EFFECTS OF THE PYRROLIZIDINE ALKALOID HELIOTRINE ON CELL DIVISION AND CHROMOSOME BREAKAGE IN CULTURES OF LEUCOCYTES FROM THE MARSUPIAL *POTOROUS TRIDACTYLUS*

By Y. A. E. Bick* and W. D. Jackson†

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Summary

Heliotrine causes an almost complete suppression of cell division in leucocyte cultures at a concentration of 2×10^{-4}M; below 1×10^{-6}M, its effect on the mitotic index is very slight. The mean interval for cells to enter first mitosis is extended by 10 hr with 5×10^{-5}M heliotrine. Both chromosome and chromatid breaks are produced at this latter concentration of heliotrine, the total damage being approximately equivalent to that produced by 300 r of X-irradiation delivered at 25 r/min. The chromosome breakage is considered to be true chromosome damage since it appears in cells in first division following treatment. Thus it would appear that heliotrine is capable of breaking the chromosomes in G_1, i.e. before DNA synthesis. Most alkylating agents do not demonstrate any capacity to induce breakage before the replicating phase. Breakage in G_1 has been demonstrated by treatment with hydroxylamine and 8-ethoxycaffeine with which the action of heliotrine is compared.

The distribution of damage by heliotrine and X-irradiation shows a non-random pattern. The overdispersion is marked in heliotrine treatments. This pattern is related to the distribution of heterochromatin.

I. Introduction

The pyrrolizidine alkaloids belong to a group of chemicals which have been known for many years to be potentially dangerous hepatotoxins to livestock, and some of them are now known to be chemical mutagens with radiomimetic properties. Two major alkaloids, heliotrine and lasiocarpine, both present in the plant *Heliotropium europaeum* L., possess considerable biological activity (Schoental, Head, and Peacock 1954; Schoental and Head 1955; Bull *et al.* 1956; Schoental 1957; Bull, Dick, and McKenzie 1958; McKenzie 1958; Schoental and Magee 1959). They are mutagenic in *Drosophila* (Clark 1959, 1960, 1963; Brink 1963) and can cause chromosomal breakage in *Allium* and *Vicia* (Avanzi 1961, 1962). Schoental (1957) found that lasiocarpine and heliotrine produced severe liver lesions in the laboratory rat, and Christie (1958) and Gallagher (1960) investigated some of the biochemical disturbances produced in liver cells *in vivo* and *in vitro*. There is still little detailed information, however, on the ability of these alkaloids to cause chromosome breakage in mammalian cells.

The radiomimetic properties of heliotrine have been investigated by treatment of short-term cultures of peripheral leucocytes from *Potorous tridactylus* (Kerr), the Tasmanian rat kangaroo or potoroo. This marsupial has a small number of chromosomes: $2n = 12\,♀,\ 13\,♂$, with two X chromosomes in the female and XY_1Y_2 sex

* Department of Zoology, University of Tasmania, Hobart, Tas. 7001.
† Department of Botany, University of Tasmania, Hobart, Tas. 7001.

chromosomes in the male (Sharman, McIntosh, and Barber 1950). The individual elements of the karyotype are readily distinguishable from one another, so that scoring at metaphase is accurate since there is no doubt as to the identity of every chromosome. The favourable nature of this material for cytological work has already been pointed out by a number of investigators (Sharman 1959; Walen and Brown 1962; Shaw and Krooth 1964).

II. Materials and Methods

The blood was obtained from the tail of the animal or by heart puncture using a syringe moistened with heparin. After the blood had been oxygenated, 10-ml aliquots were treated with several drops of phytohaemagglutinin (Wellcome), and then centrifuged at 100 *g* for 5 min. The supernatant plasma with the suspended leucocytes was then centrifuged at 150 *g* for 7 min and the cells washed twice with complete medium. The washed cells were cultured by a modified method of Moorhead *et al.* (1960), using Parker's medium 199 with 12% foetal calf serum and 2% phytohaemagglutinin. Higher concentrations of serum, especially of horse serum, sometimes proved toxic to these marsupial cells although human leucocytes grew satisfactorily under similar conditions.

