The fine structure produced in cells by primary fixatives
2. Potassium dichromate

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With 2 plates (figs. 1 and 2)

Summary

The exocrine cells of the mouse pancreas were fixed in potassium dichromate solution, embedded in araldite or other suitable medium, and examined by electron microscopy.

Almost every part of these cells is seriously distorted or destroyed by this fixative. The ergastoplasm is generally unrecognizable, the mitochondria and zymogen granules are seldom visible, and no sign of the plasma membrane, microvilli, or Golgi apparatus is seen. The contents of the nucleus are profoundly rearranged. It is seen to contain a large, dark, irregularly shaped, finely granular object; the evidence suggests that this consists of coagulated histone. The sole constituent of the cell that is well fixed is the inner nuclear membrane.

The destructive properties of potassium dichromate are much mitigated when it is mixed in suitable proportions with osmium tetroxide or formaldehyde.

Introduction

POTASSIUM dichromate was widely used as a fixative in the second half of the nineteenth century, chiefly in the form of 'Müller's fluid'. Müller himself (1860*b*) simply stated that his fluid consisted of an aqueous solution of potassium dichromate and sodium sulphate, without giving particulars as to the concentrations of these salts. Subsequent authors used the dichromate at 2 to 2½%, and the sodium sulphate (probably Glauber's salt) at 1% (Frey, 1871; Exner, 1878; Mojsvár, 1879). In the present paper Müller's fluid must be taken to mean a 2½% aqueous solution of potassium dichromate with the addition of 1% of Glauber's salt. The latter is an 'indifferent' salt, not capable of exerting any fixative effect on tissues at the concentration at which it is used (Fischer, 1899), but possibly affecting fixation in the same way as sodium chloride affects fixation by formaldehyde.

It is important to recognize that acidified potassium dichromate reacts with tissues in a very different way from the unacidified salt. The subject has attracted the attention of several authors, and has been comprehensively reviewed and investigated by Casselman (1955). It must suffice here to mention that when the pH falls below about 3·6, potassium dichromate produces the same effects as a solution of chromium trioxide. Müller himself (1860*a*) sometimes used a solution of potassium dichromate, sodium sulphate, and chromium trioxide, but this is not the fluid ordinarily known by Müller's

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name. The fine structure produced by chromium trioxide (and acidified potassium dichromate) is under investigation by myself.

From the last decade of the nineteenth century onwards potassium dichromate has been used chiefly in mixtures with other fixatives, or for *Postchromirung* (Benda, 1901) after preliminary treatment with other fixatives. In many of the mixtures (e.g. Zenker, 1894), acidification has made the salt act like chromium trioxide; but in others (e.g. Altmann, 1894; Orth, 1896; Helly, 1903; Regaud, 1910; Dalton, 1955) this is not so.

The present paper is concerned primarily with solutions of unacidified potassium dichromate used alone or with the addition of sodium sulphate, but mention is also made of the effects of certain fixative mixtures.

Material and methods

The test-object has been the same as that used before, in the investigation of the fine structure produced by mercuric chloride (Baker, 1963); namely, the exocrine pancreatic cell of the house-mouse, *Mus musculus*. The reasons for the choice of this cell were given in the earlier paper. The pancreas was cut into small pieces with scissors or a razor blade.

Fixation. The fixatives used were $1\frac{1}{2}$ and $2\frac{1}{2}\%$ aqueous solutions of potassium dichromate, and Müller's fluid. All three were used at room temperature. The period of fixation varied from 24 h to 20 days. Often the pieces of tissue were fixed for 24 h and then transferred to a 5% or a saturated solution of potassium dichromate maintained at 37° C, and left in this for 24 h.

Pieces of tissue were also fixed in a 1% aqueous solution of osmium tetroxide at about 4° C, for comparison with those fixed in potassium dichromate solution. It was found that cells of the chosen tissue fixed in this simple solution appeared similar in fine structure to those that had been fixed in the buffered osmium tetroxide solution of Palade (1952). This confirms the results of Malhotra (1962).

Experiments on fixation with mixtures containing potassium dichromate, and on the postchroming of tissues that had been subjected to the action of fixatives other than potassium dichromate, are mentioned on p. 20.

Washing out the fixative. In accordance with the usual practice in light microscopy, pieces fixed with potassium dichromate or with mixtures containing this salt were washed (usually for about 5 h) in running water or repeated changes of tap-water, and then rinsed with distilled water.

