NEW EVIDENCE ON THE MODE OF ACTION OF 'MITOTIC POISONS'

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I. Biology

IN 1925 A. P. Dustin¹ reported that certain chemical agents which he called "*poisons caryoclastiques*" were able to exert a toxic action on cell nuclei without apparently injuring the cytoplasm. It has since been claimed that a wide variety of compounds can act in this way (see, for example, reviews by Dustin² and Astaldi *et al.*³), and, since they appear to be active only on dividing cells, they have sometimes been called mitotic poisons.

Broadly speaking, the problem has been attacked from two main positions. The medical discipline, with the question of cancer therapeutics in mind, has usually chosen as its experimental material mitotically active animal tissue, either *in vivo*, as, for example, in studies of the intestinal crypts of Lieberkühn, or *in vitro*, as in work on chick fibroblasts. Empirical observations on cell effects and measurements of the growth inhibition of the tissue or tissue culture have been used as criteria for the activity of the agent under test. Geneticists and cytologists, on the other hand, have in recent years worked on quite a different line. By the use of the precise techniques that had previously been devised for the detection and quantitative estimation of X-ray effects, they have shown that certain classes of these compounds likewise are mutagenic agents, for they can cause heritable nuclear changes that are demonstrably similar to those induced by X-rays. Consequently they have used as experimental materials those, such as *Drosophila* and the classical cytological objects, which were best suited to the application of these techniques; and they have felt justified in doing this by their confidence that their results could in general be extrapolated to other cellular systems.

It has therefore come about that workers with training and research experience in these widely different fields have contributed to what is now an extensive literature. Compounds have been reported as 'active' on different criteria, on different materials, and under entirely different test conditions. Some workers may even disagree as to what results should be included as comparable at all. Nevertheless, the object of the present article is to evaluate a few of the different methods and results and to attempt to correlate the chemistry and modes of action of certain of the different classes of compounds that have been used. The article is thus designedly critical and selective and is not intended as a comprehensive review.

The observed effects of the compounds reported as active have been divided into three categories. The first includes those compounds which have been shown by genetical or cytological test to be radiomimetic. All the work in this field stems from Auerbach's original discovery that by the *CIB* test mustard gas, like X-rays, is mutagenic⁴. Mainly from the work of Auerbach, Robson, Carr, Koller and the Slizynskis⁵, both nitrogen and sulphur mustards

are now known to cause gene mutation and chromosome breakage. Other compounds which it is claimed induce either gene or chromosome changes, or both, include allyl *iso*-thiocyanate⁶, urethane⁷, phenol⁸, certain of the aminostilbenes⁹, formaldehyde¹⁰, 20-methylcholanthrene, 3:4-benzpyrene, 1:2:5:6-dibenzanthracene and β -naphthylamine¹¹.

These compounds are of interest not because of any direct toxic effects they may produce in the cell during their presence there, but because of the irreversible hereditary changes they induce in the nucleus. These hereditary changes or mutations will persist after the agent itself has been lost or destroyed, and at the subsequent mitosis will cause a loss or an asymmetric segregation of genetic material. The two daughter nuclei of this mitosis will have a deficient or unbalanced genetic complement: cells so affected will either die or will at least have a reduced viability.

Although these agents are said to be radiomimetic, it is of vital interest, from the point of view of their mode of action, to determine whether this is strictly true: whether the type and incidence of the heritable change that they induce is exactly that which is also induced by X-rays. Several differences have already been found. Auerbach has shown in her mustard gas experiments with *Drosophila* that in the progeny of a treated individual the frequency of mosaicism is very high—as much as 50 per cent of observable mutation being of this type. Thus the change induced may not manifest itself phenotypically for several cell generations at least. Such mosaics occur after X-ray treatment, though the incidence is relatively small. The *Drosophila* results also show that after mustard gas treatment the number of observed chromosome translocations is smaller per number of sex-linked lethals than after X-irradiation.

Auerbach's results do not, however, indicate that mustard gas has any selective action on the chromosomes. But Ford¹² working with a nitrogen mustard, NN-di(2-chloroethyl)methylamine, has shown that in *Vicia* root tip cells the distribution of breaks is very markedly biased towards certain chromosome regions. Ford's work has been confirmed in this laboratory¹³, and it has also been shown that these high break regions are certainly very near demonstrable regions of heterochromatin and probably are actually in them, and that the breaks seem to show a proportionality in number that corresponds to the length of the heterochromatic segments. This localization of the breakage is in marked contrast with the more generally distributed breakage that follows X-irradiation. This finding is significant in view of the fact that heterochromatin has a high nucleic acid charge in the resting nucleus, and it is known that the mustards have an affinity for acid groups. We may thus suspect that the extrinsic agent is combining directly with the deoxyribose nucleic acid at the resting stage chromocentres, and that breakage is the result. The cytological effects of di(2:3-epoxy-

propyl)ether, the chemistry of which is dealt with later, are precisely the same as those induced by NN-di(2-chloroethyl)methylamine*.

