Endrin Induced Cytological Changes in Albino Rats

by T. S. S. DIKSHITH and K. K. DATTA
Industrial Toxicology Research Centre
Lucknow, India

Pesticides are being used extensively in the control of crop pests, mosquitoes and vector-borne diseases (ANONYMOUS, 1965). Improper handling and abuse of these potentially hazardous chemicals has not only made them ubiquitous, but also induced serious health hazards among workers during manufacture, formulation and field use. Exposure to different insecticides has induced certain histopathological changes in the testes (DIKSHITH and DATTA 1972a; DIKSHITH and DATTA, in press) and also in the skin (DIKSHITH and DATTA 1972b; KAR and DIKSHITH 1970) of experimental animals. Our knowledge on the chromosomal cytology of mammals exposed to different pesticides in vivo is very meagre while much literature is available on the chromosome cytology with other chemicals (OEHLKERS 1953; KIHLMAN 1966; COHEN 1969). We, therefore, considered it interesting to report here our findings on the action of endrin on rat chromosomes in vivo.

Materials and Methods

Male albino rats (body weight 200-250 g) were treated with 0.25 mg/testis of endrin* in saline intratesticularly. The animals of the control group were injected with saline only. There was no death or clinical symptoms of poisoning in any animal during experimentation. The given dose is approximately nine times less than that of the oral LD_{50} dose (oral LD_{50} of Endrin for male while rats is 17.8 mg/kg (GAINE 1960). Similar was also the dosage used in our earlier studies (DIKSHITH and DATTA 1972a) where again the mortality of endrin-treated animals was nil. The same dose was, therefore, selected to study the effect of endrin on cells and chromosomes of the rat testes. Reference to the mode of administration, prima facie it may look that the manner of application (exposure) of endrin to the experimental rats should be similar to the one seen in humans. The purpose of intratesticular injection of endrin,

* Endrin 1,2,3,4,10,10 hexachloro-6,7, epoxy-1,4,4a,5,6,7,8,8a-octahydro-1, 4-endo-endo-5,8 dimethanonaphthalene. Recrystallized and supplied by Woodstock Agricultural Research Centre, Kent, U.K.

Bulletin of Environmental Contamination & Toxicology,
Vol. 9, No. 2, © 1973 by Springer-Verlag New York Inc.

however, was to achieve direct exposure of male germinal tissue and cells to the action of endrin in vivo and then to record its performance on the behaviour of chromosomes. The rats of the control and treated groups were killed after 10 days and their testes fixed in acetic acid:alcohol (1:3) mixture. Paraffin cut sections as well as acetocarmine and haematoxylin squash preparations were examined for cytological changes using an Olympus phase contrast microscope.

Results

The effects of endrin on the testes of albino rats were very clear. In the nucleus, the most conspicuous change was seen in the chromosomes. Endrin induced several changes and the details are given in Table 1.

Table 1

Frequency of chromosomal aberrations in the endrin-treated rat testis

Group	No. of cells scored	Number of cells with the type of aberration					Mean % of the aberration
		Stickiness	Chromatin bridges	Chromosome breaks	Fragments	Ring chromosomes	
Control	70	1	-	-	-	-	1.42
Treated	70	3	1	1	2	-	2.49
	75	4	2	2	2	-	3.56
	75	2	2	-	3	1	2.85

Figure A shows the normal chromosomes at metaphase. Among the less well defined chromosomal changes, stickiness and bizzarre configurations at metaphase was very common (Fig. 1,2). Endrin also induced the formation of chromosome fragments, abnormal restitution of chromosomes (Fig. 3). Formation of single (Figs. 4, 5) and double bridges with acentric fragments (Fig. 6) was very common. Unequal distribution of chromosomes at anaphase I was also observed (Fig. 7). Severity of cellular damage resulted in the liquification and transformation of the chromatin mass into an amorphous lump (Fig. 8).

