The negative influence of some insecticides on male fertility has been noted. We report our cytogenetic observations on a group of infertile insecticide workers. Increased chromosomal breakage was a constant finding and the Y chromosome was especially damaged. This may account for impaired spermatogenesis. Furthermore, the involvement of heterochromatic chromosomal variants both in the individual susceptibility to the chemically induced damage and in the reproductive fitness is emphasized.

MATERIAL AND METHODS

Recent reports from the United States (Whorton et al. 1977) and from the Ben Gurion University in Beer-Sheba, Israel, emphasize the deleterious influence of 1,2-dibromo-3-chloropropane on male fertility. Negative effects of DDT and other insecticides have also been observed.

We want to refer our own observations on 5 men with fertility problems, who had for long periods been frequently exposed to various chlorinate and phosphorylate organic insecticidal compounds.

MATERIAL AND METHODS

The 5 men were part of a study on a group of 130 patients from the male infertility clinic of Hasharon Hospital, Israel (Shabtai et al. 1977). All of them were unable to procreate for years, the sterility being primary or secondary. The case of a patient (Table, case 3) with secondary sterility was of particular interest. He began to work with insecticides at a very young age (14 years); nevertheless, he interrupted his work during the three years service in the Army. Then he married and his wife became pregnant. A healthy boy was born. After the Army he went back to his work and he had no more children in seven years.

All the patients underwent:
(1) Routine physical and laboratory examinations, including tests for diabetes and thyroid function evaluation;
(2) Serial spermogramme;
(3) Hormonal evaluations of FSH, LH, testosterone, 17-ketosteroids, 17-hydroxysteroids;
(4) Meiotic studies according to Sperling and Kaden (1971);
(5) Cytogenetic analysis of peripheral blood cultures using G-banding technique (Seabright 1971) for chromosome identification and C-banding (Sumner 1972) for evaluation of heterochromatic polymorphism.

The patients refused testicular biopsies to be taken.
RESULTS

All patients were found physically normal and healthy. The relevant laboratory findings are summarized in the Table. Evaluations of FSH, LH, 17-ketosteroids and 17-hydroxysteroids gave normal values. In patients 1, 2 and 5, FSH and LH were at the highest normal levels, whereas 17-ketosteroids and 17-hydroxysteroids were at the low limit of normal range. Patients 2 and 4 had a low testosterone level. Testosterone was not tested in patient 1.

Sperm counts ranged from severe oligospermia (1-8 mil/ml) to normal counts. The motility was decreased in all cases, reaching complete astheno- or necrospermia. The incidence of abnormal forms of different types was usually increased, as compared to that found in fertile men.

Meiotic studies revealed chromosomes in different stages of the first meiotic prophase. They had usually a normal appearance, but in some cells the absence of the sexual vesicle, the XY bivalent, was noted.

All the patients had a normal karyotype. High incidence of heterochromatic variants was found, especially of chromosomes 1 and 9 (see Table and Fig. 1). Increased breakage rate (10-25%), as compared to the normal range of our laboratory (2-5%), was a constant feature.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Spermiogramme</th>
<th>Karyotype</th>
<th>Chromosomal variants</th>
<th>Chromosomal breakage</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Necro-terato-oligospermia gravis</td>
<td>Normal</td>
<td>1h— 9pi 9h+ 19h+</td>
<td>Increased</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Astheno-terato-oligospermia gravis</td>
<td>Normal</td>
<td>1h + 9h+ 15p+</td>
<td>Increased</td>
<td>Low testosterone</td>
</tr>
<tr>
<td>3</td>
<td>Asthenospermia, normal count</td>
<td>Normal</td>
<td>9h+</td>
<td>Increased</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Terato-moderate oligospermia</td>
<td>Normal</td>
<td>1h+</td>
<td>Increased</td>
<td>Low testosterone</td>
</tr>
<tr>
<td>5</td>
<td>Astheno-oligospermia gravis</td>
<td>Normal</td>
<td>1h + 9h+</td>
<td>Increased</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Examples of heterochromatic variants:

- $h+$ = increased heterochromatic mass;
- $h-$ = decreased heterochromatin;
- p.i. = pericentric inversion.
Breakages were distributed over all the groups of chromosomes, with clustering in B and D groups, in chromosome 17 and especially in the Y chromosome (Fig. 2). The frequency, intensity and duration of exposure to the insecticidal compounds were different from one patient to the other. No correlation could be found between anyone of the above-mentioned parameters and the gravity of the clinical condition. Probably, individual factors of genetic backgrounds are involved in the individual response. In fact, patients 2 and 3 had the same working conditions, the first for five years, the second for seven years. The clinical state of the first patient was much worse. The incidence of chromosomal breakage in the peripheral blood cultures was higher in the second. Furthermore, other men working for years in the same place under the same working conditions were referred to have no fertility problems.