An attempt has been made, again using *Vicia* as test material, to determine where in the nucleus this combination occurs¹³. This work has been based on three premises: first, that the pair of chromosomes carrying the nucleolar organisers are at least partially in the interior of the nucleus; secondly, that the other chromosomes are predominantly disposed on the nuclear membrane; thirdly, that interchange between chromosomes indicates a proximity (in a chromocentre) of these two chromosomes at the time of breakage and reunion. Comparative estimates of the breakage and interchange frequencies in and between different chromosomes following treatment with NN-di(2-chloroethyl)methylamine are quite different from those made on the X-rayed controls, and suggest that combination occurs much more readily with the chromocentres that are on the nuclear surface than with those in the interior of the nucleus. This work will be published in detail elsewhere.

There is no evidence in *Drosophila* of any such selectivity of action; but if the nucleic acid charge and the spatial distribution of the chromosomes within the nucleus are factors influencing the position of breaks, then the nucleus of the sperm and of the plant somatic cell might be expected to react very differently.

A subsidiary group of cytological results have been presented by Cornman¹⁴ on the effects of coumarin and parascorbic acid, by Ostergren¹⁵ on coumarin, and by Levan and Tjio¹⁶ on some phenolic compounds. It appears to us, however, that any chromosome breakage seen after treatment with these compounds is not of the same type as that caused by the mustards, but is due to an induced intercalary stickiness of the chromosomes that leads to the observed anaphase bridges and fragments, an explanation which has, indeed, been suggested by Levan. Kihlman and Levan¹⁷ have published a preliminary report on chromosome breakage induced by caffeine. This result has been qualitatively confirmed by a study of *Vicia* metaphases in this laboratory, but there are at present insufficient data to indicate whether breakage is localized. It should be remembered, however, that the concentration required to produce an observable number of breaks is extremely high, and is of quite another order than that used in

the NN-di(2-chloroethyl)methylamine experiments. This suggests that we are here dealing with a different mode of action.

Urethane has been reported as active on plant cells by Oehlkers⁷ and has, of course, been used clinically in the treatment of leukaemia. Koller¹⁸ has shown that, after injection into rats implanted with Walker carcinoma, chromosome breakage occurs in the tumour and the bone marrow cells. One positive result obtained after urethane treatment on plant material in this laboratory¹³ has, however, proved to be quite irreproducible, and other workers (for example, Ludford and Koller) have also remarked on an unaccountable variation in its activity. With this compound again, relatively high concentrations are required for positive results.

Our second category includes a number of compounds for which activity of a sort has been claimed and which in our opinion require separate consideration. A. P. Dustin¹⁹ described the cytotoxic effects of tryptaflavine, and P. Dustin² in a review ascribes a tryptaflavine-type reaction on mouse intestinal crypt cells to a number of substances including urethane, various cyanates, arsenicals and mercury compounds (as well as hydroquinone and pyrocatechol). A degenerative pycnotic change that appears very shortly after treatment is induced in the nuclei of cells about to undergo division, and since nuclear pycnosis is also one of the immediate effects of X-irradiation Dustin claims that these compounds are radiomimetic. Levan has presented evidence of another kind for hydroquinone and pyrocatechol; but we do not consider Dustin's experiments sufficiently crucial to justify placing the tested compounds in the same class as those shown to be radiomimetic by genetical or cytological test. All available evidence²⁰ suggests that the truly radiomimetic poisons act on nuclei in the resting stage; consequently their chromosome effects do not manifest themselves until these cells come into division. If the observations are only made at very short intervals after treatment, chromosome breakage will not be seen, for this will only begin to appear to any extent at about eighteen to twenty-four hours. With nitrogen mustards also, as Dustin points out, effects of the tryptaflavine type in cells in division can be seen at very early stages after treatment, but these reactions are not of the radiomimetic type which appear at later stages: they are merely an expression of the generally toxic action of all these substances on any tissue and upon rapidly proliferating tissues in particular.