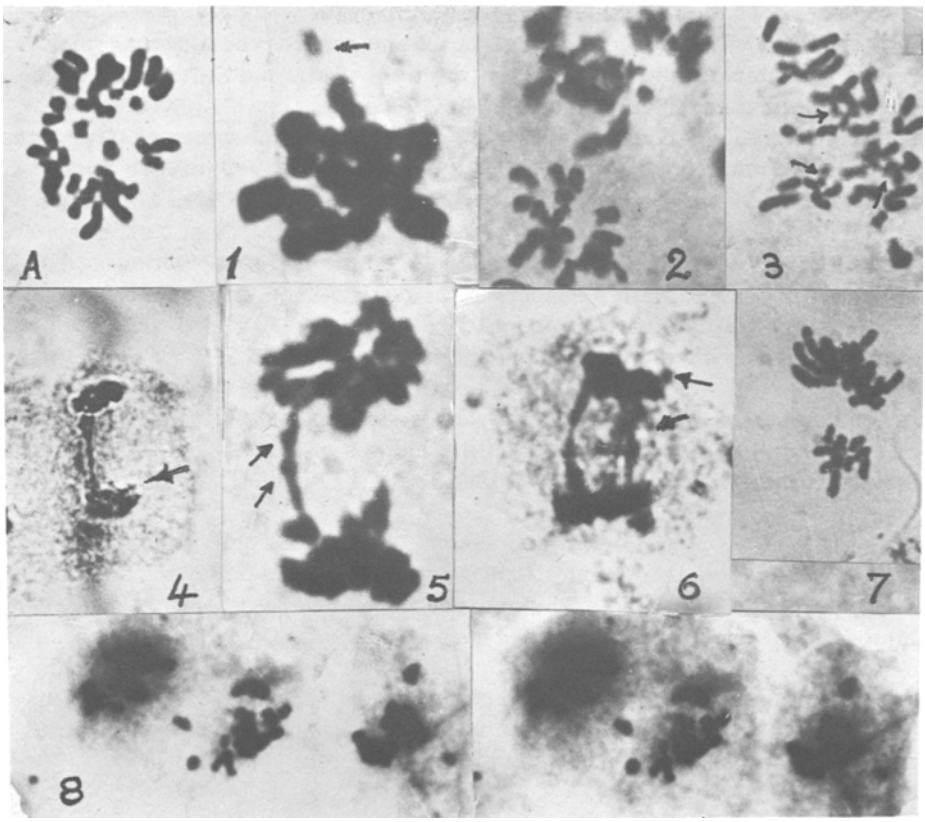

Fig. A. Normal chromosomes of the control rat X Ca. 1480
Figures 1 to 8. 1. Stickiness of chromosomes with an acentric fragment. X Ca. 1130; 2. Bizzarre configuration at metaphase. X Ca. 1165; 3. Fragmentation of chromosomes (arrow). X Ca. 1130; 4. Chromosome bridge (single) with acentric fragments (arrow). X Ca. 1070; 5. Same from the methyl parathion plus DDT treated material (arrow). X Ca. 1130; 6. Double bridge with acentric fragments (arrow). X Ca. 827; 7. Unequal separation of chromosomes at anaphase. X Ca. 1130; 8. Chromatin material in the form of an amorphous lump. X Ca. 1400.

Discussion

Stickiness and fragmentation of chromosomes in different phases of division cycle indicates symptoms of cellular degeneration. Similarly formation of chromatin bridges also disturbs the normal disjunction of chromosomes eventually affecting the chromosome complements of the division products. It has been shown that breakage, stickiness and liquification of chromosomes are intimately associated with each other (RAHIMAN and RAJASEKHARASETTY (1967).

Because of specific localization and high DNA content of chromosomes it becomes obvious that any change in the integrity of the chromosomes is due to the alteration in the DNA synthesis SHARMA and SHARMA (1960). Arrest of chromosomes at metaphase due to rotenone MEISNER and SORENSEN (1966) and progressive clumping of chromosomes at interphase due to ethidium bromide VACQUIER and BRACHET (1969) are all abnormalities linked with the inhibition of DNA synthesis. DDT also induced stickiness and liquification of chromosomes in cells of plant tissue VARAAMA (1947). This has been accounted for by the reduction in the viscosity of matrix matter rendering the chromosome more fluidy. Again another insecticide of the organophosphate group - DDVP has also been shown to induce chromosome gaps and breaks SAX and SAX (1968); LOFROTH et al. (1969). Similar seems to be the effects of endrin on the chromosomes of rat. Preliminary observation of methyl parathion plus DDT treated rat also revealed similar findings in the cells of the seminiferous tubules (Fig. 5) DIKSHITH and DATTA, (unpublished).

