Studies on Abnormal Mitosis induced in Chick Tissue Cultures by Mustard Gas ($\beta\beta'$-Dichlordiethyl Sulphide)

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With three Plates

INTRODUCTION

That mustard gas causes structural changes in chromosomes was first shown indirectly by the important genetical experiments of Auerbach and Robson (1944, 1946, 1947), who found that by exposing adult male Drosophila to mustard-gas vapour a wide range of mutations was produced. Direct cytological evidence was provided by Roller (1947), who demonstrated various types of chromosomal abnormality in the pollen grains of Tradescantia after treatment with different concentrations of the vapour. These observations have recently been elaborated and extended by Darlington and Koller (1947).

In work done during the war for the Chemical Defence Research Department of the Ministry of Supply, Fell and Allsopp (1948) noticed profound disturbances of the mitotic process in the cells of tissue cultures growing in a medium containing 5–100 $\gamma$/c.c. of mustard gas. Later they found similar abnormalities in the regenerating epidermis of mice treated with repeated applications of minute quantities of the agent. Mitotic abnormalities have also been described by Gillette and Bodenstein (1946) and Bodenstein (1947) in amphibian embryos treated with a nitrogen mustard compound.

Recent optical developments have made it possible to study cell division in living material in much greater detail than hitherto. Hughes and Swann (1948) investigated the anaphase movement in cultures of normal chick osteoblasts, using both phase-contrast and polarized-light microscopy, and are accumulating more information about the mitotic spindle by these methods. It seemed desirable to extend this work with similar observations on different types of abnormal mitosis induced by chemicals and other agents, in the hope that the results might shed further light on the physiology of normal cell division.

The extensive literature on the pathology of mitosis has been reviewed by Politzer (1934). Abnormal mitoses in tissue cultures have been produced by
very varied experimental means. Among the most notable contributions to this subject are the beautiful direct observations of reversible changes in living mitotic cells \textit{in vitro} made by M. R. Lewis (1923, 1933a and b, 1934). The effect on dividing cells in culture of many different chemicals has been studied: various acids (M. R. Lewis, 1923; Bauer, 1923), reduced neutral red (M. R. Lewis, 1923), carbon dioxide (Mottram, 1928), ether (Kemp and Juul, 1930; Rosenfeld, 1932), potassium iodide (Razzesi, 1932), ammonia (Rosenfeld, 1933), auramine, urethane, methyl sulphonal, sodium cacodylate, colchicine and some of its derivatives, quinine, atropine, aconitine (Ludford, 1936), and of certain carcinogens and related hydrocarbons (Hearne Creech, 1939). The action of heat (Kemp and Juul, 1930; M. R. Lewis, 1933a), of hypotonic culture medium (M. R. Lewis, 1934), and of radiation (Strangeways and Oakley, 1923; Strangeways, 1924a and b; Lasnitzki, 1943; and others) have also been investigated. Abnormal mitosis may be produced merely by the addition of stale plasma to the culture medium (Strangeways, 1924). Cinema films of pluripolar mitosis \textit{in vitro} have been made by W. H. Lewis (1932) and by G. Gey (1947).

The observations described in the present paper refer to cells growing in a medium containing low concentrations of mustard gas (pure $\beta\beta'$-dichlorodiethyl sulphide). The results were obtained partly by the analysis of cinema films of the living cells made by phase-contrast microscopy, and partly from the cytological study of fixed and stained cultures; these two methods of approach were found to be complementary, and each demonstrated features of abnormal mitosis not shown by the other.

A. H. was responsible for the cinematography and the quantitative data obtained from the films and H. B. F. for the tissue culture and the observations on fixed material. The general analysis of the films and their interpretation were the joint work of both authors.

**MATERIAL AND METHODS**

\textit{Tissue Culture.} Fragments of the frontal bones from 11- to 12-day fowl embryos were cultivated in a mixture of equal parts of fowl blood-plasma and tissue extract made with Tyrode from 11- to 12-day chick embryos. The explants were grown in hanging drop preparations on 1 in. square No. 2 coverslips over $3 \times 1\frac{1}{2}$ in. hollow-ground slides.

The cultures were incubated for 2-3 days, by which time the original bone fragment had become surrounded by a halo of migrating cells consisting mainly of osteoblasts. The tissue was then transferred to medium containing mustard gas in one of three concentrations: 12.5, 25.0, and 50.0 $\gamma$/c.c. The agent was introduced into the culture medium in the following way. A solution of mustard gas in absolute alcohol was prepared and a small quantity was added to a known volume of plasma in such a way that the plasma contained double the amount of the agent that was required for the final culture medium. A drop of this plasma solution was then placed on a No. 1 coverslip and mixed with an equal drop of embryo extract. The explant was transferred
to the mixture before it clotted, and for cinematography the coverslip was mounted on a special type of culture vessel; the application of phase-contrast illumination to tissue cultures and the culture chamber devised for this purpose have been described elsewhere (Hughes and Swann, 1948). Cultures required for fixation and staining were mounted on hollow-ground slides in the usual way. All the preparations were incubated for 24-48 hours before use.

In one experiment the explants were transferred to normal medium, incubated for 24 hours, then opened, and a drop of serum containing 50 γ/c.c. of mustard gas was deposited on the tissue. After this treatment the cultures were mounted on the special chambers mentioned above and examined either immediately or after 24 hours’ further incubation.

Cinematography. The phase-contrast objective (X 95) and condenser used for this work were supplied by Messrs. Cooke, Troughton & Simms, Ltd. (see Hughes and Swann, 1948). For the photomicrography of living cells in tissue cultures this apparatus should be used in conjunction with sensitized film of maximum contrast. This is particularly important for cells treated with mustard gas in which the contrast of the chromosomes is much below normal, probably owing to a diminished content of nucleic acid.

After many different types of film had been tried with the generous collaboration of the research staff of Messrs. Kodak, Ltd., a special 16-mm. negative of extremely high contrast was chosen, known as ‘film for ciné-photomicrography’. The contrast in the photograph is greater than in the direct image, so that the cellular detail can be seen much better in a print than by direct observation through the microscope. The use of this film in the study of living cells will be described elsewhere.