Embedding. After dehydration with ascending grades of ethanol, the pieces were embedded in araldite, butyl methacrylate, or plexigum (usually the first-named).

Some of the pieces were passed through toluene into paraffin, in accordance with the routine procedure of light microscopy. The cooled paraffin was dissolved away with warm toluene, and this was replaced with absolute ethanol; the pieces were then embedded in araldite. The purpose was to find out whether embedding in paraffin causes any changes in the structure of the cell.

Sectioning. A Huxley ultramicrotome was used to produce sections showing silver interference colours. The sections were mounted on carbon film over formvar.

Staining. Some of the sections were 'stained' with a 2% solution of uranyl acetate in methanol, but unstained sections were also examined to make sure that the stain had not affected the structure.

Microscopy. Sections were examined with an Akashi TRS 50 electron microscope.

A small investigation was made by light microscopy, to clarify a problem that had arisen in the course of the study by electron microscopy. (See p. 18.)

Results

Since there is no method by which the fine structure of the living cell can be established with certainty, the standard of comparison will be the structure revealed after fixation by osmium tetroxide. The chief components of the exocrine pancreatic cell of the mouse, fixed by a 1% solution of osmium tetroxide in distilled water, are shown in fig. 1, A.

It makes little difference whether $1\frac{1}{2}$ or $2\frac{1}{2}$% potassium dichromate or Müller's fluid is used as fixative, and essentially the same picture is produced whether fixation is short or long. The use of a more concentrated fixative at 37° C after initial fixation at room temperature does not produce any striking modification in the image. A single description of the fine structure produced by potassium dichromate will therefore suffice, though some small exceptions to this will be mentioned.

Fig. 1, B has been chosen to represent the structure produced when potassium dichromate is used as fixative. The disruption of the cytoplasm is almost complete. Nothing remains but a coagulum, with interspersed areas of various sizes, which appear to be empty spaces (*s*). The coagulum usually consists of granules, mostly about 20 or 30 mμ in diameter (figs. 1, B; 2, B), but their size cannot be measured accurately since they are not sharply delimited, and they often merge with one another (fig. 2, D). There are also lines in the micrographs, which must be taken to represent sections of membranes (fig. 1, B, *m*). The membranous element tends to be somewhat more conspicuous when Müller's fluid is used (fig. 2, C) instead of a simple solution of potassium dichromate, and it sometimes happens that the cisternae of the ergastoplasm are more or less intact in places, though nothing that can be interpreted as a ribosome is seen.

As a general rule mitochondria are not preserved. Occasionally they survive and appear in micrographs as grey, sausage-shaped objects with very indistinct traces of cristae. In the whole investigation no trace of the Golgi apparatus was ever seen in any preparation fixed with potassium dichromate. The zymogen granules have usually been dissolved away, and not even their former sites can be detected; but small remnants are sometimes seen in oval spaces, or spaces that have probably contained them remain.

The plasma membrane does not survive, and the limits of the cells cannot be determined. Microvilli are not seen.

The nucleus is considerably larger than in osmium preparations. It is limited by a very distinct single membrane, marked by arrows in figs. 1, B and 2, B to D. A double membrane, such as is seen in osmium preparations (fig. 2, A, E), never survives intact, but traces of a broken outer membrane sometimes occur here and there. Since the outer membrane is a part of the ergastoplasm, the disruption of the latter by potassium dichromate accounts for its complete or almost complete absence. The striking difference between the responses of the inner and outer nuclear membranes to the action of potassium dichromate is the most remarkable single fact that has emerged from this investigation.

The contents of the nucleus differ radically from those seen in osmium preparations. Very irregularly shaped dark masses (*h* in figs. 1, B; 2, B, C) lie on a pale background. The dark masses consist of a close aggregation of granules, each roughly 20 mμ in diameter. The pale background often but not always contains dark, sharply delimited granules, most of them rather less than 40 mμ in diameter; lines radiate from these, probably representing sections of membranes. These granules are particularly clearly seen in fig. 2, D at *x*. Their nature is obscure.

In an attempt to interpret the contents of the nucleus as seen in electron micrographs, pieces of pancreas were fixed for 24 h in Müller's fluid, embedded in paraffin, sectioned, and treated with Feulgen's reagent and light green. One or more irregularly shaped masses were seen in each nucleus, stained with light green; they appeared not to extend to the periphery of the nucleus. The whole of the rest of the nucleus, right up to the nuclear membrane, was filled with a Feulgen-positive substance. It seems almost certain that the substance having an affinity for the green dye is the same as the dark, finely granular substance seen in electron micrographs.