Heliotrine was dissolved in sterile Hank's balanced salt solution (B.S.S.) before being added to the cultures. In the controls an equivalent amount of B.S.S. lacking the alkaloid was used. Colchicine was added to the cultures after they had been incubated at 37°C for 72 hr (final concentration 0·004%), and 2 hr later the cells were treated for 10 min with hypotonic saline (1 vol. B.S.S. to 2 vols. water) and then fixed with glacial acetic acid and absolute ethanol (1 : 3 v/v). Air-dried films were rinsed in methanol and then placed in Leishman's stain for 10 min. Metaphases of cells in their first division were obtained by adding colcemid (to give a final concentration of 0·0007%) at the 24th hr of culture. The cells were then harvested 60 hr after the initiation of cultures.

To compare the damage produced by heliotrine and by X-irradiation, experiments were carried out in which the fully oxygenated blood samples were exposed to X-rays delivered at a dose rate of 25 r per minute (H.V.L. 0·5 mm Cu; 235 kV peak). After irradiation the leucocytes were separated and cultured as above.

III. Results

(*a*) *Despiralization of Chromosomes*

In cultures containing heliotrine, about 10% of cells in division showed a pronounced despiralization of chromosome material [Figs. 1(*a*), 1(*b*)]. Such metaphases were completely absent from the controls.

(*b*) *Suppression of Mitosis*

For cultures terminated after 72 hr, the percentage of metaphases in the controls was 4·5. The dose–effect relationship for mitotic inhibition (Fig. 2) appears to be sigmoidal for heliotrine concentrations up to 1×10^{-5}M. Below 1×10^{-6}M the effects of the alkaloid are very slight and in five replicates at 2×10^{-7}M no significant mitotic inhibition could be detected. This established a lower limit for the threshold concentration. To achieve almost complete mitotic inhibition it was necessary to increase the heliotrine concentration to 2×10^{-4}M. At this concentration only a few prophases were present when the cultures were terminated. The leucocyte cultures for the

above experiments were grown in medium containing 20% horse serum. Later supplies of horse serum proved toxic to potoroo leucocytes, when used at this concentration. In all subsequent experiments the leucocyte cultures were grown in 12% foetal calf serum.