Cytogenetic analysis was also performed on peripheral blood cultures from two aged men, working under the same conditions with various insecticidal compounds for more than 30 years. Both had normal karyotype. One was unmarried and childless, but the other had two normal children, conceived after years of exposure to the insecticidal compounds. The first, presenting a G22sat+ variant, had only a slight increase in chromosomal breakage

Fig. 2. Damage on the Y chromosome in different cells of different patients.
of the chromatid type, whereas the second, presenting the variants 16h+, 13sat+, 22h+, had a very high incidence of breaks, fragments, dicentrics, premature condensation of chromosomes and other aberrations.

DISCUSSION

The mutagenic and carcinogenic activity of some insecticides has been noted and studied (Guerzoni and Del Cupolo 1976, Bignami et al. 1977, Whorton et al. 1977). So, it is not surprising that people working with such a kind of mutagens for long periods have an increased rate of chromosomal breakage. Nevertheless, the chemically induced damage on man does not seem merely a function of exposure duration (Whorton et al. 1977), although surely this is an important factor.

Individual susceptibility seems evident from the patients studied and must be considered. In this regard, we want to point out the presence of variants of chromosomes 1 and 9. All the patients had at least one of these variants. For each of these chromosomes, polymorphism was present in 4 of the 5 patients. The incidence is much higher than in control studies from the literature and in Israel (Cohen et al. 1975, and personal observations on 800 normal newborns, unpublished data), where a maximum incidence of respectively 7% and 8% was found for these variants. A statistically significant higher frequency of such heterochromatic polymorphisms, usually connected with an increased breakage rate, has been found both in patients with malignant or premalignant hematologic diseases (personal observations) and in cases of congenital malformations without karyotypic abnormalities (personal observations and Kunze and Mau 1975). This leads us to think that heterochromatic variations, or at least some of them, may play a role in individual susceptibility to viruses and mutagens. Indeed, Meist (1975) found that the clastogenic effect in vitro of a mutagenic compound was higher in cells of people whose karyotype presented heterochromatic polymorphisms. Seabright (1976) observed increased number of X-ray-induced lesions and exchanges in individuals with variant chromosome 1.

Increased incidence of variant chromosomes 1 (15%) and 9 (25%), particularly partial inversion (9), with concomitant increased breakage rate, was also found in the whole group of infertile men studied by us (Shabtai et al. 1977).

A similar mechanism of increased susceptibility to different environmental agents, such as herpes viruses, lead, benzene, mycoplasma, toxoplasma, could be supposed in most of the cases. On the other hand, such chromosomal variations, more than others, may play a role in reproductive fitness (Jacobs et al. 1975) by a presently unknown mechanism. In this regard it is of interest that the aged man of this study with variants 16h+, 13sat+, 22h+ had apparently a high susceptibility to the chemicals, but his reproductive fitness was not disturbed (referring to his family history).

The correlations between the cytogenetic damage induced in somatic cells and in male germ cells by various mutagens have been studied in experimental animals (Tates and Natarajan 1976, Van Buul 1976, Natarajan and Tates, in press). Except for to 2-chloroethyl-1-nitrosoureido-ethanol, usually spermatogonia have been found to be more sensitive than somatic cells.

The works of Rees (1952) and of Darlington and Haque (1955) showed that chromosomal breakage may be associated with desynapsis. That may be the reason for failure in post-meiotic stages. Other meiotic studies on men (Chandley et al. 1976, Hulten et al. 1970,
Pearson et al. 1970) have shown breakdown of spermatogenesis in association with pairing failure at meiosis in infertile men with a normal somatic karyotype. In our patients it is worth noting the absence of the XY bivalent in some meiotic figures. We have never met this finding in control fertile men, neither in infertile men of our study (Shabtai et al. 1977) except for patients with structural abnormalities of the Y chromosome (para and pericentric inversions) and another patient with a very high breakage rate. The frequent damage on the Y long arm observed in the patients reported here could be a cause for XY pairing defect. Pairing failure of the X and Y chromosomes in spermatocytes was seen in 100% of cells of an azoospermic patient with a ring Y chromosome (Chandley et al. 1976) and in an azoospermic patient with a dicentric Y (Mcllree et al. 1966). Miklos (1974) suggested that saturation of pairing sites between homologous chromosomes may be essential for normal postmeiotic development. Alternatively, spermatogenic arrest and X-Y dissociation could be both due, as suggested by Beechey (1973), to a prior defect in the distal pairing segment probably carrying gene(s) necessary for normal spermiogenesis.

The decreased motility in presence of normal sperm count and apparent normal morphology could also be due to chromosomal breakage producing slightly genetically unbalanced gametes. In conclusion, mutagenic compounds may induce male infertility and increase the risk of genetic mutations in men who maintain their fertility. To keep away men in their reproductive age from frequent exposure to mutagenic agents should be recommended. From the reproductive history of patient 3, who fathered after three years interruption of the work, we may deduce that the chemically-induced damage may be reversible. In this regard some patients are under investigation. During the last few months they changed their work, and some improvement seems to appear.

Acknowledgement
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