The ciné records were studied exhaustively by projection in both directions, by examination frame by frame, and by comparing paper enlargements of selected series of frames. The sequences described below were analysed by means of all three methods. The rates of anaphase movement were obtained by measuring the distances between the daughter chromosome groups in successive frames projected on paper (Hughes and Swann, 1948).

Fixation and Staining. In most of the experiments some of the cultures were used for ordinary cytological study. They were fixed for 4-5 min. in Maximow’s solution (10 parts Zenker’s fluid: 1 part formol: 1 part 2 per cent. osmium tetroxide) freshly prepared for each occasion, washed overnight in distilled water, then treated with alcoholic iodine and washed with 70 per cent. alcohol. Some of the cultures were stained by Feulgen’s method. Others were hydrolysed for 8 min. at 60° C. as for the Feulgen technique and then stained for 10-15 min. in well-ripened Ehrlich’s haematoxylin; this method gave a very clear picture of the cells and of the chromosome structure and was particularly suitable for photography; the distribution of the stain was precisely the same as with the Feulgen technique. Preparations stained with haematoxylin without previous hydrolysis were much inferior in clarity to the hydrolysed specimens. The stained cultures were dehydrated, cleared, and mounted whole in Canada balsam.
Hughes and Fell—Studies on Abnormal Mitosis induced in

For certain purposes fixation for 3-4 min. in Zenker's fluid without acetic acid followed by hydrolysis and staining with Ehrlich's haematoxylin was useful. This method was particularly suitable for demonstrating the smaller micronuclei in the many multi-nucleate cells which mustard gas produced in the cultures (Pl. III, fig. 31); it also rendered the spindle of mitotic cells very distinct (Pl. III, fig. 30).

RESULTS

Normal Mitosis as seen by Phase-contrast Microscopy

In normal living osteoblast cultures the intermitotic cell (Pl. I, fig. 3) is much flattened and usually of triangular or spindle-shaped outline; the oval nucleus, which is rather paler than the cytoplasm, contains one or more irregular nucleoli which appear nearly black. In the cytoplasm are seen the dark mitochondrial filaments, many small granules, and the highly refractile fat globules. Both the cell and its contents are in continual slow movement.

When prophase begins the cytoplasmic processes are largely withdrawn, the nucleoli vanish, and faintly grey, diffuse chromosomes materialize throughout the nuclear area. At the same time the nuclear membrane disappears, but cytoplasmic inclusions remain outside the nuclear area until late anaphase. The chromosomes contract, become increasingly distinct, and assume a radial orientation in the plane of the coverslip (Pl. I, fig. 1a); they probably lie in this plane as the mechanical result of the flatness of the cell. Whether the spindle has already begun to form is not yet known, and since it is therefore uncertain whether this stage should be regarded as late prophase or early metaphase, we have termed it the radial stage.

As the cell becomes more nearly spherical, the chromosomes rotate from the plane of the coverslip to one at right angles to it, presumably under the influence of the spindle elements, but in normal cells the spindle is indistinct with phase-contrast microscopy and the details of its formation cannot be seen. During metaphase (Pl. I, fig. 1b) the chromosomes move to and fro in the equatorial region of the spindle with unsynchronized linear motion (W. H. Lewis, 1939; Hughes and Swann, 1948). Without warning the chromatids suddenly separate and pass quickly to opposite poles (Pl. I, fig. 1c, d). Details of the anaphase movement have been described by Hughes and Swan (1948).

About 3 min. after the beginning of anaphase, the granules and mitochondria of the surrounding cytoplasm bulge into the inter-zonal region and constriction into daughter cells begins. 'Bubbling' of the peripheral cytoplasm, spreading from the poles to the equator (cf. Chambers, 1938), may occur at any stage of mitosis but always becomes increasingly vigorous during telophase (Pl. I, fig. 1e). The contrast of the chromosomes falls during telophase so that usually nothing is clearly visible in the nuclear area until 12-20 min. after anaphase, by which time the nuclear membrane and nucleoli are present and the daughter cells are flattening (Pl. I, fig. 1f). A connecting thread of cytoplasm persists for a very variable time and then snaps.
Chick Tissue Cultures by Mustard Gas

The stages of normal mitosis as they appear in fixed and stained cultures are shown in Pl. III, figs. 12-17.

The Effect of Mustard Gas on Mitosis

I. General Effects on the Cells (Table 1)

In living cells grown in the presence of small quantities of mustard gas and examined by phase-contrast microscopy, the cytoplasmic structures and particularly the mitochondria are abnormally distinct, but, as stated above, the contrast of the chromosomes is subnormal. Owing to the high contrast of the negative used, however, the general form of the chromosomes can be distinguished in cinema films of the living cells, but suitably fixed and stained preparations are required for a more precise study of chromosome structure.

At the higher concentrations of mustard gas the volume of the cell, as judged by its surface area, is increased. The fat content, even after 48 hours' cultivation, is abnormally low at all three concentrations, and the cytoplasm may be free of all but the smallest lipoid granules. These small lipoid granules are seen to be in Brownian movement by direct observation, whereas in the normal cell movement can only be directly appreciated in a minority of the globules. From this it may be inferred that the water-content of the cells treated with mustard gas is abnormally high and the viscosity of the cytoplasm relatively low.

The degree of mitotic disturbance caused by the presence of mustard gas in the culture medium varies in the same culture from a slight deviation from the normal to great irregularity. The proportion of extreme abnormalities is naturally much larger at the two higher concentrations, but even at the 12.5 γ/cc. level a few greatly distorted mitotic figures are seen (Pl. III, fig. 28).

Three main types of abnormal mitosis may be distinguished, which are bipolar, tripolar, and apolar, respectively; they will be considered in detail in the next section.

II. Observations on the Three Main Types of Abnormal Mitosis

1. Bipolar Mitosis. At the lowest concentration of mustard gas (12.5 γ/cc.), some mitoses are nearly normal. This is illustrated by one of the ciné records:

   Record 1 (Pl. I, fig. 2). In this film mitosis, and in particular the radial stage, is unduly prolonged (see Table II), but otherwise division proceeds normally.

   The abnormal bipolar mitoses may be divided arbitrarily into two groups, in one of which (group A) the abnormality is much less extreme than in the other (group B); in life the dividing cells of group B are usually larger than those of group A.