To illustrate this point, Figs. 2 and 3 show cell divisions in root-tip preparations of *Vicia faba* at short intervals after treatment with ethyl alcohol and nitrogen mustard respectively. In both cases division is inhibited and the chromosomes are seen in an adherent condition usually described as stickiness, and there are in the same preparation numerous cells in various stages of the degeneration and pycnosis which follow. The meristems treated with alcohol eventually resumed normal proliferation with no sign that any chromosome rearrangements had been induced. The material treated with nitrogen mustard, re-examined at twenty hours, had also recovered from the general toxic effects of the treatment and was dividing actively; but these divisions showed a high frequency of unequivocal chromosome breaks which at a later stage again completely, and this time permanently, inhibited cell proliferation. The significance of this demonstration is that in both

* Prof. A. Haddow has provided the following note:

(1) In the course of an investigation being conducted here into the mechanism of action of the nitrogen mustards, Dr. W. C. J. Ross of this Institute had originally suggested a series of di-epoxides which it would be desirable to examine, among other types, as biological alkylating agents; this proposal was incorporated in his chemical programme forming part of the investigation as a whole.

(2) Before this suggestion could be put to the test, it reached us again from a different quarter. In connexion with the hypothesis of Goldacre, Loveless and Ross (*Nature*, 163, 667; 1949), that cross-linking may play an important part in the action of the nitrogen mustards, Prof. J. B. Speakman, of the Department of Textile Industries, University of Leeds, kindly agreed to test a series of halo-alkylarylamines (Haddow, Kon and Ross, *Nature*, 162, 824; 1948) for their cross-linking potentialities in the reduced keratin fibre. At the same time, Prof. Speakman indicated we should do well to study the biological properties of a number of known cross-linking substances not hitherto so examined, and particularly suggested those di-epoxides which have recently come into prominence as effective cross-linking agents in textile practice. On biological test it at once became evident that a number of di-epoxides, including the di(epoxypropyl)ether referred to in the above text do, in fact produce, in both normal and tumour tissues, effects which are largely indistinguishable from those due to the nitrogen mustards.

(3) Meantime, an independent and contemporary interest in these effects of the di-epoxides had been developed by the Research Department of Imperial Chemical Industries, Ltd. (Dyestuffs Division). By mutual arrangement, separate accounts will be published concurrently at a later stage.

Cells at anaphase of mitosis from *Vicia faba* root-tip preparations. Acetic-alcohol and Feulgen ($\times 3,000$)

(1) Anaphase showing two dicentric bridges and two fragments, after 1 hr. of treatment with $M/10^4$ NN-di(2-chloroethyl)methylamine, and 22 hr. in water. (2) Anaphase showing chromosome lagging and 'stickiness' after 1 hr. of treatment in 1 per cent ethyl alcohol and 2 hr. in water. (3) Anaphase showing chromosome 'stickiness' after 1 hr. of treatment in $M/10^4$ NN-di(2-chloroethyl)methylamine and $5\frac{1}{2}$ hr. in water

cases the initial effects observed indicated that there was a toxic action on the cell but not a genetic alteration in the nucleus.

Toxicity of this type is in itself of interest, but must be regarded as an entirely separate problem. For this reason, in the second part of this paper, where consideration is given to the mode of action of radiomimetic agents, the theory proposed does not attempt to accommodate some of the compounds considered by Dustin to be of the tryptaflavine type, since it is not considered that their activity has been substantiated *sensu stricto*.

The last group of compounds includes colchicine and those substances which act in a similar way. Dixon²¹ first observed the cytological effects of colchicine on dividing leucocytes. Later A. P. Dustin²², Ludford²³ and Brues and Cohen²⁴ studied its effects on various animal tissues. With the discovery by Blakeslee and Avery²⁵ that colchicine can produce polyploidy in plants, it became apparent that the drug might be of great use to the plant breeder, and much work has since been published on its uses in this direction. Levan²⁶, in a careful analysis made on *Allium* root-tip cells, showed exactly how colchicine was able to act on the plant cell to inhibit spindle formation, causing the accumulation of metaphases and the formation of polyploid nuclei. Acenaphthene²⁷ was also found to cause spindle failure and polyploidy in plant tissues. More recently Ostergren and Levan²⁸ have claimed that a wide variety of compounds can at a critical concentration cause spindle suppression. Ostergren²⁹ has pointed out that their activity appears to be inversely proportional to their water solubility, and he advances a physical hypothesis for their mode of action similar to that of Meyer and Overton for narcotics. The colchicine-type activity is, however, different from that of the other chemical classes we shall consider, for, although it produces a heritable nuclear change,

this change is a quantitative one and is not of the qualitative kind characteristic of the 'radiomimetics': colchicine does not induce gene mutation or chromosome breakage. Any growth-inhibitory action depends first upon its effect in delaying metaphases and so causing a doubling in chromosome number, and secondly, upon the various abnormalities that may arise in the polyploid animal cell at subsequent divisions³⁰.

