methods, give excellent results and the detail demonstrated surpasses that observed in ground sections. With the exception of a few departments of dental histology, neither of these methods is in general use in American laboratories. I have been unable to find Schmorl's original description of his methods but they are repeated in a more recent work of 1909. An excellent discussion of the methods is also found in a paper on the structure of bone of Fasoli[2] and adequate directions for the successful use of these methods are given by Carleton[3] in his recent book on histological technique. References to Schmorl's methods may also be found in the works of Lange[4] and Fischer.[5] It seems unnecessary to completely outline the method since it can be readily obtained in English in a modern text-book on histological technique. Formol, Orth's, Müller's or Regaud's fluids may be used for fixing. Fluids containing mercuric chloride should be avoided. Best results are obtained with celloidin or frozen sections. If nuclear patterns are desired, the tissue should be first stained in alum-carmine or hemalum, as the success of the picro-thionin method depends entirely on the precipitation of the thionin in the lacunae and canaliculi. The picro-thionin method is best adapted to work with old bone, while the phosphotungstic acid method is more useful for demonstrating the histology of young bone and the process of ossification.

ALDEN B. DAWSON

DEPARTMENT OF BIOLOGY,
NEW YORK UNIVERSITY

SOME FIXATIVES FOR BOTH NUCLEI AND MITOCHONDRIA

A 2.5 per cent. solution of copper bichromate C. P. (Eimer and Amend) has a pH of 2.0. When root tips of Zea are fixed in it the fixation image is that of chromic acid, *i.e.*, the nucleolus appears as a spherical, darkly staining body in a hollow nucleus whose surface is composed of the chromatin reticulum. The mitochondria are either dissolved by the fixative or by the dehydrating alcohol. If, however, a slight excess of cupric oxide is added to the solution, the pH is altered to about 4.6 and the fixation image is greatly changed. There is here no hollow space around the nucleolus; the nucleus is a solid body, and in the resting stages the chromatin reticulum is much less distinct. In the dividing nucleus the spireme shows up distinctly and the chromosomes are well preserved. While the spindle fibers are not distinguishable individually, collectively they are well delineated. The mitochondria are well fixed and mordanted and can be followed through each of the mitotic stages. This fixative has the following faults: the resting nuclei show little detail, the cytoplasm is somewhat distorted and the outer layer of cells is generally over fixed. The addition of .05 per cent. acetic acid causes the resting nuclei to show more detail, though one must be cautious in the use of this acid, for a slight excess of copper acetate will dissolve the mitochondria. The most successful formula for the fixative is:

copper bichromate	5 grams
cupric oxide	1 gram
10 per cent. sol. acetic acid	1 c.c.
water	200 c.c.

The material should be left in the solution for from 36 hours to six days, and when thus fixed both chromosomes and mitochondria are well stained with Heidenhain's haematoxylin. Destaining should not proceed as far as is usual for an examination of the nuclei, for the mitochondria do not hold the stain as well as the chromosomes and can be completely decolorized before the chromosomes have started to fade.

It is very important to make up the fixative at least 24 hours before it is to be used. It must be shaken frequently in the interval and the excess copper oxide allowed to settle. If it is used too soon the fixation image will be that of chromic acid. It is best to wash out the fixative with 70 per cent. alcohol. If the dehydration is too prolonged the mitochondria will be dissolved out of the peripheral cells. A half hour in each of 70 per cent., 85 per cent. and 95 per cent. alcohol, and an hour in each of two changes of absolute, are sufficient for the dehydration.

Another solution which fixes both chromosomes and mitochondria is:

chromium trioxide	5 grams
glucinum carbonate	3 grams
water	200 c.c.

This also has a pH about 4.6. If there is no excess of glucinum carbonate a little more should be added, for otherwise the fixation image will be that of chromic acid. The fixed material should be dehydrated as described above. Material fixed in this solution appears very much like that fixed in the copper bichromate mixture; the cytoplasm is perhaps a trifle more granular and the mitochondria are thicker, otherwise the two fixatives are alike.

A third solution which fixes both chromosomes and

[2] 1905. Fasoli, G. "Ueber die feinere Struktur des Knochengewebes." *Arch. mikr. Anat.*, Bd. 66, S, 471.

[3] 1926. Carleton, H. M. "Histological Technique." Oxford University Press.

[4] 1913. Lange, W. "Histologische Technik für Zahnärzte." Springer, Berlin.

