CHROMOSOME BREAKAGE INDUCED BY VEGETABLE OILS AND EDIBLE FATS

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DURING the course of mutation research in progress at the Indian Agricultural Research Institute, the earlier findings of Gustafsson[1] and other workers that there are marked differences in sensitivity to radiation among different crop plants was confirmed.[2] It was found that some oilseed plants like linseed are much less sensitive to radiation with X-rays and β-particles from ^{32}P and ^{35}S than cereals like wheat and paddy. It was considered likely that the presence of oil in seeds might have a buffering effect on radiation, and in an experiment designed to elicit information on this problem, seeds of several plants were first soaked in some vegetable oils and edible fats and later subjected to irradiation. In this experiment, controls with no treatment and with seeds soaked in oils for various durations but not subsequently irradiated were kept. Observations on preparations of root tip mitosis made from this material showed that immersion in the oils alone caused chromosome breakage in many cells. A summary of the results is presented is this paper.

Dry seeds of *Triticum monococcum*, *T. dicoccum*, *T. aestivum*, *Oryza sativa* and *Vicia faba* were soaked for different durations extending from 6 minutes to 6 hours in the following oils and fats (the pure oils had been extracted at the Division of Chemistry of this Institute): mustard oil from *Brassica compestris* var. *toria* groundnut oil, castor oil, gingelly oil, coconut oil, linseed oil, hydrogenated groundnut oil and ghee. After being soaked in oil, the seeds were thoroughly wiped with cloth and sown in petri-dishes over moist filter-paper. Soaking for two or more hours in linseed oil and groundnut oil completely inhibited the germination of seeds. Germination was reduced to varying extents in the other treatments. Root tips were fixed 24, 48 and 96 hours after germination in a mixture of 3 parts of alcohol and 1 part of acetic acid for a day and later stained with leuco-basic

FIGS. 1–3. Mitosis in *T. monococcum* root tips treated with oils. Fig. 1. Mustard oil treatment for 2 hours showing extreme chromosome fragmentation. Fig. 2. Anaphase in mustard oil treated cell with one dicentric bridge and a point error configuration. Fig. 3. Castor oil treatment for two hours showing 12 chromosomes and 4 fragments (arrows). Note that the treatment leads to the contraction of chromosomes, thus facilitating their spread.

(Figs. 1 and 3, × 900 ; Fig. 2, × 1 800.)

fuchsin and squashed. Preparations were also made from control seeds sown either dry or after soaking in water for 2 to 6 hours.

Cell division was normal in the root tips of control seeds. Analysis of metaphase and anaphase plates in preparations made from treated root tips showed several aberrations like chromosome and chromatid breakages, minute fragments, dicentric bridges and acentric fragments and 'error' bridges probably arising from subchromatid breakages[3] (Figs. 1 to 3). Treatment with the oils, especially castor oil, led to the contraction of chromosomes thereby facilitating their clear spread in squash preparations.[4] No stickiness among the chromosomes was caused by the treatment. In several cells, the two chromatids of a chromosome were asymmetrically broken, thus indicating that they were separately and independently affected. There was also practically no reunion among the chromosome fragments. These observations would indicate that breakage occurs near to the time of chromosome reduplication and also is spread over a period of time. There were some tetraploid cells in the treated root tips, which appeared to have arisen by failure of cytokinesis, since there was no evidence of interference with anaphase movement. Binucleate cells were present lending support to the view that cell division was disturbed. Some binucleate cells showed non-synchronised mitosis, one nucleus being at the resting stage and the other at prophase or metaphase.

The standard *Allium* test of Levan[5] was performed using mustard and gingelly oils and it was found that mustard oil treatment for half to one hour caused the formation of tumours in *Allium* root tips and also induced chromosome breakage. The recovery of the cells from the effects of the treatment was, however, rapid and meristems developed subsequently to the treatment hardly showed aberrant cells. In comparison with mustard oil, gingelly oil had no adverse effects on *Allium* root tip cells.