Meiosis is a complex phenomenon. Accurate replication of the chromosomes requires uninterrupted and coordinated de novo synthesis of the protein. Any kind of disturbance in the synthesis of the types of histones or the nature of bonds between histone and DNA induces several anomalies ANSLEY (1957). The formation of chromosome breaks and acentric fragments are the resultants of disturbance in RNA metabolism due to endrin. This wave of distrubance affects the protein synthesis of the cytoplasm which in turn alters the DNA protein synthesis of the chromosomes.

As mentioned earlier, none of the animals exhibited any clinical symptoms of poisoning. Careful microscopic examination, however, showed nuclear and cytoplasmic alterations at the cellular level. Similar are the findings of our earlier report DIKSHITH and DATTA (1972b). In view of the increasing number of pesticides present in our environment and since several organophosphate insecticides are of the triester type and possibly possess alkylating properties, cytotoxicity tests using submammalian test systems or human cell cultures in vitro becomes necessary. This aspect of study receives further emphasis in the light of the recent report on pesticides and their relationship to environmental health (ANONYMOUS, 1969). Different mammalian test systems such as bone marrow, peripheral blood leukocytes, spermatogenic tissues and spleen offer valuable media to carry out such a test. It must, however, be emphasized that great caution is needed to interpret the data obtained from cell populations grown in vitro since conditions of in vivo are fundamentally different from those of the former. In this context cytological changes observed in spermatogenic tissue of rats under in vivo conditions is of interest. Extrapolation of the chromosomal abnormalities induced by endrin in the cells of the rat testes has indicated the mechanism of action of the pesticide at subcellular level.

Acknowledgment

The authors are grateful to Dr. S. H. Zaidi for his keen interest and encouragement. They also thank Mr. M. Ahmed for the photomicrographs.

References

ABDUL RAHIMAN, M. and M. R. RAJASEKARASETTY, J. Cytol. and Genet. 2, 32 (1967).

ANONYMOUS, The war that never ends. Office of Information USDA 1-2 May, 1965.

ANONYMOUS, Report of the Secretary's Commission on pesticides and their relationship to environmental health, Part II, U.S. Dept. of Health, Education and Welfare P. 612 (1969).

ANSLEY, H., Chromosoma 8, 380 (1957).

COHEN, M. M., Can. J. Genet. Cytol. 11, (1969).

DIKSHITH, T. S. S. and K. K. DATTA, Acta Pharmacol. et Toxicol. 31, 1 (1972a).

DIKSHITH, T. S. S. and K. K. DATTA, Experientia 28, 169 (1972b).

DIKSHITH, T. S. S. and K. K. DATTA, Exp. Path. (in press).

GAINES, T. B., Toxicol. Appl. Pharmacol. 2, 88 (1960).

KAR, P. O. and T. S. S. DIKSHITH, Experientia 26, 634 (1970).

KIHLMAN, B. A., Actions of chemicals on dividing cells. Prentice-Hall, Inc. Englewood Cliffs, New Jersey, 1966.

LOFROTH, G., C. KIM and S. HUSSAIN, Environ. Mutagen Soc. Newslet. 2, 21 (1969).

MEISNER, H. M. and L. SORENSEN, Exp. Cell. Res. 42, 291 (1966).

OEHLKERS, F., Heredity 6, 95 (1953).

SAX, K. and H. J. SAX, Japan J. Genet. 43, 89 (1968).

SHARMA, A. K. and A. SHARMA, Inter. Rev. Cytol. 10, 101 (1960).

VACQUIER, V. D. and J. BRACHET, Nature 222, 193 (1969).

VARAAMA, A., Hereditas 33, 191 (1947).