0	56.25
IV a)	Control	15 days	5743	17.17	59	3.38	0	5.08	0	8.46
b)	Irradiated (0.50 mega rads)	15 days	2832	0.67	19	5.26	21.05	36.84	13.60	63.15

1. Stone, W.S., Wyss, O. and Haas, F., Proc. Natl. Acad. Sci. U.S., ~~1947~~ 1947, 33, 59.
2. Natarajan, A.T. and Swaminathan, M.S.,  
Ind. J. Genet., 1958, 18, 220.
3. Swaminathan, M.S., Chopra, V.L. and  
Bhaskaran, S., Radiation Research, 1962, 16, 183.
4. Swaminathan, M.S., Satya Nirula., Natarajan, A.T.  
and Sharma, R.P., Science, 1963, 141, 637.
5. Chopra, V.L., Natarajan, A.T. and  
Swaminathan, M.S., Radiation Botany, 1963, 3, 1.
6. Chopra, V.L. and Swaminathan, M.S.,  
Die Naturwissenschaften, 1963, 10, 374.
7. Holsten, R.D., Sugii, M. and Steward, F.C.,  
Nature, 1965, 208, 850.
8. Moorhead, P.S., Nowell, P.C., Mellman, W.J.,  
Batipps, L.M. and Hungerford, D.A., Exp. cell Res.,  
1960, 90, 613.
9. Reinhardt, R.R. and Ratty, F.J., Genetics, 1965, 52, 1119.
10. Chopra, V.L., Microbial genetics Bulletin, 1965, 23, 8.
11. Om Parkash., Nature, 1965, 205, 312.
12. Molin, N. and Ehrenberg, L., Int. J. Rad. Biol.,  
~~1965~~ 1965, 8, 223.
13. Berry, R.J., Hills, P.R. and Trillwood, W.,  
Int. J. Rad. Biol., 1965, 9, 559.

14. Moutseken, J. and Matagne, R., Radiation Botany, 1965, 5, 23.

15. Chopra, V.L., Nature, 1965, 208, 699.

16. Reddy, O.S., Reddy, G.M., Rao, J.I., Ebenezer, D.N. and Rao, M.S., Nature, 1965, 208, 702.

17. Khan, A.H. and Alderson, T., Nature, 1965, 208, 700.

18. Goldblith, S.A., Nature, 1966, 210, 433.

~~19. Russel, W.L., Radiation Biology ~~vol 1~~, Part 2, 1954, ~~Edited~~ Edited by Alexander Hollaender, McGraw-Hill Book Company, Inc.~~

19. Russel, W.L., Cold Spring Harbor Symposia Quant. Biol., 1951, 16, 327.