In view of the much wider appreciation than hitherto of the important part that induced chromosome changes can play in growth inhibition, we wish to direct attention to the limitations of what we may call the 'bridge and fragment method'. In this type of analysis, anaphases are scored either as normal or as showing a varying number of dicentric and acentric chromosomes. In mammalian tumour tissue and in chick fibroblast cultures, it is impossible to make the necessary detailed observations on the changes in number and morphology of the metaphase chromosomes which, in more favourable material, would be the method adopted to score breakage. The scoring of anaphase effects is thus the only means available. Such an analysis can at best give only an index and never an absolute estimate of the amount of chromosome breakage induced. But it should also be realized that, especially in unfavourable material, the analysis itself requires some experience for its interpretation: the mere observation of disordered anaphases (accompanied perhaps by growth inhibition) is not necessarily an indication that chromosome breakage has occurred. During chromosome segregation at mitosis, the cell performs a mechanical feat which must be, like other cellular functions, a highly organised process. The presence of any extrinsic agent within the mitotic cell may interfere with the division cycle and produce effects that are not easily distinguishable from the effects of breakage and reunion (cf. Figs. 2 and 3 with Fig. 1). Such

tests should therefore be scored with care, and their results accepted with reserve.

II. Chemistry

It appears we must regard the so-called 'mitotic poisons' as comprising at least three distinct classes of compound. Moreover, on examination, each class seems to show a chemical homogeneity correlated with biological activity.

The first consists of a number of compounds well known for some other physiological activity, and these are characterized by their ability to undergo substitution or addition reactions with mercaptans. They include the lachrymators, bacteriostatic and anti-protozoal agents and anti-growth substances of plant origin. They exert a highly toxic, non-specific effect upon the cell and are *non-radiomimetic* in the properly defined sense.

The second class comprises the vesicants such as sulphur and nitrogen mustards, di-epoxides and protoanemonin. Although reaction with —SH compounds has been demonstrated, it is becoming apparent that reaction at other sites, almost certainly acid groups, underlies their important biological effects. Reaction in the case of the mustards is almost entirely by the S_N^1 mechanism, which permits alkylation of free amino-groups and anions, and esterification of acid groups is therefore favoured. The value of these compounds lies in the highly specific effect upon the resting nucleus, manifested in subsequent mitosis by the appearance of chromosome breakage and rearrangements of various kinds: for this reason they may be correctly termed 'radio-mimetic' and are probably the only class of 'mitotic poisons' of potential value in the chemotherapy of malignant disease.

The last group comprises those compounds capable of destroying or preventing the formation of the mitotic spindle without any undue toxic effect upon the cell. Colchicine shows a far higher degree of specificity in this respect than any other substance known, although a very large number of chemically unrelated compounds have been reported as showing some degree of activity. The difficulty of correlating these chemically has led some authors to conclude that their properties are the consequence of physical rather than chemical attributes and that they may be likened in this respect to narcotics. It is difficult to believe, however, that colchicine is a 'structure non-specific' substance, and work reported herein may shed new light on this problem.

In addition to these three groups, there is a large number of other substances which have from time to time been reported as 'mitotic poisons'. No attempt is made to review or classify these chemically since, as we have already seen, the biological criteria upon which they have been judged are obscure and a critical appraisal is impossible. References are available in reviews by Lettré²¹ and Astaldi *et al.*³.

Class I: Non-Radiomimetic Mitotic Poisons

Dixon²² has pointed out that lachrymators are of three types: (a) compounds possessing a positive halogen atom, that is, where the normal displacement of electrons in the carbon-halogen bond is reversed by an adjacent electron-attracting group such as the carbonyl group; (b) unsaturated compounds having an electron-attracting group adjacent to the double bond, thus permitting addition of nucleophilic reagents; (c) some arsenicals. Each type of com-

pound will readily react with —SH groups, the arsenicals reversibly.

A series of 'mitotic poisons' may also be classified in this way, including the following: iodoacetic ester (I) and acid amide, maleic acid (II), unsaturated lactones such as parasorbic acid (III) and coumarin (IV), the quinones such as benzoquinone (V) with the quinonimines and diimines and arsenicals of the type of cacodylates and arsenoxides.

In Levan's¹⁶ study of the cytological effects of a series of mono- and polyphenols, the greatest activity was found for those which were disubstituted in the *ortho* or *para* positions. It was left to Dustin²³ to direct attention to the fact that these are the ones which can oxidize to quinones, and there seems little doubt that this underlies their activity. Ostergren¹⁵ has recently shown that coumarin and parasorbic acid have similar activity, but does not recognize the unsaturated character of both as the basis of activity. Haynes²⁴, in an excellent review of physiologically active unsaturated lactones, notes that —SH addition had been postulated as the mechanism of action, but points out that not all are antagonized by extrinsic thiols. Two possible explanations seem significant, namely, (1) the compound may react at two points, either the double bond and the carbonyl group (*cf.* reactions of unsaturated ketones²⁵) or as an open-chain doubly unsaturated compound, or (2) reaction may occur with groups other than —SH, a faculty possessed by virtue of the electrophilic character of the double bond.