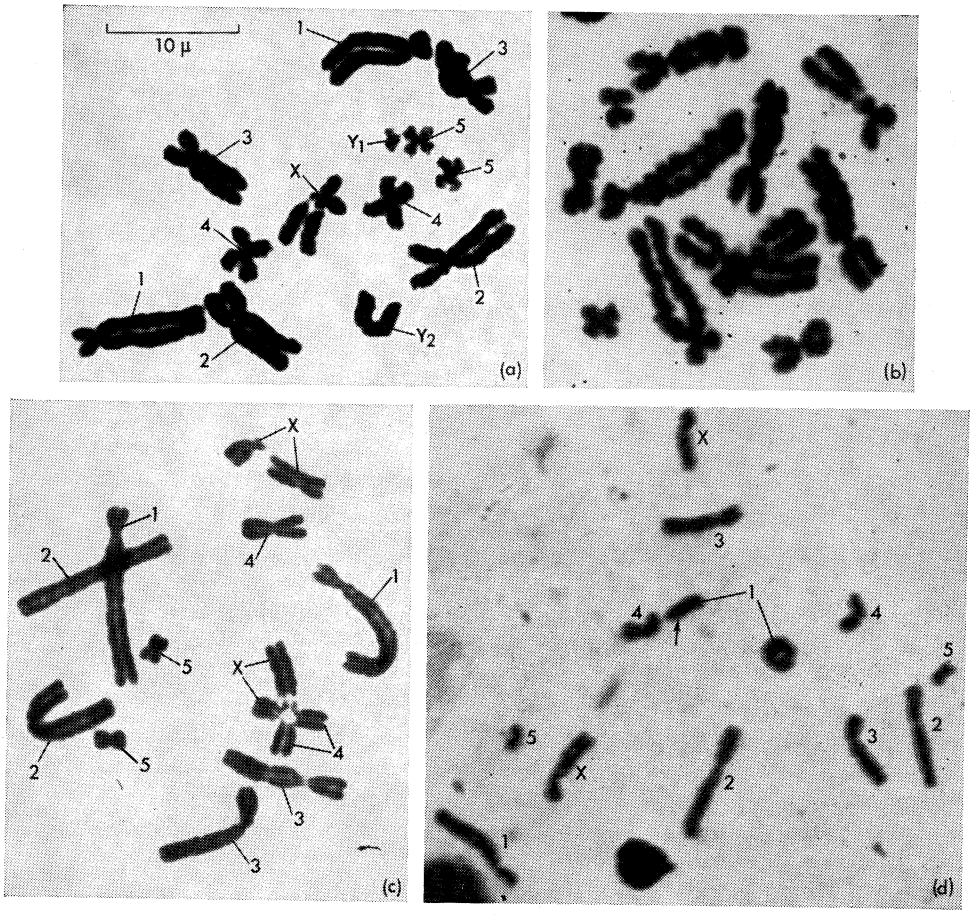


Fig. 1.—(*a*) Normal cell in mitosis from control culture. (*b*) Despiralization of chromatin and secondary constrictions produced by heliotrine. (*c*) Chromatid interchange at the centromeric regions between chromosomes *X* and 4, and an acentric pair formed by a break in the heterochromatic region of *X*. (*d*) A centric ring and an acentric pair (arrow) from chromosome 1 formed by heliotrine treatment.

To determine the interval between the initiation of the culture and the onset of mitosis and to estimate the increase of the mitotic index in normal leucocyte cultures, two parallel cultures were set up and samples were removed periodically. A few divisions appeared as early as 24–26 hr of the culture period, and then the mitotic index rose sharply to give two peaks, one at *c.* 60 hr and a second at *c.* 72 hr [Fig. 3(*a*)].

Cultures of leucocytes with heliotrine added to a final concentration of 5×10^{-5}M showed a considerable depression of the mitotic index at all stages. It can be seen from Figure 3(*b*) that a concentration of 5×10^{-5}M heliotrine represses the

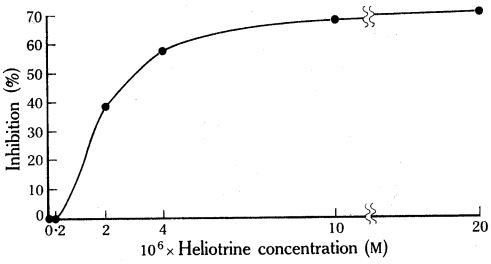


Fig. 2.—Percentage mitotic inhibition compared with control of potoroo leucocytes by heliotrine; colchicine treatment from 72nd to 74th hr of culture.

multiplication rate of leucocytes, causing a shift of the mitotic index so that an additional period of at least 10 hr is necessary for the mitotic index to reach the level present in controls.

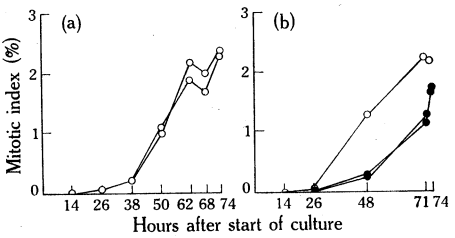


Fig. 3.—Mitotic index relative to the period of incubation (*a*) for two parallel leucocyte cultures without heliotrine, (*b*) for leucocyte cultures without (○) and with (●) 5×10^{-5}M heliotrine; colcemid treatment given for final 2 hr prior to each sampling. All mitotic indices estimated by counting 5000 cells except in 14- and 26-hr samples where 10,000 cells were counted.

To study the effect of heliotrine on the mitotic index of leucocytes in their first division *in vitro*, colcemid was added 24 hr after the initiation of cultures, and metaphases were scored at the 60th hr of the culture period. The effects of three concentrations of the alkaloid on the entry of cells into mitosis is given in the following tabulation, where all mitotic indices were estimated by counting 5000 cells:

Heliotrine concn. (M)	1×10^{-4}	5×10^{-5}	1×10^{-5}	0
Mitotic index (%)	0·78	2·0	4·2	5·0
Inhibition (%)	84·8	60·0	16·0	0·0

(*c*) *Chromosome Breakage*

(i) *Heliotrine Damage in 72-hr Cultures*

When cultures treated with heliotrine were stopped after 72 hr by colchicine treatment for a final 2-hr period, chromatid and isochromatid deletions, chromatid and chromosome interchanges [Fig. 1(*c*)], rings [Fig. 1(*d*)], and dicentrics were observed (Table 1). At concentrations above 1×10^{-4}M scoring became difficult because of the scarcity of metaphases and, after several attempts, was discontinued.