   Mitoses of group A are characterized by the delayed arrival of certain chromosomes at the equatorial plate or their failure to reach it (Pl. III, figs. 18 and 19), by lag at anaphase and telophase (Pl. III, figs. 20-22), and by the formation of one or more micronuclei derived from the lagging chromosomes in addition to two daughter nuclei of nearly normal size (Pl. III, figs. 23 and 24).
**TABLE I. Summary of Observations, mainly from Cinema Records, of Mitotic Abnormalities produced by Mustard Gas**

<table>
<thead>
<tr>
<th>Number of examples recorded by cinemas</th>
<th>Nearly normal</th>
<th>Bipolar</th>
<th>Triplet</th>
<th>Apolar</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5 y/c.c.: 3</td>
<td>12.5 y/c.c.: 3</td>
<td>12.5 y/c.c.: 3</td>
<td>12.5 y/c.c.: 3</td>
<td>12.5 y/c.c.: 3</td>
</tr>
<tr>
<td>25.0 y/c.c.: 2</td>
<td>25.0 y/c.c.: 2</td>
<td>25.0 y/c.c.: 2</td>
<td>25.0 y/c.c.: 2</td>
<td>25.0 y/c.c.: 2</td>
</tr>
<tr>
<td>50.0 y/c.c.: 1</td>
<td>50.0 y/c.c.: 1</td>
<td>50.0 y/c.c.: 1</td>
<td>50.0 y/c.c.: 1</td>
<td>50.0 y/c.c.: 1</td>
</tr>
<tr>
<td>(in serum)</td>
<td>(in serum)</td>
<td>(in serum)</td>
<td>(in serum)</td>
<td>(in serum)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Illustrations</th>
<th>Pl. I, figs. 2, 4, 5</th>
<th>Pl. III, figs. 18-24</th>
<th>Pl. I, fig. 6</th>
<th>Pl. II, figs. 9, 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration</td>
<td>4 cells: N.</td>
<td>2 cells: prolonged</td>
<td>4 cells: prolonged</td>
<td>1 cell: prolonged</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Metaphase</td>
<td></td>
<td>Fig. no.</td>
<td></td>
<td>Fig. no.</td>
</tr>
<tr>
<td>Duration</td>
<td>3 cells: prolonged</td>
<td>Prolonged up to twice</td>
<td>4 cells: prolonged</td>
<td>1 cell: R.I.</td>
</tr>
<tr>
<td>Delay or failure in reaching metaphase plate</td>
<td>1 cell: N.</td>
<td>N.</td>
<td>2 cells: R.I.</td>
<td>1 cell: R.I.</td>
</tr>
<tr>
<td>Linear movements on spindle</td>
<td>+</td>
<td>+</td>
<td>1 cell: +</td>
<td>+</td>
</tr>
<tr>
<td>Condition of chromosomes</td>
<td>N.</td>
<td>N. or slightly beaded</td>
<td>6, 8</td>
<td>1 cell: R.I.</td>
</tr>
<tr>
<td>Movement of anaphase groups</td>
<td>N. rate</td>
<td>N. rate</td>
<td>6, 8</td>
<td>1 cell: N. rate</td>
</tr>
<tr>
<td>Lag</td>
<td>2 cells: +</td>
<td>5 cells: + +</td>
<td>9</td>
<td>No recognizable anaphase</td>
</tr>
<tr>
<td></td>
<td>1 cell: -</td>
<td>1 cell: -</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Type of cleavage</td>
<td>2 daughters</td>
<td>2 daughters</td>
<td>2 daughters</td>
<td>1 cell: 3 daughters</td>
</tr>
<tr>
<td>Rate of cleavage</td>
<td>N.</td>
<td>About half N. rate</td>
<td>About half N. rate</td>
<td>1 cell: at first 3 daughters</td>
</tr>
<tr>
<td>Cytoplasmic streaming</td>
<td>N.</td>
<td>N.</td>
<td>4 cells: + +</td>
<td>About half N. rate</td>
</tr>
<tr>
<td>Daughter nuclei</td>
<td>2 of N. size</td>
<td>2 of N. size</td>
<td>Several nuclei of various sizes</td>
<td>6, 8</td>
</tr>
<tr>
<td></td>
<td>2 of N. size +</td>
<td>micronuclei</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 cell: 2 of N. size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Telophase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Some of the chromosomes appear normal in stained preparations, but in others the nucleic acid charge is localized in granules, the intergranular material being nearly colourless when stained by Feulgen's method or by Ehrlich's haematoxylin (Pl. III, fig. 20). Some interesting features of these mitotic abnormalities are seen in two of the ciné records (Records 2 and 3).

TABLE 2. Showing Phase Times in Minutes of Bi- and Tri-polar Mitoses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Type of division</th>
<th>Pre-phase</th>
<th>Meta-phase</th>
<th>Early cleavage</th>
<th>End of cleavage</th>
<th>Nucleoli in daughter cells</th>
<th>Record no. in text</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 controls</td>
<td>Normal</td>
<td></td>
<td>c. 4</td>
<td>2:3-5:0</td>
<td>3:5-6:0</td>
<td>11-20</td>
<td>..</td>
<td></td>
</tr>
<tr>
<td>12.5 γ/cc.</td>
<td>N.N.</td>
<td>&gt; 2:4</td>
<td>9:4</td>
<td>3:6</td>
<td>7:0</td>
<td>13:2</td>
<td>..</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N.N.</td>
<td></td>
<td>3:0</td>
<td>6:8</td>
<td>13:4</td>
<td></td>
<td>..</td>
<td></td>
</tr>
<tr>
<td>25.0 γ/cc.</td>
<td>N.N.</td>
<td>&gt; 6:7</td>
<td>3:4</td>
<td>6:2</td>
<td>9:1</td>
<td></td>
<td>..</td>
<td></td>
</tr>
<tr>
<td>50.0 γ/cc. (serum)</td>
<td>N.N.</td>
<td>&gt; 4:7</td>
<td>9:9</td>
<td>6:1</td>
<td>9:4</td>
<td></td>
<td>..</td>
<td>Nucleoli still present when record begins</td>
</tr>
<tr>
<td>12.5 γ/cc.</td>
<td>A</td>
<td>&gt; 36:6</td>
<td>6:4</td>
<td>9:6</td>
<td>18:2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td></td>
<td>4:8</td>
<td>9:6</td>
<td>18:2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.0 γ/cc.</td>
<td>B</td>
<td>12:0</td>
<td>..</td>
<td>5:8</td>
<td>8:4</td>
<td>13:0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td></td>
<td>8:4</td>
<td>8:4</td>
<td>13:0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50.0 γ/cc. (serum)</td>
<td>B</td>
<td>13:0</td>
<td>3:2</td>
<td>5:5</td>
<td>13:9</td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td></td>
<td>3:2</td>
<td>5:5</td>
<td>13:9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.0 γ/cc. (serum)</td>
<td>Tripolar</td>
<td>&gt; 18:8</td>
<td>9:0</td>
<td>10:7</td>
<td>17:2</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9:0</td>
<td>10:7</td>
<td>17:2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Record 2** (Pl. I, fig. 4). This cell shows a simple lag of chromosomes at anaphase within the spindle area; it finally divides into two daughter cells, one of which contains a single nucleus, while the other forms one nucleus of nearly normal size and two micronuclei.

