

TABLE I—(Contd.)

1	2	3	4	5
10. Acetaaminophen		4:1, 2:3, 1:9, 1:4.	9:1, 1:3.	..
11. Tween 80		1:4.	4:1, 1:1, 3:2, 1:9.	..
12. Sodium Carboxy Methyl Cellulose		9:1, 1:1, 2:3, 1:4.	9:1, 2:3.	..
13. Serotonin		1:4.	1:4, 2:3.	..
14. Methyl Paraben		No Interaction.	No Interaction.	..
15. Propyl Paraben		4:1, 2:3, 1:9.	4:1, 3:2, 2:3.	..
16. Benzalkonium Chloride		3:2, 3:7.	n4:1, 1:1.	..
17. Cetyl Trimethyl Ammonium Bromide		4:1, 1:1, 1:4.	3:2, 2:3.	..
18. Cetyl Pyridinium Chloride		4:1, 3:2, 1:9.	4:1, 3:2, 1:9.	..
19. Sodium Citrate		7:3, 1:9.	7:3, 1:9.	..
20. Citric Acid		9:1, 4:1, 3:2.	3:2, 2:3.	..
21. Benzoic Acid		4:1, 7:3, 1:4.	4:1, 7:3, 1:4, 1:1·7.	..
22. Sodium Benzoate		4:1, 3:2, 3:7, 1:9.	4:1, 3:2, 3:7, 1:9.	..
23. Sodium Pyruvate		9:1, 7:3, 2:3, 1:9.	9:1, 3:7, 1:9.	..
24. Lactic Acid		4:1, 1:1, 2:3, 1:4.	9:1, 2:3.	..
25. Para Amino Benzoic Acid		No Interaction	3:2.	..

The complexation reactions of Diazepam and chemical agents mentioned in Table I are being subjected to detailed investigations in this laboratory.

Our thanks are due to University Grants Commission, New Delhi, for the award of a Junior Research Fellowship to one of us (A. B. C.).

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8. Podesva, C. and Burford, R. G., *Chim. Therap.*, 1971, 6, 472.
9. Job, P., *Ann. Chim.*, 1928, 9, 113.
10. Vosburgh, W. C. and Cooper, G. R., *J. Am. Chem. Soc.*, 1941, 63, 437.
11. Comors, K. A. and Mollica, Jr., J. A., *J. Pharm. Sci.*, 1966, 55, 772.

EFFECT OF STREPTOLYSIN 'O' ON SUB-CELLULAR ORGANELLE OF TISSUE CULTURE

DURING exposure of tissue cultures to enteroviruses and ultraviolet irradiation, damage to subcellular organelles (lysosomes, mitochondria and plasma membrane, etc.) occurs long before the appearance of the morphological change^{1,2}. Cytotoxic changes in tissue cultures are also produced by bacterial toxins^{3,4} including streptolysin 'O'^{5,6}. In the present study an effort was made to investigate the effect of streptolysin 'O' on the subcellular structures of monkey kidney tissue culture.

Primary monkey kidney tissue culture was prepared in flat bottles. On the 10th day, the fluid was changed with M. Earle's solution without calf serum. Three International Units of streptolysin 'O' (Wellcome

1. Sterabach, L. H. and Reeder, E., *J. Org. Chem.*, 1961, 26, 4936.
2. Kaplan, S. A., Jack, M. L., Alexander, K. and Weinfeld, R. E., *J. Pharm. Sci.*, 1973, 62, 1789.
3. Carstensen, J. T. *et al.*, *Bull. Parent. Drug Ass.*, 1971, 25, 193.
4. Vander Kleij, E., *Arch. Int. Pharmac. Therap.*, 1969, 179, 225.
5. Windorfer, A. and Mihailova, K., *Z. Kinderheilk.*, 1973, 114, 83.
6. Schiff, D. and Chun, G., *Pediatrics*, 1971, 48, 139.
7. Dunphy, R. F. and Lyaton, H., *Can. J. Chem.*, 1971, 49, 3401.