There seems little doubt that the activity of this group is in the main due to reaction with tissue —SH groups, and the high general toxicity and non-specific action is thus to be expected. The salts of heavy metals and organic mercurials as well as arsenicals likewise fall into the same category.

The reversibility of reaction of —SH groups with mapharside (3-amino-4-hydroxyphenyl arsenoxide) may explain the somewhat different cytological effects which it produces in comparison with compounds reacting irreversibly. Thus roots of *Vicia faba* treated with a sufficient dose to bring about complete arrest of mitosis show an apparently complete recovery. In experiments using *Liridurus* larvae

and already reported briefly²⁶, recovery was often accompanied by a stickiness of the chromosomes, producing terminal and intercalary bridges at anaphase. The latter occasionally produced fragments by rupture at the point of adhesion, an effect also noticed by Levan (*loc. cit.*) in *Allium* treated with phenols and by D'Amato²⁷ as the result of cold treatment. Barber and Callan³⁰ have also reported similar effects in *Triturus* as the result of cold treatment. It is highly important to distinguish between this effect and the type of breakage produced by radiation and vesicants which occurs in the resting nucleus.

Tests with hydroquinone performed in this laboratory on *Vicia* roots have not corroborated the results of Levan, and no evidence whatsoever of chromosome breakage was obtained.

Class II: Radiomimetic Compounds

The sulphur and nitrogen mustards owe their special reactivity to the electron-repelling character of the sulphur and nitrogen atoms which is derived from the lone pair(s) of electrons which they carry. As a consequence, ionization of the halogen is facilitated in aqueous solution, resulting in the production of a positive carbonium ion (VI) which is the initial reactant. In the case of the nitrogen mustards attention has recently been directed to this by Ross²⁸. It may be depicted thus:

The behaviour of the second halogen will depend upon the manner of reaction of VI, but it is probable that it will follow the same course. The mustards are thus bifunctional alkylating agents, reacting readily with nucleophilic groups, notably ionized $-\text{SH}$, $-\text{COOH}$ and phosphate groups, and also free $-\text{NH}_2$ by the S_N^1 mechanism and suffering rapid hydrolysis in water. They thus stand contrasted with compounds of the iodoacetate type, where the group adjacent to the halogen atom is electron-attracting and reaction is by the S_N^2 mechanism.

The requirement for 'radiomimetic' activity in compounds of this type appears to be the presence in the molecule of two alkylating groups having a minimum degree of activity³⁹. In the case of the nitrogen mustards this has been measured by the rate of hydrolysis (Ross, *loc. cit.*). Since amino-groups are largely ionized and $-\text{SH}$ un-ionized at physiological pH, it seems probable that the significant reaction is esterification of acid groups, possibly thereby bringing about cross-linkage between protein or nucleoprotein molecules³⁹. Reaction with acid groups has been suggested by Mrs. Pirie⁴⁰ from a consideration of the binding of collagen by S-mustard and by Herriott *et al.*⁴¹ working with a number of proteins. Furthermore, Elmore *et al.*⁴² found that mustard gas reacted readily with the phosphoryl groups of nucleic acids, giving in the case of thymus nucleic acid one compound in which two groups were blocked per mol. of mustard.

Of great significance are the results of Herriott⁴³ on the inactivation of enzymes and viruses by

S-mustard. He finds that viruses are more readily inactivated than enzymes and suggests this is due to reaction with nucleic acid. Further, those which contained deoxyribonucleic acid were more susceptible than those having only ribonucleic acid.

These results achieve a new significance in the light of Revell's¹⁸ findings that the regions of breakage of *Vicia* chromosomes induced by N-mustards and di-epoxides are constant and bear a close relation to the distribution of heterochromatin; that is, those regions which retain a 'nucleic acid charge' in the resting nucleus.

Following up the idea of cross-linkage of acid groups, Mr. J. L. Everett and Dr. W. C. J. Ross of this Institute have been preparing a series of di-epoxides, of which VII and VIII are typical:

Di-epoxides were of particular interest among the various esterifying agents considered, since it was learned that compounds of this type are already in use in the textile industry for bringing about cross-linkage between carboxyl groups of wool fibres⁴⁴ (see footnote on p. 939).

These compounds show precisely the same cytological effects as the nitrogen mustards. Moreover, VIII is said to be vesicant⁴⁵, and there is evidence of its reacting by the S_N^1 mechanism⁴⁶.

Unsubstituted NN-dichlorodiethylamine, which does not appear to have been tested previously, is also active, though less so than NN-di(2-chloroethyl)-methylamine.