**Record 3** (Pl. I, fig. 5). The cell is in prophase when the film begins. Most of the chromosomes pass to the equator in the normal way, but two fail to join the others and remain near one pole of the spindle (Pl. I, fig. 5b). Within 2 min., however, the tardy chromosomes are drawn to the equator (Pl. I, fig. 5c) and soon afterwards anaphase begins. Several chromosomes on each side of the metaphase plate do not divide and separate with the rest, but lag behind at the equator (Pl. I, fig. 5d). As the peripheral cytoplasm bulges into the interzonal region preparatory to cleavage, the lagging chromosomes from each side are pushed towards each other and are finally brought together by the equatorial constriction (Pl. I, fig. 5e); this lateral form of lag will be discussed in more detail below. After anaphase the fate of the laggards is obscured by the bubbling of the cytoplasm. Eventually one daughter cell is seen to contain a single large nucleus and the other one large nucleus and a micronucleus.
The divisions of group B are much more abundant at the higher concentrations than at the 72.5 γ/c.c. level and the mitotic abnormality is an exaggeration of that described above in group A. The spindle seems relatively normal, but in stained preparations the chromosomes appear very deficient in nucleic acid and have not contracted properly; there is very pronounced lag at anaphase and the daughter cells contain more micronuclei than are formed in the divisions of group A.

In prophase (examined in fixed preparations only) the filaments are somewhat vaguely defined and stain lightly as compared with the normal; they often vary in thickness along their length and are sometimes beaded; they may be unevenly distributed in the nuclear area and in places entwined to form long, tangled, granular skeins (Pl. III, cf. figs. 12 and 25). At metaphase (Pl. III, figs. 26 and 27) the chromosomes are seen to vary enormously in size and appearance, some being minute granules, others long, beaded, and often attenuated filaments; in Feulgen preparations, the beads on the long chromosomes are Feulgen-positive while the rest of the thread is nearly colourless, indicating severe nucleic acid deficiency. Other chromosomes have an irregular outline and the Feulgen-positive material is aggregated in lumps here and there on the nearly unstained thread. Some chromosomes may fail to reach the equatorial plate and lie in the cytoplasm.

In many of the cells at metaphase most of the chromosomal material is aggregated into one or more large tangled skeins (Pl. III, figs. 26 and 27) similar to, but usually larger and more compact than, those sometimes seen at prophase (Pl. III, fig. 25). The filamentous structure of these masses varies in distinctness in different cells; in some they are quite clearly composed of entwined, beaded threads, while in others they appear as almost homogeneous bodies. These masses may lie on each side of the spindle at the equator or freely in the cytoplasm (Pl. III, fig. 27). Sometimes the large chromatinic masses move to the surface of the cell, where a strictly localized bubbling of the cytoplasm takes place (Pl. I, fig. 6a-c; Pl. III, fig. 27).

Anaphase (Pl. III, fig. 28) is characterized by a pronounced equatorial lag, usually of several chromosomes, though the two main daughter groups pass to the poles at the normal rate (Text-fig. 1). This failure of individual chromosomes to move normally to the poles of the spindle is of two types: a simple median lag, usually of small chromosomes, within the spindle, and a lateral lag, to which reference has already been made, which is associated with a somewhat diffuse structure of the metaphase plate, especially when the chromosomes are incompletely contracted. In the latter types, in which the equatorial plate is disproportionately large for the spindle, the long lateral chromosomes and sometimes large chromosomal masses (Pl. III, fig. 28) fail to move during anaphase and remain flanking the interzonal region, the interior of which is clear except for the small chromosomes undergoing median lag (Pl. II, fig. 8b–e).

When cleavage begins, constriction of the interzonal region pushes the mitochondria and the lateral lagging chromosomes into an axial position, mid-
way between the groups of daughter chromosomes; here they remain, until
the completion of cleavage incorporates them in one or other of the daughter
cells where they form micronuclei (Pl. II, fig. 8e-g). Such lagging filamentous
chromosomes often produce a bridge of chromatin uniting the two daughter
cells, and expanding at either end into an oblong or pear-shaped nucleus.
Sometimes this bridge is surprisingly long and attenuated (Pl. III, fig. 29)
indicating considerable plasticity of the chromosomes.

The multinucleate condition of the daughter cells in group B is not always
due entirely to the lagging chromosomes. In some cells the daughter chromo-
somes of the two anaphase groups are very loosely arranged (Pl. III, fig. 28)
and instead of forming a single large nucleus, give rise to a nest of micronuclei;
there is some evidence both from the films and from fixed material that there
may be secondary fusion in such nests, so that one or more larger nuclei
are later formed. It is probable that chromosomal material which fails to
reach the spindle at metaphase (Pl. III, fig. 27) also forms micronuclei at
telophase.

Most of the phenomena described above are illustrated by two of the film
records (Nos. 4 and 5).