TABLE I
Biochemical findings of enzymes in streptolysin 'O' treated MKTC

Time		Acid phosphatase (KAU)	Alkaline phosphatase (KAU)	Glutamic oxaloacetic transaminase (Units)	Adenosine triphosphatase (Units)	Lactate dehydrogenase (Units)
5 min.	C	0.53	0.56	27	1.06	70
	T	1.04	1.4	14	2.13	100
10 min.	C	0.53	0.50	27	1.06	70
	T	0.94	1.2	10	2.13	120
15 min.	C	0.53	0.45	27	1.06	70
	T	1.12	1.5	12	2.06	105
30 min.	C	0.53	0.40	26	1.02	70
	T	1.32	1.5	22	2.93	120
1 hr.	C	0.53	0.37	25	0.80	70
	T	1.55	1.42	27	4.26	150
2 hrs.	C	0.50	0.37	25	0.80	65
	T	1.6	1.88	33	4.00	165
3 hrs.	C	0.53	0.37	24	0.86	70
	T	0.94	0.95	20	3.60	140
6 hrs.	C	0.50	0.36	26	0.93	60
	T	0.82	0.75	24	3.20	120
24 hrs.	C	0.50	0.37	27	1.07	75
	T	0.94	0.56	20	2.40	135
42 hrs.	C	0.53	0.37	27	1.07	65
	T	1.05	0.47	22	2.13	115
72 hrs.	C	0.75	0.37	26	1.06	70
	T	1.12	0.00	20	1.67	145

T—streptolysin 'O' treated MKTC.

C—MKTC—not treated with streptolysin 'O'.

Research Laboratory, Backenham, U. K.) was inoculated in each bottle. To the control bottles, equal volumes of the diluent was added in place of streptolysin 'O'. The bottles were incubated at 37° C. The streptolysin 'O' inoculated and the equal number of control bottles were removed at 5, 10, 15 and 30 minutes and 1, 2, 3, 6, 24, 42 and 72 hours and biochemical estimation of acid and alkaline phosphatase and glutamic oxaloacetic transaminase were carried out on the cells as reported earlier². The lactate dehydrogenase was estimated by the colorimetric method of King and Wootton⁷. Adenosine triphosphatase enzyme activity was measured by allowing the 0.5 ml cell homogenate to react on 0.4 ml substrate adenosine triphosphate (0.01 M) in the presence of 0.5 ml tris-buffer (pH 7.4, 0.125 M) and 0.6 ml distilled water for 30 minutes at 37° C. The reaction was then stopped by adding 0.5 ml of 10% trichloroacetic acid. The liberated inorganic phosphorus was determined by the method of Fiske and Subbarow⁸.

The details of the biochemical findings in streptolysin 'O' treated and untreated tissue cultures have been presented in Table I. It was observed that streptolysin 'O' damages the mitochondria (as shown by the increase of adenosine triphosphatase and decrease of GOT), lysosomes (increase of acid phosphatase), plasma membrane (increase of alkaline phosphates) and microsomes (increase of lactate dehydrogenase) of the monkey kidney tissue culture. The increase of the acid phosphatase, adenosine triphosphatase and lactate dehydrogenase started at 5 minutes reaching a peak value between 1 and 2 hours. At later periods elevated levels of these enzymes persisted. Alkaline phosphatase gradually diminished and was not detectable at terminal periods.

The cell damage, as observed in the present study, was the net result of injury to different subcellular organelles and not due to that to any particular structure as suggested by different workers⁹⁻¹¹. Similar damage to subcellular organelle of monkey kidney tissue culture

has also been produced by viruses¹ and ultraviolet irradiation², in spite of vast difference in their nature. The changes at the molecular or biochemical level start in the cell almost immediately (5 min.) after exposure to all these three types of injury. Though involvement of same subcellular organelles occurred with all the three types of injuries, yet the morphological expression of the injury was totally different. This difference could be due to selective involvement of a particular metabolic pathway in the cell by the injurious agent which may be the deciding factor of the physical expression of the injury.