Tests have also been made upon the other known vesicants, protoanemonin (IX) and divinyl sulphone (X). Attention was directed to these compounds since the double bonds might be expected to react strongly with nucleophilic reagents on the theory given earlier. In addition, however, they have two double bonds, that is, are bifunctional. Again, the cytological effects were similar to the mustards, though the compounds had an obviously high general toxicity, divinyl sulphone more so than protoanemonin. Patulin, which is non-vesicant and for which the structure (XI) has been proposed⁴⁷, has been found inactive. It must be considered as a member of Class I.

It seems a reasonable conclusion that all vesicants typified by slow healing of the primary lesion also

cause chromosome breakage, and that the latter is responsible for the healing difficulty. Also it might be held that the site and nature of the chemical reaction determining the cytological effect is the same in all cases, but that those which are less active, such as protoanemonin, are so because of the greater competition of the other reactive groups in the tissues, notably —SH. This is in agreement with the observed greater toxicity of the latter substance.

A number of substances of other types has been described as producing chromosome breakage similar to radiation, including aminostilbenes, carcinogenic hydrocarbons, urethane and caffeine. These observations, in the case of the first three, have largely been made on the Walker rat carcinoma⁹ and it is impossible to say what was the ultimate mechanism. Certainly the evidence of tests upon *Vicia* and *Triturus* larvæ with urethane does not indicate any similarity of effect with the vesicants. Kihlman and Levan's¹⁷ work with caffeine and other methyl xanthines has only so far been briefly reported. If, indeed, the effects prove to be similar, an entirely different mode of action, though one possibly associated with nucleic acid metabolism, must be sought. In addition, certain compounds have been described as mutagenic which do not fall into this class. A proper chemical appraisal of these findings is not at present possible.

Class III: Colchicine-type Compounds

A great deal has been written concerning the action of colchicine and other compounds having like effects, and it is not intended to attempt any comprehensive review. As stated earlier, some authors, notably Ostergren²⁹, have concluded that nearly all organic substances show some degree of 'C-mitotic' action and suggest that spindle suppression is an aspect of narcosis.

It is rash to consider the relationship of structure to activity while that of colchicine is undecided; however, most authors agree that activity is lost if ring C becomes degraded. Thus, Steinegger and Levan^{48,49} find 'isocolchicine' and trimethyl colchicine acid active, but loss of activity accompanies alteration of the C ring.

On Windaus's formulation⁵⁰ this ring has an o-quinonoid or quinone methide form (XII), while Dewar⁵¹ has suggested, on theoretical grounds, a seven membered ring structure (XIII):

More recently Cook and Somerville have succeeded in synthesizing 3:4 benzotropolone⁵² akin to XIII, but the biological properties do not appear to have been studied.

The methoxymethylene group of XII might be expected to be very reactive, and, indeed, the quinone-methides that have been described undergo rapid polymerization. If this be the structure, therefore, one could not regard colchicine as an 'inert' substance.

XIII, on the other hand, could not be considered highly reactive.

In this connexion two results may be of significance: (1) Cornman¹⁴ reports the occurrence of colchicine-type metaphases and the production of tetraploid cells in *Zea mays* after treatment with coumarin (IV). (2) In the experiments with protoanemonin (which possesses an exomethylene group as well as a second double bond) a very high incidence — up to 50 per cent — of spindle suppression was observed immediately after treatment, and a large number of tetraploid divisions after twenty-four hours. These observations on the effects of protoanemonin were not made in a previous study of its cytological effects⁵³ owing to the use of an experimental procedure unsuited to this type of investigation.

It appears that colchicine-like effects can be brought about by compounds which are both reasonably soluble and reactive. A series of compounds structurally analogous to components of the C ring according to both formulations are now being tested and details will be reported in due course.

The original work reported briefly above will also be published in full elsewhere.

III. Summary and Conclusions

Confusion in the use of the term 'radiomimetic' has led to erroneous judgments on the possible chemical basis of this action. Thus Dustin³³, taking 'pycnosis' as his criterion, has concluded that radiomimetic activity is characteristic of —SH-active compounds, whereas we believe that it is the non-specific 'toxic' effects which are predominantly characteristic of such substances. It is, moreover, suggested that the selective, truly radiomimetic action of such compounds as the mustards and other vesicants is due to their ability to react at sites other than —SH, probably by the esterification of acid groups. Possibly this work may also cast light on the problem of vesication.

It is, of course, well known that protein sulphur metabolism is of great importance in growth and cell division, and, clearly, substances blocking —SH groups have a profound effect upon the dividing cell. Whether the influence is exerted directly on the chromosome, through interference in the $2SH \rightleftharpoons S-S$ redox system or by enzyme immobilization, is not known.