Record 4 (Pl. II, fig. 8). The cell is in metaphase when photography begins.
During anaphase a group of chromosomes moves to each pole in the usual
way, but several chromosomes, in some of which a beaded structure is very
distinct, lag behind (Pl. II, fig. 8c). When the cell constricts at cleavage,
chromosomes previously lateral to the spindle and possibly not incorporated
in the metaphase plate, together with some of the lagging chromosomes men-
tioned above, are pushed into an axial position (Pl. II, fig. 8f, e). Thus at
telophase there are three groups of chromosomes in course of nuclear recon-
struction—one equatorial and two polar (Pl. II, fig. 8f). Each polar group
forms a single oval nucleus of normal size and appearance which moves
freely in the cytoplasm. The equatorial group, however, produces a bouquet
of small pear-shaped nuclei in one daughter cell (Pl. II, fig. 8g), which are
unable to move freely until liberated from their attachment to the interzonal
strand by the completion of cleavage. Finally, one daughter cell is seen to be
mononucleate, while the other contains one nucleus of normal size and several
micronuclei derived from the interzonal group.

Record 5 (Pl. I, fig. 6). In this cell both the spindle and the chromosomes
are very distinct. When the record begins, there are several large, irregular
granular masses and small chromatinc granules and filaments distributed over
the spindle (Pl. I, fig. 6a). The smaller structures display very active, un-
synchronized linear movement up and down the spindle, but the larger masses
drift outwards, apparently under the influence of cytoplasmic currents. The
filaments alternately stretch and retract as they move up and down, and the
larger chromosomal bodies continually change shape. Some of the larger
masses, though lying in the cytoplasm, retain their connexion with the spindle,
but the largest body moves right out of the spindle area and slowly works its
way to the surface of the cell, where an active and strictly localized bubbling
begins (Pl. I, fig. 6a-c). A small cytoplasmic protuberance is formed at this point into which the chromosomal body passes (Pl. I, fig. 6c). Metaphase was enormously prolonged and anaphase did not take place until 135 min. after the beginning of observation.

The rate of anaphase movement is normal, but the groups of daughter chromosomes represent a relatively small proportion of the total chromatin content of the cell (Pl. I, fig. 6e). Much of the material is incorporated in the large irregular bodies described above; other chromosomal structures remain scattered near the poles of the spindle, never having reached the equatorial plate, while others again lag behind during anaphase. When cleavage begins both daughter cells show a spiral streaming of the cytoplasm which is much more active in one cell than in the other. Eventually this mitosis produces two daughter cells, each containing many medium-sized and small nuclei (Pl. I, fig. 6f, g).

2. Tripolar Mitosis. In tripolar mitosis there may be a typical triradiate metaphase (Pl. II, fig. 9a and Pl. III, fig. 30), or a recognizable metaphase may be entirely omitted, the cell passing straight from the radial stage to a tripolar anaphase and telophase (Pl. II, fig. 10). An example of both types of tripolar division was recorded by the cinema (Records 6 and 7).

Record 6 (Pl. II, fig. 9). A regular triradiate metaphase with active linear movement up and down the spindle axes is seen. Not all the chromosomes are incorporated in the spindle area, many being scattered throughout the surrounding cytoplasm. Suddenly the cell enters anaphase (Pl. II, 9b) and a group of chromosomes passes to each of the three poles, leaving a fourth group of laggards moving irregularly in the centre of the cell (Pl. II, fig. 9c). Meanwhile the outline of the cell becomes triangular and the cytoplasm begins to bubble. Bubbling increases in violence and the cell divides into three multinucleate daughters (Pl. II, fig. 9d-e).

Record 7 (Pl. II, fig. 10). This shows a much less regular tripolar division. An incomplete ring of chromosomes (Pl. II, fig. 10a) in active radial movement passes directly into a tripolar anaphase (Pl. II, fig. 10b), without forming a recognizable metaphase. All the chromosomal material seems to be in the form of granules; some are double and the two constituents are pulled apart at anaphase. Telophase, with violent bubbling and streaming of the cytoplasm, produces three daughter cells united by narrow bridges (Pl. II, fig. 10c), but before cleavage is complete two of the daughter cells reunite, so that finally one large and one smaller cell are formed, both of which are multinucleate (Pl. II, fig. 10d).

3. Apolar Mitosis. In mitosis of the apolar type there is no recognizable metaphase or anaphase. In some cells the chromosomal material is in the form of distorted filaments and rods, but in others most of it appears as granules (Pl. III, fig. 32), some of which are fairly large but others so small as to be only just visible. These dust-like particles are often arranged in radiating lines, but it is impossible to see whether they are discrete bodies or minute beads on a continuous unstained thread. Usually these cells also
contain one or more much larger chromatinic bodies, in some of which a closely tangled filamentous structure is distinguishable in fixed preparations while others appear as homogeneous globules (Pl. III, fig. 32). This modified radial stage may continue for a fairly long time, to be succeeded by a phase of nuclear reconstruction in which the scattered chromosomal material forms a host of nuclei of widely varying sizes (Pl. III, fig. 31). There is no cleavage of the cytoplasm.

Cinema films were made of three cells of this type, two of which are described below (Records 8 and 9):

Record 8 (Pl. II, fig. 11). When the film begins, radially arranged granules of chromatin are seen in active radial movement. Suddenly this scattered group of chromosomal bodies is churned round by a rapid spiral streaming of the cytoplasm which continues for a short time and then gradually subsides. The cell begins to flatten and a number of nuclei develop (Pl. II, fig. 11d). Finally, a single large multinucleate cell is formed (Pl. II, fig. 11e); one nucleus is much larger than the rest and appears to be the fusion product of several smaller nuclei.

Record 9 (Pl. I, fig. 7). In this cell the chromosomal material is very dispersed (Pl. I, fig. 7a), but two large lumps of chromatin are clearly seen at one stage (Pl. I, fig. 7b). At first the cell is fairly quiescent, then an active spiral movement of the cytoplasm, associated with violent bubbling, begins (Pl. I, fig. 7b); this commotion gradually dies away and a single cell results in which about 17 nuclei were counted in life (Pl. I, fig. 7c).

III. Time Relations in Abnormal Mitosis (Table 2).

The duration of each mitotic phase in cells dividing under the influence of mustard gas was measured from the photographic records. The figures obtained, with comparable data from normal material, are presented in Table 2.