The chromosomal damage was estimated as the number of breaks per nucleus; chromatid and isochromatid deletions were scored as one-hit events, while large

interstitial deletions, dicentrics, rings, and chromosome interchanges were scored as two-hit events. The following tabulation shows the damage per nucleus produced by different concentrations of heliotrine:

Heliotrine concn. (M)	5×10^{-6}	1×10^{-5}	$2{\cdot}5\times10^{-5}$	5×10^{-5}	1×10^{-4}
No. of hits/cell	0·10	0·29	0·52	0·95	1·52

(ii) Heliotrine Damage in Cells in First Divisions

When heliotrine-treated leucocyte cultures were stopped in the 72nd hr, both chromosome and chromatid aberrations were observed. The chromosome breakage could be attributed to:

(1) True chromosome breakage present in cells arrested in first division after treatment.

(2) Spurious breakage resulting from a proportion of cells with chromatid breakage entering a second mitosis before the time of fixation. Most alkylating agents are able to produce breakage in chromosomes only during S (DNA-synthetic phase) or later.

It was important to distinguish between these alternatives. The culture period was therefore reduced from 72 to 60 hr and an extended period of spindle suppression

TABLE 1

CHROMOSOME DAMAGE PRODUCED BY HELIOTRINE IN POTOROO LEUCOCYTE CULTURES

Culture period 72 hr with colchicine added for final 2 hr. 100 cells scored in each treatment

Chromosome Damage	Heliotrine Concentration (M): 5×10^{-6}	1×10^{-5}	$2{\cdot}5\times10^{-5}$	5×10^{-5}	1×10^{-4}	0
Chromatid deletions	7	7	15	40	66	1
Isochromatid deletions:						
Non-union in centric and acentric parts	4	15	35	26	52	3
Sister union in both centric and acentric parts	0	4	0	2	0	0
Non-union in proximal (i.e. centric) part	1 } 5	3 } 11	1 } 1	0 } 4	4 } 12	0 } 2
Non-union in distal (i.e. acentric) part	4	4	0	2	8	2
Total isochromatid deletions	9	26	36	30	64	5
Two-hit events:						
Chromatid	0	1	1	8	8	0
Chromosome	0	0	1	6	0	0
Three-hit events:						
Chromatid	0	0	1	0	4	0
Chromosome	0	0	0	1	0	0

was included to ensure that cells examined were in their first mitosis following heliotrine treatment. The procedure of Sasaki and Norman (1966) was followed: colcemid was added to cultures after 24 hr and all cultures were terminated at the 60th hr. The damage produced by three concentrations of heliotrine when only first divisions were scored is given in Table 2.

If the isochromatid deletions for all heliotrine experiments are pooled, then there are 74 closed or partially closed deletions [i.e. sister unions in both centric and acentric parts, non-unions in proximal (i.e. centric) and distal (acentric) parts] and

230 open deletions (i.e. non-unions in both centric and acentric parts). This means that 76% of isochromatid deletions are of the open type.

TABLE 2

CHROMOSOME DAMAGE PRODUCED BY HELIOTRINE IN POTOROO LEUCOCYTE CULTURES

Cells arrested in their first division *in vitro* by addition of colcemid from 24th to 60th hr. 100 cells scored for each treatment

Chromosome Damage	Heliotrine Concentration (M): 1×10^{-4}	5×10^{-5}	1×10^{-5}	0
Chromatid deletions	36	28	18	3
Isochromatid deletions:				
Non-union in centric and acentric parts	43	34	21	4
Sister union in both centric and acentric parts	11	3	2	1
Non-union in proximal (i.e. centric) part	8 } 25	4 } 11	1 } 4	0 } 1
Non-union in distal (i.e. acentric) part	6	4	1	0
Total isochromatid deletions	68	45	25	5
Two-hit events:				
Chromatid	3	5	1	0
Chromosome	2	1	0	0
Multiple-hit events:				
Chromatid	3×3; 1×8	2×3; 2×4; 1×6; 1×8	—	—

(d) Comparison of Heliotrine Damage to Chromosomes with X-irradiation Damage

(i) *Dose Equivalence*

For X-irradiated cultures, the damage was scored for cells in division after 72 hr and the dose-effect relationship is shown in Figure 4. The deviation from linearity in the dose-effect relation is possibly due to the greater mitotic delay with higher doses.