The theoretical chemistry of the first two classes has been dealt with, largely bearing in mind the needs of the biologist who may not be aware of the principles involved.

If this analysis is accepted, the term 'mitotic poison' becomes invalid both biologically and chemically. The cell in division reveals the consequences of physiological derangement which remain undetected microscopically (though not biochemically) in the resting state. This is no justification for using the expression 'mitotic poison' in respect of unspecific toxic agents simply because their effect on the dividing cell has been described. The term might perhaps be applied correctly only to colchicine where there is an apparently selective action on an apparatus, the spindle, present only during cell division and essential to that process.

The main aim of work in this field must be to elucidate the possible mechanisms of mitotic inhibition and nuclear derangement, first, for the information they may give on the process itself, and secondly, for the discovery of useful growth-inhibitors.

As we have briefly indicated, compounds of the second class can serve both these functions and it may be in this direction that future research should be concentrated if useful progress is to be made in the chemotherapy of malignant disease.

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- ¹ Dustin, A. P., *C.R. Soc. Biol.*, **93**, 465 (1925).
- ² Dustin, P., *Nature*, **159**, 794 (1947).
- ³ Aastaldi, G. Mauri, C., and Allegrì, E., *Il Farmaco Scienza e Technica*, **2**, 256 (1947).
- ⁴ Auerbach, C., *Drosophila Inform. Service*, No. 17, 48 (1943).
- ⁵ Auerbach, C., *Proc. 8th Int. Cong. Genet.*, p. 128 (1943).
- ⁶ Auerbach, C., and Robson, J. M., *Nature*, **154**, 81 (1944).
- ⁷ Oehlkers, F., *Z. Vererbshg.*, **81**, 313 (1943).
- ⁸ Hadorn, E., and Niggli, H., *Nature*, **157**, 162 (1946).
- ⁹ Koller, P. C., *Symp. Soc. Exp. Biol.*, I. Nucleic Acid, p. 270 (1947).
- ¹⁰ Rapoport, I. A., *C.R. Acad. Sci. U.R.S.S.*, **54**, 65 (1946).
- ¹¹ Demerec, M., *Science*, **105**, 634 (1947).
- ¹² Ford, C. E., *Proc. 8th Int. Cong. Genet.*, p. 570 (1948).
- ¹³ Revell, S. H. (unpublished).
- ¹⁴ Cornman, I., *J. Exp. Biol.*, **23**, 292 (1947).
- ¹⁵ Ostergren, G., "Botaniska Notiser", 376 (1948).
- ¹⁶ Levan, A., and Tjio, Joe Hin, *Hereditas*, **34**, 250 (1948).
- ¹⁷ Kihlman, B., and Levan, A., *Hereditas*, **35**, 109 (1949).
- ¹⁸ Koller, P. C. (unpublished).
- ¹⁹ Dustin, A. P., *Arch. exp. Zellf.*, **22**, 395 (1939).
- ²⁰ Darlington, C. D., and Koller, P. C., *Heredity*, **1**, 187 (1947).
- ²¹ Dixon, W. E., "A Manual of Pharmacology" (1905).
- ²² Dustin, A. P., *Bull. Acad. Roy. Med. Belg.*, **74**, 487 (1934).
- ²³ Ludford, R. J., *Arch. exp. Zellf.*, **18**, 411 (1936).
- ²⁴ Brues, A. M., and Cohen, A., *Biochem. J.*, **30**, 1363 (1936).
- ²⁵ Blakeslee, A. F., and Avery, A. G., *J. Hered.*, **28**, 393 (1937).
- ²⁶ Levan, A., *Hereditas*, **24**, 471 (1938).
- ²⁷ Schmuck, A., *C.R. Acad. Sci. U.R.S.S.*, **19**, 189 (1938).
- ²⁸ Ostergren, G., and Levan, A., *Hereditas*, **29**, 496 (1943).
- ²⁹ Ostergren, G., *Hereditas*, **30**, 429 (1944).
- ³⁰ Barber, H. N., and Callan, H. G., *Proc. Roy. Soc., B*, **131**, 258 (1943).
- ³¹ Lettré, H., F.I.A.T. Report of German Science: Bioch. II, 139.
- ³² Dixon, M., *Symp. Biochem. Soc.*, **2** (1948).
- ³³ Dustin, P., *C.R. Soc. Biol.*, **142**, 1433 (1948).
- ³⁴ Haynes, L. J., *Quart. Rev. Chem. Soc.*, **2**, 46 (1948).
- ³⁵ Posner, T., *Ber.*, **35**, 799 (1902).
- ³⁶ Goldacre, E. J., Loveless, A., and Ross, W. C. J., *Nature*, **163**, 667 (1949).
- ³⁷ D'Amato, F., *Nuovo Giornale Bot. It.*, **54**, 1 (1948).
- ³⁸ Ross, W. C. J., *J. Chem. Soc.*, **43**, 183 (1949).
- ³⁹ Haddow, A., Kon, G. A. R., and Ross, W. C. J., *Nature*, **162**, 824 (1948).
- ⁴⁰ Pirie, A., *Biochem. J.*, **41**, 185 (1947).
- ⁴¹ Herriott, R. M., Anson, T. L., and Northrop, J. H., *J. Gen. Physiol.*, **30**, 185 (1946).
- ⁴² Elmore, D. T., Gulland, (the late) J. M., Jordan, D. O., and Taylor, H. F. W., *Biochem. J.*, **42**, 308 (1948).
- ⁴³ Herriott, R. M., *J. Gen. Physiol.*, **32**, 221 (1948).
- ⁴⁴ Speakman, J. B., Fibre Science, The Textile Institute, Manchester, 299 (1949).
- ⁴⁵ Werner, E. G. G., and Farenhorst, E., *Rec. Trav. Chim.*, **67**, 438 (1948).
- ⁴⁶ Werner, E. G. G., *Rec. Trav. Chim.*, **67**, 446 (1948).
- ⁴⁷ Woodward, R. B., *J. Amer. Chem. Soc.*, **71**, 758 (1949).
- ⁴⁸ Steinegger, E., and Levan, A., *Hereditas*, **33**, 385 (1947).
- ⁴⁹ Steinegger, E., and Levan, A., *Hereditas*, **34**, 194 (1948).
- ⁵⁰ Windaus, A., *Ann.*, **439**, 59 (1924).
- ⁵¹ Dewar, M. J. S., *Nature*, **155**, 141 (1945).
- ⁵² Cook, J. W., and Somerville, A. R., *Nature*, **163**, 410 (1949).
- ⁵³ Erickson, R. O., *Science*, **108**, 533 (1948).