The results so far mentioned suggested the possibility that potassium dichromate might not be a fixative at all: for such fixation as occurred might have been due to the ethanol used for dehydration. To test this possibility a version of Müller's fluid was prepared, in which the potassium dichromate was replaced by an equimolar amount of a substance known to have no fixative effect, namely potassium chloride. Pieces of pancreas were left in this fluid for 24 h and washed as usual. Sections were prepared as before for study by light microscopy. They were treated with Feulgen's reagent. No signs of nuclei were seen anywhere: the tissue was unrecognizable as cellular material

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FIG. 1 (plate). Electron micrographs of parts of pancreatic exocrine cells of the mouse.

A, fixed with osmium tetroxide (stained in bulk with uranyl nitrate and on the grid with uranyl acetate; araldite). ×24,000.

B, fixed with potassium dichromate (araldite; stained with uranyl acetate). ×24,000. The arrow points to the inner nuclear membrane.

er, ergastoplasm; *g*, Golgi apparatus; *h*, substance regarded as coagulated histone; *m*, mitochondrion; *me*, membrane; *n*, nucleus; *s*, space; *x*, unidentified granule; *z*, zymogen granule.

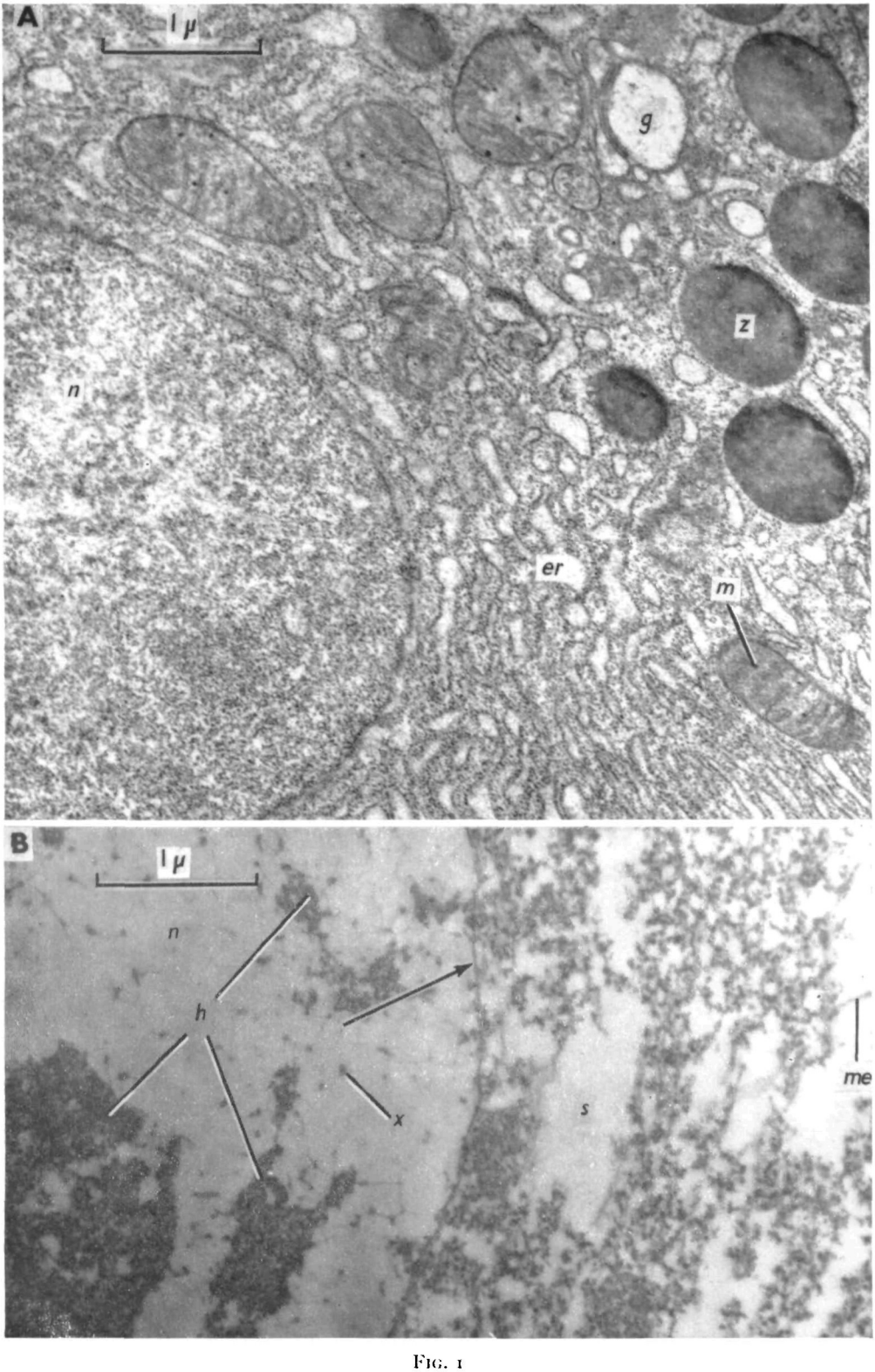

FIG. 1

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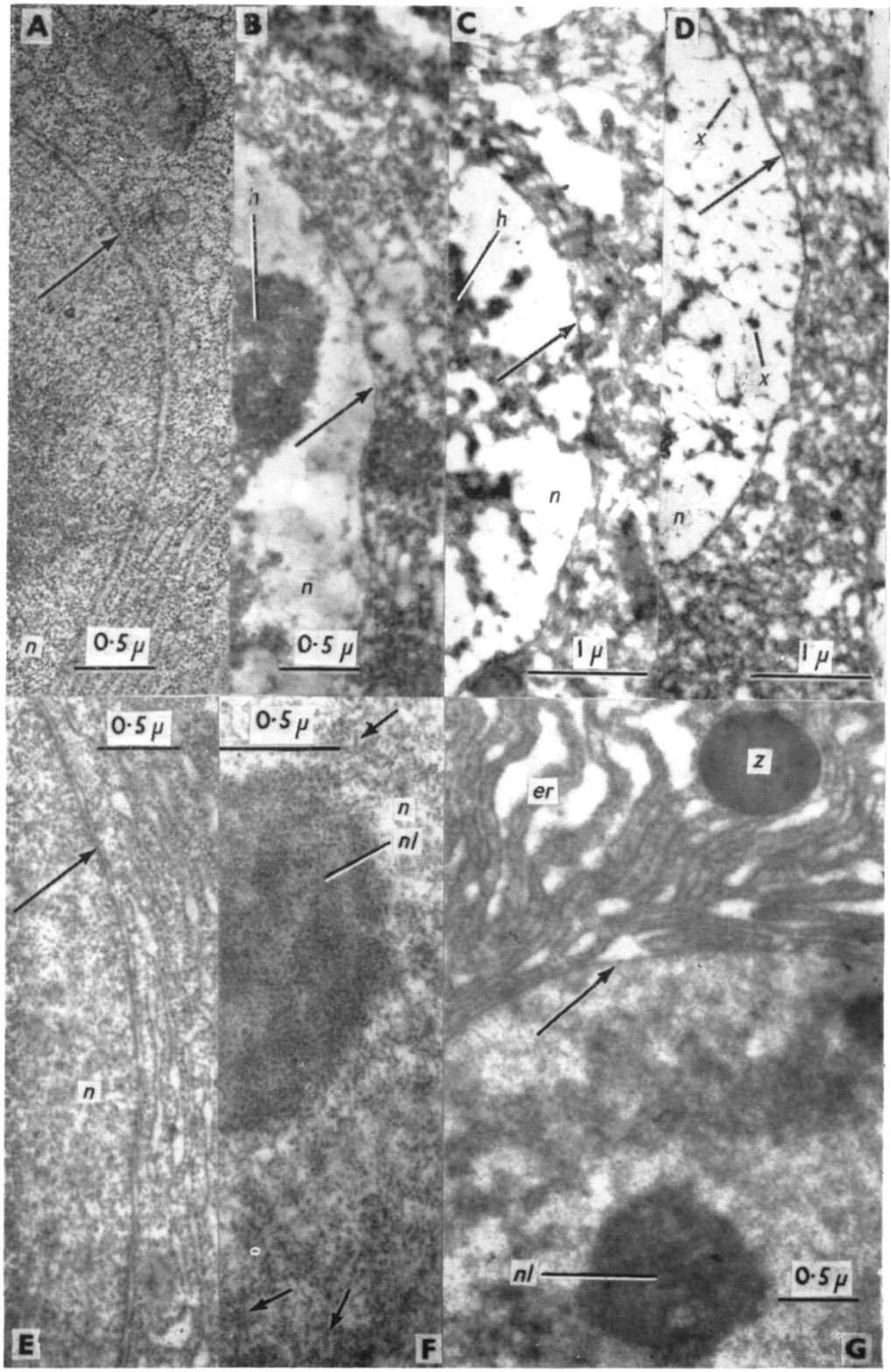

FIG. 2

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of any kind. Nothing was present except an extremely coarse reticulum, *positive* to Feulgen.