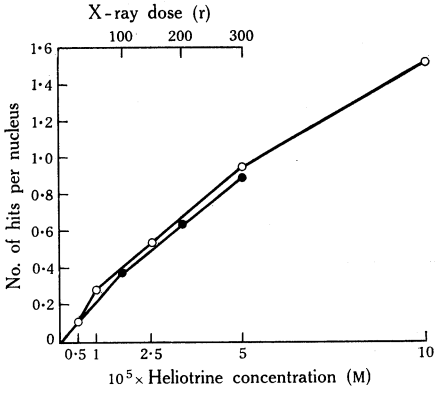


Fig. 4.—Dose–effect comparison for chromosomal damage produced by heliotrine (○) and X-irradiation (●); colchicine from 72nd to 74th hr of culture period.

Cultures of potoroo leucocytes irradiated with 200 r in the 60th hr of culture showed virtually no cells in division till at least 6 hr after the X-ray dose was given. This increase in mitotic inhibition with larger doses of X-rays would prevent the more heavily damaged cells reaching metaphase at the time of fixation. The slope of the

dose–effect curve produced by X-rays is almost identical with the dose–effect curve for heliotrine (Fig. 4).

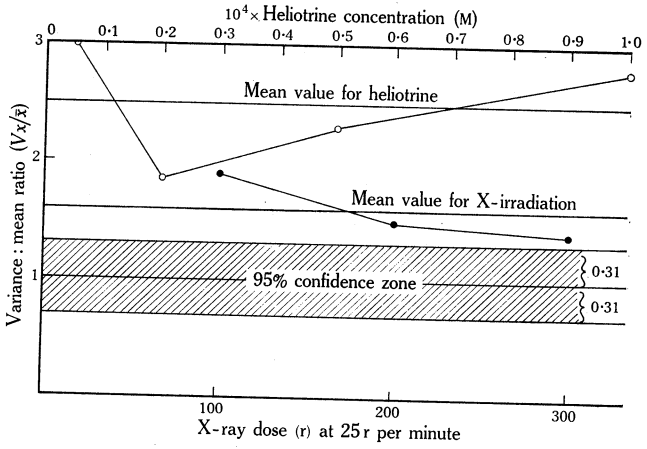


Fig. 5.—Overdispersed distribution of chromosomal breakage between cells—ratio of observed to expected variance for different doses of X-rays and heliotrine. The shaded area represents the 95% confidence zone for the Poissonian expectation of random distribution (variance: mean = 1).

An average value of 0·0033 breaks/cell/roentgen was found for X-irradiation treatments of potoroo leucocytes. This value is somewhat lower than 0·0039 found by Bell and Baker (1962) and compares well with the value of 0·0031 obtained by