BIRD BEHAVIOUR

ON September 6, Section D (Zoology) of the British Association held at Newcastle upon Tyne a symposium on the subject of bird behaviour.

Mr. P. H. Hartley, speaking on "Routines in Bird Life", gave an account of some activities concerned in the maintenance of the individual bird. The value of such studies is three-fold: they are part of the basic ethology of birds; they provide the setting in which more complex actions must be studied and compared; and they throw light on certain ecological problems which are difficult of direct attack. In illustration of daily routines in feeding activity, herring gulls *Larus argentatus* in west Cornwall have three feeding periods in the day in the second half of September, separated by two resting periods; in the second half of October there are two feeds separated by a single rest. Blackbirds *Turdus merula* in Berkshire in winter feed all through the day, but are especially active in the hours after sunrise. The study of the feeding routines of two congeneric species, chaff-chaff *Phylloscopus collybita* and willow warbler *Ph. trochilus*, living in one wood showed that the chaff-chaffs forage consistently higher than willow warblers; this implies that the two species are not in close competition, although living on the same ground. The discovery of the same foods in two species does not necessarily imply competition between them. The different titmice of the genus *Parus* show ecological isolation, each species having its own feeding levels. The fact that any breakdown of species segregation is explicable by the occurrence of a temporarily superabundant food (such as caterpillars in early summer, and nuts or wild fruits in autumn) suggests that the possession of a separate feeding niche by each species at other times points to a genuine need to avoid interspecific competitions.

Mr. Hartley also gave examples of variations in routines of song utterance. In discussing the work of D. R. Barber on the song of the chaffinch *Fringilla caelebs* (see *Nature*, **161**, 277; 1948), he pointed out that any study of local variations in phrase length would be stultified if allowance were not made for the stage in the annual song cycle.

In his survey "Some Aspects of the Relationship between the Pair Bond and Breeding Economy of Birds", the Rev. E. A. Armstrong urged the need for an integrative approach to the study, in which psychological, ethological, morphological and physiological factors should be equally considered. He pointed out that at the time of the formation of the pair bond, there takes place a revolutionary change in the approach of each bird to another individual. Hostility or indifference must be replaced first by tolerance and later by co-operation, often with a fixed allotment of the roles in the breeding cycle.

When both sexes of a species are conspicuous, both share in the rearing of the young; and when there is colonial nesting the bright colours of the adults may function as a warning. In such colonial species as are conspicuous in coloration, it is almost inevitable that monogamy should be the rule of life, as there must be one parent always on duty at the nest. An unattended nest in a conspicuous colony offers an invitation to the raids of predators. In species wherein the male is conspicuous and the female cryptically coloured, the nest is hidden, and the hen usually bears the major part of the care of eggs or young. In such cases the species may be promiscuous or polygynous. In promiscuous species a 'lek' is used