Since nucleoprotein is not coagulated by potassium dichromate, and DNA is readily dissolved by solutions of this salt (Fischer, 1899; Berg, 1905; Baker, 1958), the facts can only be understood on the assumption that *potassium dichromate is primarily a fixative for the inner nuclear membrane.* The Feulgen-positive material is retained within the nucleus, dispersed throughout all parts of it except those occupied by the substance having an affinity for light green, simply because the preservation of the inner nuclear membrane prevents its escape.

It is known that potassium dichromate is a strong coagulant of histone (Pischinger, 1937). The evidence therefore suggests that the substance in the nucleus that has an affinity for light green and appears in electron micrographs as the dark, finely granular material, is coagulated histone.

Nucleoli cannot be recognized with certainty in electron micrographs of pancreatic exocrine cells fixed with potassium dichromate. The three characteristic components of the nucleolus (Hay and Revel, 1962) are seen in osmium preparations, namely the dark nucleolonema, granules having the characters of ribosomes, and pale intervening spaces (fig. 2, F); but nothing of this sort is seen after fixation with potassium dichromate. Roughly circular objects of about the right size are often seen, but their fine structure suggests that they are merely parts of the dark, finely granular substance.

Embedding in paraffin before araldite does not result in any characteristic change in the fine structure of cells fixed by potassium dichromate. It follows that cells fixed by this substance and studied by the routine processes of light microscopy have the same fine structure as that revealed in sections that have been embedded in araldite, with or without previous embedding in paraffin.

The question next arose whether potassium dichromate is itself destructive, or whether the damage is caused by washing, dehydration, or embedding after the fixative has acted. To answer this, pieces of pancreas were transferred to osmium tetroxide solution after fixation by potassium dichromate or Müller's fluid, in order to stabilize the structure when the action of the dichromate was complete. Electron micrographs showed the usual appearance

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FIG. 2 (plate). Electron micrographs of parts of pancreatic exocrine cells of the mouse.
A, fixed with osmium tetroxide (stained with uranyl nitrate and acetate; araldite). ×24,000.
B, fixed with potassium dichromate (araldite; stained with uranyl acetate). ×24,000.
C, D, fixed with Müller's fluid (plexigum). ×18,000.
E, fixed with osmium tetroxide (stained with uranyl nitrate and acetate; araldite). ×24,000.
F, ditto. ×36,000.
G, fixed with osmium tetroxide and transferred directly to potassium dichromate (araldite; stained with uranyl acetate). ×24,000.
The long arrows point to the inner nuclear membrane. The short arrows in F point to filaments in the nucleus, arranged in pairs.
er, ergastoplasm; *h*, substance identified as coagulated histone; *n*, nucleus; *nl*, nucleolus; *x*, unidentified granule; *z*, zymogen granule.

produced by potassium dichromate, except that the zymogen granules were better preserved.

It still remained to find out whether potassium dichromate would exert its destructive effects on pancreatic exocrine cells if used in conjunction with a better fixative. To answer this, pieces of pancreas were fixed in Altmann's (1894) fluid (potassium dichromate and osmium tetroxide). The resulting preparations show great variations in fine structure. Ergastoplasm (with ribosomes), mitochondria (with very indistinct cristae), zymogen granules (with membranes), and inner and outer nuclear membranes are visible in some of them, but the contents of the nucleus always show a general resemblance to what is seen after fixation by potassium dichromate alone. In many micrographs of Altmann material the cytoplasm is seen to be almost completely disorganized.

Preparations made by Regaud's method of fixation and postchroming show a general resemblance to those fixed by formaldehyde alone. The ergastoplasm is preserved, but the space between one cisterna and the next is narrow. Ribosomes are seen, but are indistinct. The zymogen granules (with their membranes) and the vacuoles of the Golgi apparatus are preserved. The mitochondria show little internal structure. The double nuclear membrane persists; the nuclear pores are widely open. There are aggregations of granules in the nucleus, reminiscent of those seen in preparations fixed with dichromate alone, but mostly situated at the periphery.

When pieces of pancreas are fixed with osmium tetroxide and then postchromed, the resulting picture resembles in general that produced by osmium tetroxide alone, but there is less sharpness in the micrographs and the ribosomes are indistinct (fig. 1, G).