The most sharply defined stages in mitosis are the beginning of anaphase and the end of cleavage; the transition from the radial stage to metaphase is more gradual but can usually be estimated to within half a minute. Photography began either at prophase or metaphase, and the duration of the phase in which the cell was first observed is indicated as more than (> ) the recorded time. The late phases of chromosome division are reckoned from the beginning of anaphase; the beginning of cleavage is defined as the constriction inside the cell of the interzonal region of the spindle or, when this is not visible, as the first appearance of an external cleavage furrow.

In general, mitosis is prolonged by the influence of mustard gas, and prophase (Pl. I, fig. 2) and metaphase (Pl. I, fig. 6) in particular may be greatly protracted. The maximum duration of a prophase that was known to be succeeded by a metaphase was >36·6 min. Twice we followed what appeared to be a prophase for 78 min. (12·5 γ/c.c. mustard gas) and 144 min. (50 γ/c.c.) respectively, but during these periods the cells did not enter metaphase and observation was discontinued.
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The maximum duration of a metaphase that we have recorded was \( >135 \) min. (Pl. I, fig. 6), a period several times as long as that of any other metaphase that we have studied. Such an extreme prolongation seems to be exceptional.

Anaphase proceeds at the normal rate. Curves of anaphase movement plotted against time were obtained from our cinema records (Text-fig. 1); when compared with curves from similar records of normal osteoblast cultures (Hughes and Swann, 1948) they are seen to fall within the normal range as regards rate of movement, distance traversed by the chromosomes, and general shape. At the higher dosage levels, however, the curves tend to move towards the lower limit of the normal range. The curves in the text-figure do not, of course, refer to the lagging chromosomes, which either remain stationary or move more slowly than the others.

Cleavage is somewhat, but not greatly, protracted, but the reconstruction of daughter nuclei, as indicated by the interval between the beginning of anaphase and the first appearance of nucleoli, is not greater than normal and sometimes may possibly even be less. Comparison with the corresponding period in the division of normal cells, however, is complicated by the fact that reconstruction of the daughter nuclei is less obscure in the
treated than in the untreated cells, so that possibly the formation of nucleoli is visible at an earlier stage in the abnormal mitoses.

A normal rate of chromosome movement at anaphase with a somewhat prolonged cleavage are also found in cells growing in normal medium, but subjected to the intense illumination necessary for polarized light microscopy

TEXT-FIG. 2. Measurements of the outward displacement of chromosomes in a tripolar mitosis in an osteoblast culture treated with 29 γ/c.c. mustard gas (Record 6). The three symbols refer to each of the three half-spindles. The continuous curve is the average displacement of one group of daughter chromosomes in eight normal osteoblast mitoses.

(Hughes and Swann, 1948), or to γ-radiation, as well as in cultures treated with other chemicals (Hughes, unpublished).

As stated above, we have one cinema record of a fairly orderly anaphase movement in a tripolar mitosis (Pl. II, fig. 9). In this cell a group of chromosomes remains in the centre, and the distance between this central group and each of the three daughter groups during anaphase was plotted (Text-fig. 2). It will be seen that the rate of movement of each chromosome group in the tripolar anaphase corresponds roughly with that of a single daughter group in a normal bipolar anaphase.
DISCUSSION

The effects of mustard gas on mitosis in vitro are not specific. Such minor abnormalities as failure of one or more chromosomes to reach the equatorial plate, slight lag at anaphase, and the production of one or two micronuclei, are produced by many mildly unfavourable conditions. The more severe abnormalities described above most nearly resemble those resulting from irradiation (Strangeways and Oakley, 1923; Strangeways, 1924b; Lasnitzki, 1943), though they are also rather similar to those caused by ammonia (Rosenfeld, 1933).

Some of the phenomena we have described are essentially the same as those observed by Koller (1947) and Darlington and Koller (1947) in the pollen grains of Tradescantia treated with mustard gas, though mitosis seems to have attained a more extreme abnormality in the tissue cultures than in Tradescantia. Darlington and Koller were chiefly concerned with the breakage of chromosomes in relation to genetical problems, while our interest has been focused on the effects of the agent on the general physiology of cell division. These authors stress the close resemblance between the changes produced in their material by mustard gas and by X-rays.

Darlington and Koller describe and figure nucleic acid deficiency ('nucleic acid starvation', Darlington and Koller, 1947), similar to that seen in many of the mitotic figures in the treated tissue cultures; they also record imperfect contraction of the chromosomes; fragmentation, sometimes into small particles ('minutes' and 'subminutes'); failure or delay in reaching the metaphase plate ('errors of congression'); lag in anaphase, and the formation of micronuclei from chromosome fragments. They noted a correlation between centromere defects and errors of chromosome movement in Tradescantia, and it is probable that in the osteoblast cultures failure or delay of certain chromosomes to take part in the normal movements of metaphase and anaphase are due to defects in the attachment of the chromosomes to the spindle elements.

Whether chromosome breakage and reunion such as that seen in Tradescantia occurs also in our cultures is not known; we hope to extend our investigations to amphibian tissue cultures which are much more favourable for detailed cytological study than those of the chick.

Bodenstein (1947), in his interesting studies of the effects of a nitrogen mustard compound on amphibian development, describes cytological abnormalities in the ectoderm of Amblystoma embryos, which resemble those seen in the osteoblast cultures treated with mustard gas. He records the enlargement of those cells which normally divide actively, 'metaphases with irregularly arranged chromosomes' and anaphase lag; in the ectoderm, as in the tissue cultures, normal and abnormal mitotic figures occurred in close proximity. Multinucleate cells appeared in the ectoderm 7-8 days after exposure to the agent when mitosis had ceased; Bodenstein believes that the nuclei of these cells were formed by the fragmentation of enlarged interphase nuclei. In our material such cells were seen to arise by abnormal mitosis. There is
also strong cytological evidence that in mouse skin treated with repeated applications of mustard gas in very dilute solution the many multinucleate cells which appeared in the regenerating epidermis were the result of abnormal mitosis (Fell and Allsopp, 1948). While it is of course possible that the ectodermal cells of the Amblystoma embryos reacted rather differently from those of mouse epidermis or of chick osteoblast cultures, it would be interesting to know whether anything similar to the apolar mitosis described above (cf. Pl. I, fig. 7, Pl. II, fig. 11, Pl. III, fig. 32) was present in the amphibian tissue. The interpretation of such grossly abnormal mitotic figures is difficult in material which precludes the direct observation of the living cells.