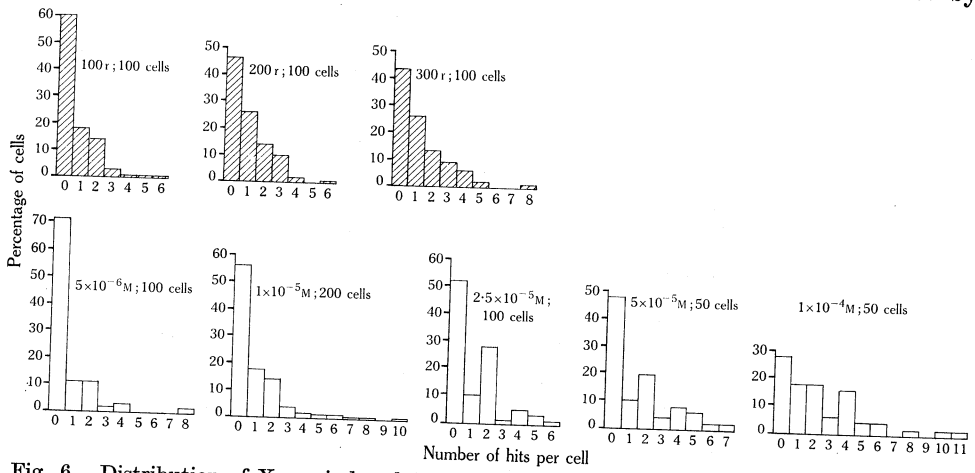


Fig. 6.—Distribution of X-ray induced (shaded histograms) and heliotrine-induced (open histograms) chromosomal damage in potoroo leucocytes. Colchicine added from 72nd to 74th hr of culture. X-ray dose rate 25 r/min. X-ray dosage, heliotrine concentration, and number of cells scored in each case are shown on histograms.

Bender and Gooch (1963) from experiments with human leucocytes. The latter authors used doses of 25 and 50 r, so that with the higher doses in the above experiments, the damage per roentgen could be expected to be somewhat higher.

In so far as comparison can be made between the effects of X-rays delivered to the cells before the culture period and of heliotrine present in the medium during the whole of the period up to metaphase arrest, a heliotrine concentration of 5×10^{-5}M produces about the same amount of final damage as 300 r delivered at 25 r per minute.

TABLE 3

DISTRIBUTION OF BREAKS BETWEEN CHROMOSOMES RELATIVE TO CHROMOSOME LENGTH

Relative length is taken from values given by Shaw and Krooth (1964). For both X-rays and heliotrine, the discrepancy between the number of hits expected in terms of physical length and the number observed is significant at less than the 1% level

Chromosome	Relative Length (*A*)	No. of Hits	Percentage of Total Hits (*B*)	*B*/*A*
		X-Rays		
1	24·4	115	30·1	1·23
2	22·7	97	25·4	1·12
3	18·3	65	17·0	0·93
4	10·4	23	6·0	0·58
5	6·7	16	4·2	0·62
X	17·3	65	17·3	1·00
Total	100·0	381	100·0	
		Heliotrine		
1	24·4	162	34·8	1·40
2	22·7	96	20·6	0·91
3	18·3	57	12·2	0·67
4	10·4	40	8·6	0·82
5	6·7	23	4·9	0·72
X	17·3	88	18·7	1·08
Total	100·0	466	100·0	

(ii) *Distribution of Damage*

The distribution of breaks between nuclei for both X-irradiation and heliotrine treatments was found to be non-Poissonian. In all experiments, the variance : mean ratio proved to be significantly greater than unity, indicating considerable over-dispersion and an aggregation of damaging events. Figure 5 shows the variance : mean ratios calculated for the three doses of X-rays and for the different concentrations of heliotrine; the distributions themselves are presented in Figure 6.

A non-random distribution of breaks between the individual chromosomes of a nucleus has also been detected (Table 3). The distribution of breaks induced by heliotrine is significantly different from that produced by X-rays (Fig. 7) and suggests some specificity of action either on initial breakage or subsequent process of repair.

A noticeable difference between X-irradiated cultures and those treated with heliotrine is in the number of damaging events that involve the centromeric or other heterochromatic regions. The proportion of breakage involving heterochromatic regions of the complement relative to the total was 2–3% in X-irradiated cultures and 10-fold greater in heliotrine treatments. For heliotrine concentrations of 1×10^{-5}M, 5×10^{-5}M, and 1×10^{-4}M the total number of damaging events were 45, 113, and 131, and the percentage of events involving heterochromatin were 33, 26, and 22 respectively.

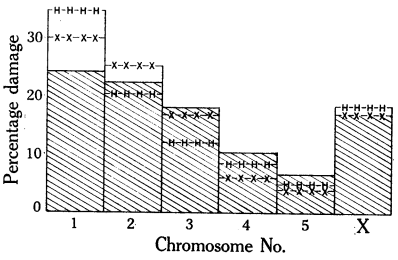


Fig. 7.—Distribution of damage between chromosomes of the potoroo complement produced by X-rays (X) and heliotrine (H). Shaded area represents relative size of chromosomes.