The data obtained from T. *monococcum* seeds treated with the different oils and fats are summarised in Table I. It will be seen from this table that groundnut and mustard oils produce the largest and gingelly oil and ghee the lowest number of aberrant cells. Such diverse action probably arises from the differences in the fatty acid components of these oils, since we found that very few cells are affected when seeds are treated with glycerol. Auerbach[6] reported that the essential oil of mustard (allyl-iso-thiocyanate) had mutagenic properties but she was doubtful whether it produced chromosome aberrations. It was subsequently shown by D'Amato and Avanzi[7] that allyl-isothiocyanate is not capable of producing chromosome breakage. It is reported from studies in Drosophila that gingelly oil[8] and groundnut oil[9] have no mutagenic properties. We are now engaged in studying the number of division cycles over which the aberrations are carried forward and the cytological effects of the fractionated components of different fats and oils.

TABLE I

Chromosome breakage induced in Triticum monococcum

Treatment	Duration of treatment	Percentage of germination	Metaphase			Anaphase		Cells with aberrations (per cent.)
			No. of cells studied	No. of cells with aberrations	Range of breaks per cell	No. of cells studied	No. of cells with aberrations	
Water	..	2 hrs.	100	Numerous	nil	..	..	..
Mustard oil	..	6 mts.	70	267	29	1–18	115	12	10·7
"	..	2 hrs.	40	213	52	1–21	141	20	20·3
"	..	6 hrs.	20	486	102	1–24	245	49	22·6
Groundnut oil	..	1 hr.	20	149	46	1–28	61	6	24·8
"	..	2 hrs.	0	..	..	..	..	..	..
Castor oil	..	2 hrs.	40	97	15	1–16	24	4	16·5
Linseed oil	..	1 hr.	20	75	13	1–7	61	4	12·5
"	..	2 hrs.	0	..	..	..	..	..	..
Hydrogenated groundnut oil		2 hrs.	20	60	10	1–21	53	3	11·8
Coconut oil	..	2 hrs.	80	205	29	1–38	294	27	9·2
Gingelly oil	..	2 hrs.	50	123	3	1–2	137	2	1·9
"	..	6 hrs.	20	182	16	1–26	168	3	5·4
Ghee	..	2 hrs.	80	107	7	1–5	109	3	4·7

The results will help to find out whether oils like those of mustard, groundnut and castor can be used for inducing mutations in plants and to isolate the component of the oil which causes the chromosome breakage. Since the mustard oil group consists of several types with varying pungency, the action of each of them is being separately estimated.

The results presented in this paper are of great interest in view of the relationship between mutagenicity and carcinogenecity. The discovery of the mutagenic effect of ionizing radiation, an agent already known to be carcinogenic, provided the first evidence in support of the somatic mutation hypothesis of malignancies. Muller[10] has pointed out that a comparative survey of the results not only with radiations of different types but also with chemical mutagens suggests the view that the effects of these agents on genes and chromosomes forms the basis of their effects in producing malignancies. The role of nutrition with reference to the incidence of cancer is now widely realised and there are indications that a search for carcinogenic compounds in human dietary regimens might be worthwhile. Many fats and oils are known to be capable of promoting and inducing the formation of skin and mammary tumours in rats. Differences in the action of the different oils have also been observed. Thus, increasing the corn oil content from 5% to 20% of the diet strikingly enhanced tumour formation in rats but replacing the 5% corn oil with 20% partially hydrogenated cotton seed oil or 20% lard produced no appreciable augmentation.[11] Such differential action of different fats has not yet been explained in terms of their chemical, physical or biological properties. Whether there are differences in the action of these oils on the nucleus, similar to the observations recorded by us, remains to be ascertained.

The present study has been carried out only in plant meristems, and it is not known whether comparable effects will be produced in dividing animal tissues. There is, however, evidence that the action of chemical mutagens such as nitrogen mustard, like that of radiation, is general and organisms of various kinds like bacteria, fungi, higher plants and mammals are equally affected.[10] In view of the fact that some of the oils and fats mentioned in this paper are widely used as cooking media in tropical countries, the present data deserve the attention of medical and cancer research workers.

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