Discussion

Potassium dichromate preserves the inner nuclear membrane intact, but it distorts or destroys almost every other part of the cell. The facts reveal a remarkable difference in chemical composition between the inner and outer nuclear membranes.

When mixed with formaldehyde, potassium dichromate only reveals its destructive tendencies to a limited extent, and in suitable mixtures with osmium tetroxide (e.g. Dalton's fluid (1955)), it permits good fixation of fine structure.

The rearrangement of the chromatin under the influence of potassium dichromate was well described by Flemming (1882) more than 80 years ago. He wrote that it partly destroys the original nuclear structure 'with solution or swelling of the preformed framework (*Gerüstbalken*) and re-precipitation of the substance thus dissolved or swollen in the form of a homogeneous network'.

The mitochondria of certain tissues of animals and plants have been shown by light microscopy to withstand fixation by potassium dichromate (Strangeways and Canti, 1927; Zirkle, 1928; Casselman, 1955). Even when they are

not destroyed, however, there is a tendency for rod-shaped mitochondria to become ovoid or spherical.

It was already known from studies by light microscopy that the zymogen granules of the pancreas are not fixed by potassium dichromate alone, but are fixed by mixtures of this substance with formaldehyde (Levene and Feng, 1962).

The fact that potassium dichromate has scarcely any fixative effect on the cells chosen for this investigation does not mean that it is necessarily a useless ingredient in fixative mixtures for light microscopy. Mixtures of potassium dichromate with osmium tetroxide give preparations that are much more readily stainable by anionic dyes than those fixed by osmium tetroxide alone.

It is a pleasure to acknowledge the skilful practical assistance of Miss E. G. M. Collins and Mr. J. M. McCrae. The Akashi electron microscope and Edwards vacuum evaporator used in this investigation were granted to me by the Wellcome Trustees, and the Huxley ultramicrotome by the Government Grants Committee of the Royal Society.

References

ALTMANN, R., 1894. *Die Elementarorganismen und ihre Beziehungen zu den Zellen.* Leipzig (Veit).
BAKER, J. R., 1958. *Principles of biological microtechnique.* London (Methuen).
BAKER, J. R., 1963. Quart. J. micr. Sci., **104**, 101.
BENDA, C., 1901. Verh. Anat. Ges. (Bonn), **15**, 155.
BERG, W., 1905. Arch. mikr. Anat., **65**, 298.
CASSELMAN, W. G. B., 1955. Quart. J. micr. Sci., **96**, 203.
DALTON, A. J., 1955. Anat. Rec., **121**, 281.
EXNER, S., 1878. *Leitfaden bei der mikroskopischen Untersuchung thierischer Gewebe.* Leipzig (Engelmann).
FISCHER, A., 1899. *Fixirung, Färbung und Bau des Protoplasmas.* Jena (Fischer).
FLEMMING, W., 1882. *Zellsubstanz, Kern und Zelltheilung.* Leipzig (Vogel).
FREY, H., 1871. *Das Mikroskop und die mikroskopische Technik.* Leipzig (Engelmann).
HAY, E. D., and REVEL, J. P., 1962. Fifth internat. Congr. Elekt. Micr. Philadelphia, **2**, p. O–8.
HELLY, K., 1903. Zeitschr. wiss. Mikr., **20**, 413.
LEVENE, C., and FENG, P., 1962. Quart. J. micr. Sci., **103**, 461.
MALHOTRA, S. K., 1962. Quart. J. micr. Sci., **103**, 5.
MOJSVÁR, A. M. E. VON, 1879. *Leitfaden bei zoologisch-zootomischen Präparirübungen für Studierende.* Leipzig (Engelmann).
MÜLLER, H., 1860*a*. Verh. Physik.-med. Ges. Würzburg, **10**, 138.
MÜLLER, H., 1860*b*. Verh. Physik.-med. Ges. Würzburg, **10**, 179.
ORTH, J., 1896. Berl. Klin. Woch., **33**, 273.
PALADE, G. E., 1952. J. exp. Med., **95**, 285.
PISCHINGER, A., 1937. Z. Zellforsch. mikr. Anat., **26**, 249.
REGAUD, C., 1910. Arch. d'Anat. micr., **11**, 291.
STRANGEWAYS, T. S. P., and CANTI, R. G., 1927. Quart. J. micr. Sci., **71**, 1.
ZENKER, K., 1894. Münch. Med. Woch., **41**, 532.
ZIRKLE, C., 1928. Protoplasma, **4**, 201.