Precisely how mustard gas disturbs the physiology of dividing cells is not clear. The agent is known to have several biochemical effects: on proteins in general (Banks et al., 1946), on nucleo-proteins (Berenblum and Schoental, 1947), and on carbohydrate metabolism through the inactivation of hexokinase (Dixon and Needham, 1946). Which of these effects operate in living cells exposed to very low concentrations of the agent, and to what extent, remains to be discovered.

Study of the cinema records emphasizes the fact that the various phenomena of mitosis are not rigidly linked to each other, and that in the same cell one process may be grossly distorted, or even omitted, while another proceeds almost normally. Thus the chromosomes may be extremely abnormal in structure, as in group B of the bipolar divisions, and yet at anaphase a considerable proportion of them move to the poles of the spindle at the normal rate: in one of the tripolar mitoses described above there is no metaphase, but the cell is able to pass directly from the radial stage into a tripolar anaphase; in the apolar mitoses not only metaphase but anaphase and telophase also are omitted, but nevertheless the general cytoplasmic upheaval normally characteristic of these phases takes place and the scattered and distorted chromosomal material is able to form nuclei.

These observations suggest that in mitosis there are parallel series of reactions, in the chromosomes, in the spindle, and in the cytoplasm, which in normal division are closely co-ordinated but which under the influence of mustard gas and other agents may partially disengage and to some extent proceed independently of each other.

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**Summary**

1. The cytological effects produced in cultures of embryonic fowl osteoblasts by low concentrations of mustard gas in the nutritive medium have
been studied both in the living cells by means of cinematography and phase-contrast microscopy, and in fixed and stained preparations.

2. Under the influence of mustard gas the movements of the cell and its contents are exaggerated and, especially at the higher concentrations, its water-content appears to be increased.

3. The treated cultures contain many abnormal mitotic figures which are more abundant and more distorted at the higher concentrations.

4. Three main types of abnormal mitosis have been observed:
   (i) **Bipolar.** Group A: these cells are characterized by failure or delay of certain chromosomes in reaching the equatorial plate, by lag at anaphase and telophase, and by the formation from the lagging chromosomes of one or more micronuclei, in addition to two nuclei of normal size. Most of the chromosomes appear normal, but in some the nucleic acid charge is localized in granules. Group B: the mitotic abnormality is an exaggeration of that seen in group A. The spindle is relatively normal; the chromosomes fail to contract properly and have a beaded structure; some chromosomes may break up into small granules while others aggregate into large, granular, skein-like masses; there is an equatorial lag, usually of several chromosomes; multinucleate daughter cells are formed.

   (ii) **Tripolar.** Two forms of tripolar mitosis have been observed: (a) a fairly regular triradiate metaphase plate was succeeded by a tripolar anaphase and the formation of three multinucleate daughter cells; (b) a recognizable metaphase was omitted, the cell passing straight from the radial stage (see p. 40) to a tri-polar anaphase and telophase.

   (iii) **Apolar.** There is no recognizable metaphase, anaphase, or telophase; the chromosomal material has a radial orientation; in some cells it is in the form of distorted filaments and rods, while in others it appears as granules of different sizes. After a period of intense cytoplasmic turmoil the cell spreads out without cleavage and many small and medium-sized nuclei have been formed from the diffuse chromosomal material.

5. In the abnormal cells the duration of mitosis is prolonged; at the higher concentrations prophase and metaphase may last for 2 hours or more; when a spindle is formed, some of the chromosomes move apart during anaphase at the normal rate; cytoplasmic cleavage may occupy 2–3 times the normal period; reconstruction of the daughter nuclei proceeds at the normal rate.

6. The cytological effects of mustard gas resemble those of irradiation.

7. The observations indicate that many phenomena of mitosis, though normally closely co-ordinated, under abnormal conditions can to some extent disengage and proceed independently of each other.
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DESCRIPTION OF PLATES

The figures in Plates I and II are of living cells photographed by phase-contrast microscopy. All were enlarged to the same magnification (×1000) from single frames of ciné records. In each series the times are reckoned from that of the first picture. The photographs in Plate III are of fixed and stained preparations and were taken by Mr. V. C. Norfield, head assistant at the Strangeways Research Laboratory.

PLATE I

Fig. 1a-f. Stages in the normal division of an osteoblast; a. radial stage; b. 8½ min., end of metaphase; c. 9½ min., early anaphase; d. 12 min., late anaphase; e. 13½ min., telophase: general cytoplasmic bubbling; f. 22 min., reconstruction of daughter cells: nucleoli have appeared.

Fig. 2a-f. 12½ y/c.c. mustard gas in the culture medium. Bipolar mitosis. Prolonged prophase, division otherwise normal. a. radial stage; b. 32 min., early metaphase; c. 36½ min., end of metaphase; d. 40 min., anaphase; e. 45½ min., late telophase; f. 62½ min., apparently normal mononucleate daughter cells.

Fig. 3. Normal intermitotic cell. Note the two irregular nucleoli in the large oval nucleus, and the very refractile cytoplasmic fat globules.
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Fig. 4a-e. 12.5 γ/c.c. mustard gas. Abnormal bipolar mitosis (Group A) showing chromosome lag in anaphase. a. end of metaphase; b. 14 min., early anaphase; c. 34 min., late anaphase; d. 8 min., telophase; e. 32 min., daughter cells: in the left-hand cell one large nucleus and two micronuclei are forming.

Fig. 5a-f. 12.5 γ/c.c. mustard gas. Abnormal bipolar mitosis (Group A) showing delay of two chromosomes in reaching metaphase plate and lag in anaphase. a. prophase; b. 54 min., metaphase: X not yet in equatorial plate; c. 17 min., metaphase: X nearly on the plate; d. 24 min., early anaphase: X incorporated in right chromosome group, lateral lag of two small chromosomes (seen as small black rods at the equator); e. 27 min., early telophase: lagging chromosomes being pushed into an axial position; f. 29 min., late telophase; g. 35 min., h. 44 min., i. 55 min., stages in nuclear reconstruction: in the left daughter cell a micronucleus develops as well as a normal nucleus.