IV. Discussion

(a) *Type of Breakage*

The production of both chromosome and chromatid abberations in potoroo leucocytes is not in harmony with data available from plant material. Avanzi (1961, 1962) found that pyrrolizidine alkaloids produce only chromatid-type aberrations in *Allium* and *Vicia*. These results agree with the general finding for the action of alkylating agents (Scott and Evans 1964). In contrast Somers and Hsu (1962) found that both chromosome and chromatid aberrations were produced by treatment of cultures of Chinese hamster cells with hydroxylamine. A high percentage of the damage was localized in the centromeric regions of the chromosomes; constrictions and despiralization of chromosomes were also present. These authors suggest that the chromosomal aberrations induced by hydroxylamine and its derivatives might be the result of main-chain scission of DNA rather than of a reaction with the cytosine of DNA. Kihlman (1966), however, proposes that hydroxylamine reacts primarily with the cytosine.

Another radiomimetic substance which has been shown to produce chromosome as well as chromatid aberrations is 8-ethoxycaffeine (Kihlman and Eriksson 1962). Scott and Evans (1964) report that cells are arrested by 8-ethoxycaffeine mainly in the G_2 (post-DNA synthetic phase) stage of interphase, which is prolonged to about twice its normal length.

That the chromosome aberrations produced by heliotrine are true chromosome breaks rather than aberrant chromosomal damage resulting from the entry of cells with chromatid damage into a second division is substantiated by the following observations:

(1) Control cultures without heliotrine show only 1–2 cells in division per 10,000 cells at the 26th hr of the culture period, and about 1% of cells in division

at the 50th hr; thus it is possible that in untreated cultures a very low number enter a second mitosis before the 60th hr.

(2) However, treatments with heliotrine at a concentration of 5×10^{-5}M produce a mitotic delay of at least 10 hr, and no divisions are observed after 26 hr of culture. At about the 50th hr, the number of dividing cells is only 0·2%. Thus it is improbable that any cells in these cultures could enter a second mitosis before the 60th hr.

(3) The addition of colcemid to the heliotrine-treated cultures at the 24th hr, i.e. before any cell comes up for division for the first time, ensures that no cells enter division for the second time before the 60th hr of the culture period.

(4) Cells which showed apparent chromosome breaks, such as rings and dicentrics, invariably contained all the expected deleted fragments; this observation strongly supports the assumption that these aberrations are in fact true chromosome breaks.

Autoradiographic studies in conjunction with heliotrine experiments would confirm the conclusion that heliotrine produces chromosome breakage. By using higher concentrations, it might be possible to induce a larger proportion of chromosome aberrations [e.g. 8-ethoxycaffeine has to be used in concentrations of 2×10^{-3}M to produce non-delayed effects (Kihlman and Eriksson 1962)]. However, the higher concentrations of the alkaloid would have to be introduced for short periods in G_1, since a concentration of 2×10^{-4}M for the total period of culture suppresses the mitotic index of potoroo leucocytes to such an extent that none reach metaphase in the alloted time.

(*b*) *Distribution of Damage*

Both X-irradiation and heliotrine treatments produced a non-random distribution of breakage between nuclei, although the overdispersion was more pronounced in treatments with heliotrine. The distribution of breaks between individual chromosomes after X-irradiation and heliotrine treatment was also found to be non-random. In irradiation treatments the damage in the larger autosomes was greater than that expected on the basis of relative length; however, damage in the larger chromosomes is easier to detect, and it is possible that it is somewhat overestimated. The distribution of breaks induced by heliotrine is significantly different from that in X-ray treatments and there is a predominance of damaging events affecting the centromeres and the heterochromatic region of the *X* chromosome. This suggests some specificity of action of the alkaloid on initial breakage or on subsequent process of repair.

A non-random distribution of damage between chromosomes in *Vicia faba* following X-irradiation has been observed by Reiger and Michaelis (1959). In reports of similar findings by Sire and Nilan (1959) and Evans and Bigger (1961) attention has been drawn to the correlation between the distributions of breakage and heterochromatin in the chromosomes. A near-random distribution of heterochromatin may account for the random distribution of damage observed in some organisms.