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1. Chaturvedi, U. C., Bahuguna, L. M. and Mehrotra, R. M. I., *Indian J. Med. Res.*, 1972, 60, 589.
2. Bahuguna, L. M. and Chaturvedi, U. C., *Brit. J. Exp. Path.*, 1974, 55, 314.
3. Gabliks, J. and Falconer, M., *J. Exp. Med.*, 1966, 123, 723.
4. Chaturvedi, U. C., Mathur, A., Khan, A. M. and Mehrotra, R. M. L., *J. Med. Microbiol.*, 1969, 2, 211.
5. Mathur, A., Chaturvedi, U. C. and Mehrotra, R. M. L., *Indian J. Med. Res.*, 1969, 57, 1670.
6. —, — and —, *Indian J. Path. Bact.*, 1970, 13, 153.
7. King, E. J. and Wootton, I. D. P., *Micro-analysis in Medical Biochemistry*, Third Ed, J. and A. Churchill Ltd., London, 1959.
8. Fiske, C. M. and Subbarow, V., *J. Biol. Chem.*, 1925, 66, 375.
9. Keiser, H., Weissman, G. and Bernheimer, A. W., *J. Cell. Biol.*, 1964, 22, 101.
10. Fauve, R. M., Alouf, J. E., Delauney, A. and Raynaud, M., *J. Bact.*, 1966, 92, 1150.
11. Thompson, A., Halbert, S. P. and Smith, U., *J. Exp. Med.*, 1970, 131, 745.

DISINFESTATION OF FRUIT FLIES IN MANGO BY GAMMA IRRADIATION

Introduction

EARLIER studies in this laboratory have shown that low dose gamma irradiation at 25 Krad either alone¹ or in conjunction with skin coating² can delay the ripening process as well as improve the transportability³ of Alphonso mangoes. The present investigation was undertaken to ascertain the effect of gamma irradiation at dose levels normally employed for delaying the ripening process in mangoes, on the prevention of emergence of adult fruit flies from infested fruits and to establish the radiosensitivity of fruit flies at different stages of their life cycle.

Materials and Methods

Two fruit fly species, the oriental fruit fly (*Dacus dorsalis*, Hendel) and the melon fly (*Dacus cucurbitae*, Coquillett) commonly found infesting mangoes were selected for these studies. The melon fly was reared under laboratory conditions using pumpkin as the rearing medium. Various life stages of the oriental fruit fly were obtained from a large number of naturally infested mango fruits.

Naked eggs, larvae and pupae of both the species were exposed to 0, 15, 25, 40 and 100 Krad of gamma rays from a cobalt-60 source at a dose rate of 75 Krad/hour. Eggs were carefully transferred to slits made on pumpkin or semi-ripe mangoes as the case may be and irradiated. In some experiments eggs were placed on moist filter paper, and after exposure to the required dose were transferred to fruit slices. Sixty eggs (20 × 3 replications) each from both the species were exposed to each of the above doses. The experiment was repeated.

Forty larvae (20 × 2 replications) of 3 to 4 days old from both species were placed on fruit pieces and irradiated with the above doses. The experiment was repeated. After irradiation, the fruit slices containing eggs and larval stages were placed over moist sand in beakers covered with muslin cloth and were held at ambient temperature (25 to 28° C) to determine hatching, larval growth rate, pupation and emergence of adults.

Forty pupae (2 to 3 days old) from each species were exposed to 0 to 100 Krad and were held in moist sand under ambient conditions until adult emergence was complete as judged from the breaking of the puparium at the eclosional suture and partial or full emergence of flies.

Results and Discussion

In both melon and oriental fruit flies, the percentage of eggs hatched after exposure to 15 and 25 Krad were 45 ± 5 and 27.5 ± 2.5 respectively as against 75 ± 5 in unirradiated control groups (Table I). Irradiation at 40 and 100 Krad prevented hatching of the eggs in both the species. The larvae emerging from eggs exposed to 15 and 25 Krad were rather sluggish and showed slower growth rate as compared to controls. Only 45 ± 5% and 17.5 ± 2.5% of the emerged larvae formed puparia in 15 and 25 Krad treated groups respectively, as against 100% in controls. No adult emergence was noticed in any of the irradiated groups as against 90% in controls. Irradiating the eggs either on moist filter paper or on fruit pieces did not show any differences in their hatchability.

Larvae exposed to 15 and 25 Krad showed slower growth rates and sluggishness while higher doses caused increased mortality and poorer growth rates. These surviving larvae, after exposure to 40 and 100 Krad,