Fig. 6a-i. 50 γ/c.c. mustard gas added in serum (see p. 39). Abnormal bipolar mitosis (Group B) showing large, granular chromosomal masses, one of which (X) is expelled from the spindle, and the formation of two multinucleate daughter cells. a. metaphase: X in spindle area; b. 54 min., metaphase: X leaving spindle area; c. 45 min., metaphase: X in cytoplasmic bubble; d. 134 min., end of metaphase; e. 136 min., anaphase; f. 139 min., telophase; g. 153 min., multinucleate daughter cells: note wide range of nuclear size.

Fig. 7a-c. 25 γ/c.c. mustard gas. Apolar mitosis forming single multinucleate cell. a. diffuse chromosomal material: absence of spindle; b. 10 min., two chromosomal masses visible, violent cytoplasmatic bubbling; c. 35 min., multinucleate cell.

PLATE II

Fig. 8a-g. 25 γ/c.c. mustard gas; abnormal bipolar mitosis (Group B) showing median and lateral anaphase lag, beading of the chromosomes and the formation of one mono- and one pluri-nucleate daughter cell. a. end of metaphase; b. 14 min., early anaphase; c. 24 min., anaphase: lagging beaded chromosomes; d. 34 min., late anaphase: lateral lagging chromosomes being pushed into an axial position; e. 45 min., telophase: lateral chromosomes now axial; f. 15 min., daughter cells: abnormal persistence of cytoplasmic bridge, in lower daughter cell, bouquet of micronuclei formed from lagging chromosomes; g. 20 min., daughter cells: one mono- and one pluri-nucleate.

Fig. 9a-g. 25 γ/c.c. mustard gas. Tripolar mitosis forming three multinucleate daughter cells. a. end of triradiate metaphase; b. 14 min., early anaphase; c. 4 min., late anaphase: some chromosomes left in the middle of the spindle area; d. 6 min., telophase; e. 10 min., f. 16 min., g. 18 min., stages in the formation of three multinucleate daughter cells.

Fig. 10a-d. 25 γ/c.c. mustard gas. Irregular tripolar mitosis with no recognizable metaphase, forming two daughter cells. a. radial stage: incomplete ring of chromosomes; b. 10 min., tripolar anaphase; c. 14 min., tripolar telophase; d. 28 min., coalescence of two of the three daughter cells.

Fig. 11a-e. 50 γ/c.c. mustard gas. Apolar mitosis forming single multinucleate cell. a. radial stage showing ring of chromosomes; b. 36 min.; c. 40 min., expansion of cell processes; d. 100 min., a nest of nuclei have been formed, note the nucleoli; e. 115 min., cell fixed and stained, the nest of nuclei are clearly seen. (× 1300.)

PLATE III

All the photographs, except Figs. 25 and 31, were taken at the same magnification as that of Fig. 12. The cells shown in Figs. 30 and 31 are from preparations fixed in Zenker's solution without acetic acid; the rest are from cultures fixed in Maximow's fluid. Figs. 13, 14, 19, and 26 were made from preparations stained by Feulgen's method and the remainder from preparations hydrolysed as for the Feulgen technique and then stained with Ehrlich's haematoxylin.

Fig. 12. Normal prophase. (× 1700.)
Fig. 13. Late normal prophase.
Fig. 14. Normal radial stage.
Fig. 15. Normal metaphase.
Fig. 16. Normal anaphase.
Fig. 17. Normal late telophase.
Fig. 18. 25 γ/c.c. mustard gas. Abnormal bipolar metaphase (Group A), showing delay of one chromosome in reaching the equatorial plate; note the beaded end of this chromosome.
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Fig. 19. 25 y/c.c. mustard gas. Abnormal bipolar metaphase (Group A), showing chromosome right outside the spindle area.

Fig. 20. 12.5 y/c.c. mustard gas. Abnormal bipolar anaphase (Group A), showing chromosome lag. Note the beaded structure of some of the chromosomes indicating nucleic acid deficiency.

Fig. 21. 12.5 y/c.c. mustard gas. Abnormal bipolar mitosis (Group A), early telophase with single lagging chromosome.

Fig. 22. 12.5 y/c.c. mustard gas. Abnormal bipolar telophase (Group A) with several lagging chromosomes.

Fig. 23. 12.5 y/c.c. mustard gas. Abnormal bipolar mitosis (Group A); early telophase with three lagging chromosomes in course of reconstruction into micronuclei.

Fig. 24. 12.5 y/c.c. mustard gas. Abnormal bipolar mitosis (Group A); daughter cells, one containing a single large nucleus and two micronuclei and the other one large and one small nucleus.

Fig. 25. 25 y/c.c. mustard gas. Abnormal bipolar mitosis (Group B); prophase (cf. Figs. 12 and 13) showing granular chromosomes of irregular shape and distribution; at the chromosome material forms a granular, skein-like mass.

Fig. 26. 25 y/c.c. mustard gas. Abnormal bipolar metaphase (Group B); most of the chromosomal material is included in two large, granular masses on either side of the fairly normal spindle. The chromosomes in the spindle area are very deficient in nucleic acid and irregular in form.

Fig. 27. 50 y/c.c. mustard gas. Abnormal bipolar metaphase (Group B) similar to that seen in Fig. 26. This cell contains three large chromosomal masses, two of which are lateral to the spindle, while the third lies freely in the cytoplasm near the surface of the cell. Note the localized cytoplasmic bubbling in the neighbourhood of all three masses.

Fig. 28. 12.5 y/c.c. mustard gas. Abnormal bipolar anaphase (Group B) showing two diffuse groups of daughter chromosomes and at the equator several beaded lagging chromosomes and large chromosomal masses.

Fig. 29. 25 y/c.c. mustard gas. Abnormal bipolar mitosis (Group B) showing multinucleate daughter cells united by a long chromatinic bridge, each end of which has expanded into a nucleus. (×1250.)

Fig. 30. 50 y/c.c. mustard gas. Tripolar metaphase.

Fig. 31. 50 y/c.c. mustard gas. Multinucleate cell derived from an apolar mitosis. (×880.)

Fig. 32. 50 y/c.c. mustard gas. Apolar mitosis showing radially arranged, granular chromosomes and homogeneous chromatinic body.