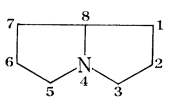
In contrast, the distribution of damage after treatment with most radiomimetic substances is markedly non-random. Kihlman and Eriksson (1962) showed that treatments with radiomimetic agents gave much greater deviation from the Poissonian expectation than X-ray treatments, and McLeish (1953) found the heterochromatic regions of the chromosomes of *V. faba* to be more susceptible to breakage by chemical treatment. In addition to any heterogeneity produced by the distribution of heterochromatin, a cumulative action resulting in an overdispersion of damage is likely in treatments with chemical agents. Overdispersion of a lower intensity can also be expected in irradiation treatments as a result of the cumulative action of the active radicals produced (Jackson and Barber 1958). Physiological variation between cells making up the population must also contribute to overdispersion, especially in treatments with radiomimetic agents.

A further factor influencing the distribution of aberrations is the organisation of the heterochromatin into chromocentres in the interphase nucleus. As a result the proportion of two-hit events is modified; restitution and reunion may also be affected. The DNA of heterochromatic regions is usually late in replicating; thus the concentration of breakage in these regions is likely to vary with the time and extent of treatment relative to the mitotic cycle.

In the heliotrine experiments 76% of isochromatid deletions showed no sister reunion of any kind. In comparative X-irradiation of potoroo kidney cells (Bick and Brown, unpublished data) only 43% of the isochromatid deletions were of the open kind. It is likely that heliotrine-treatment prevents a proportion of broken chromatids from rejoining; alternatively, a larger proportion of heliotrine-formed isochromatid breaks may result not from a single event but from two events separated in time. On the other hand, if true chromosome deletions occur in heliotrine treatments, they will always appear as the open type.

(c) *Mode of Action*

Heliotrine has a pyrrolizidine nucleus (I) in common with a number of other alkaloids which cause liver damage or produce mutations. Not all pyrrolizidine alkaloids produce these effects; Schoental (1957) observed that hepatotoxic activity could be correlated with the presence of a double bond in the 1 : 2 position. Further-



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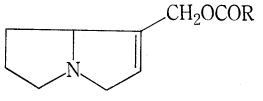

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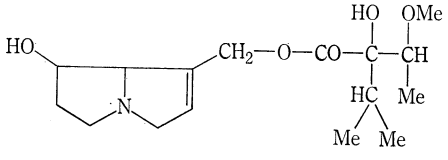

(III)

more, Culvenor, Dann, and Dick (1962) have shown that the hepatotoxic pyrrolizidine alkaloids have an alkyl ester group in position 1 (II) and have suggested that such alkaloids can act as biological alkylating agents. Heliotrine (III) has the required structural features to act in this way; moreover, its mutagenic activity is associated with the intact molecule, since neither the acid not basic fragments obtained on hydrolysis of the ester link have proved to be mutagenic (Clark 1959).

Alkylating agents can react with the tertiary phosphoric acid groups of DNA to give phosphate triesters (Stacey *et al.* 1958); since trialkyl phosphates are unstable and readily hydrolysed in weakly alkaline solutions, the alkylated DNA tends to disintegrate into smaller fragments (Brown and Todd 1952). Alkylating agents are also able to react with the base moieties of the DNA molecule; Brookes and Lawley (1964) found guanine the most susceptible base and according to Somers and Hsu (1962), the proportion of guanine and cytosine is high in the centromeric regions of Chinese hamster chromosomes. If this is also true for the centromeres and the heterochromatin of the *X* chromosome in the potoroo, then the preferential attack by heliotrine on its heterochromatic regions could be explained on this basis.

Whether heliotrine or any of the other pyrrolizidine alkaloids which show mutagenic properties act primarily as alkylating agents of the DNA molecule still remains to be determined. Recent observations by Alderson and Clark (1966) on *Aspergillus nidulans* indicate unexpected differences in specificity between alkylating agents such as diethyl sulphate and mutagenic alkaloids such as heliotrine.

The action of heliotrine resembles that of hydroxylamine in many respects. They both produce secondary constrictions, despiralization of chromosomal material, and a high percentage of damage localized in centromeric regions. Both are capable of reacting with the base moieties of DNA — hydroxylamine with cytosine and heliotrine with guanine; in consequence, the preferential attack of these bases would result in damage to the same region of the chromosomes. Part of the damage produced by these agents is considered to be due to the scission of the DNA chain which leads to visible cytological gaps in the chromosomal structure.

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VI. References

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