[5] 1910. Fischer, Bau und Entwicklung der Mundhöhle. höhle.

mitochondria apparently functions quite differently from the two just described. It is:

10 per cent. sol. chromic sulphate..... 1 part
8 " " " formalin neutralized with an excess of $CaCO_3$ or Li_2CO_3..... 1 "

If calcium carbonate is used the pH is about 2.2; if lithium carbonate is used it is about 4.8.

When this fixative is washed out with water and the dehydration proceeds slowly, the dividing nuclei and the cytoplasm appear beautifully fixed. The chromosomes are a trifle shrunken so that in the metaphase they show the split very clearly. The cytoplasm appears quite smooth with sharply delineated vacuoles. In the root tip the growth of the vacuoles and their behavior during mitosis can be easily followed. Unfortunately the mitochondria are dissolved out of the epidermis and cortex and remain only in the central cylinder. If the fixative is washed out with 70 per cent. alcohol and the dehydration is relatively rapid, the cytoplasm appears more granular and the mitochondria are preserved in nearly the whole tissue.

It is evident that there is an important relation between the pH of a bichromate and its fixation image. If it is too acid it will fix the chromatin but not the mitochondria, if it is too basic it will fix the mitochondria but not the chromatin. Certain bichromates in the presence of an oxide or a carbonate of the element which furnishes the cation will buffer at a point where they will fix both nuclear and cytoplasmic elements. Others, as their pH number is raised, suddenly change from nuclear to cytoplasmic fixatives. The pH of this point of change shows quite a range for the various bichromates. Thus ammonium bichromate pH 4.2 and potassium bichromate pH 4.4 are much too basic to fix the chromatin, while lithium bichromate pH 4.6 has the fixation image of chromic acid. Zinc bichromate pH 5.2 will fix both chromosomes and mitochondria with its characteristic fixation image.

A detailed description of the fixation images of various bichromates is being prepared.

CONWAY ZIRKLE

BUSSEY INSTITUTION,
HARVARD UNIVERSITY

SPECIAL ARTICLES

THE MnII SPECTRUM EXCITED BY RARE GAS IONS

THE MnII spectrum was excited by the method recently described by Duffendack and Smith[1] and tested by the writers[2] on the CuII spectrum. In this method rare gas ions on contact with Mn atoms ionize them and simultaneously excite them to the degree that the ionizing potential of the rare gas exceeds that of manganese, 7.4 volts.

An argon ion on contact with a Mn atom can ionize it and excite the resulting Mn^+ ion to the extent of $15.4 - 7.4 = 8.0$ volts. In the process the argon ion is neutralized by combination with an electron taken from the Mn atom and energy to the amount of 15.4 equivalent volts is made available. 7.4 equivalent volts of this is expended in extracting the electron from the Mn atom, leaving eight equivalent volts to be accounted for. Smyth and Harnwell and Hogness and Lunn[3] have demonstrated by positive ray analyses that ionization may occur upon contact between an ion and a molecule. In the investigations cited above[1,2] it has been demonstrated that the excess energy may go toward exciting the ion formed. Hence, when argon ions are used, one may expect to produce by this process Mn^+ ions excited to states whose levels are less than eight volts or 84,800 cm^{-1} above the normal state of Mn^+ but none excited to a higher degree. Consequently, lines of the MnII spectrum whose initial states are below 64,800 cm^{-1} should appear and lines originating in higher states should be absent from the spectrum thus excited. If, however, neon (ionizing potential 21.5 volts) is substituted for argon, Mn^+ ions excited to states whose levels are less than 14.1 volts or 114,210 cm^{-1} are produced and lines from these levels should appear in the spectrum.

The experimental procedure consisted in photographing the spectra of low voltage arcs in mixtures of argon and Mn vapor and neon and Mn vapor in a tungsten furnace apparatus. The manganese was put into a molybdenum trough mounted inside a cylinder of thin sheet tungsten and insulated from it. This trough constituted the anode of the arc, and the cathode was a tungsten filament mounted inside the cylinder and parallel to its axis. The tungsten cylinder was itself mounted inside a metal water-cooled vacuum chamber, filled to the desired pressure with argon or neon, and was heated by passing a sufficiently large current through it. The manganese in the trough was thus vaporized and any desired vapor pressure could be maintained inside the cylinder. The filament was then heated and a low voltage arc maintained in the mixture of argon or neon and manganese vapor within the furnace. The spectrum of the arc was photographed through quartz windows sealed onto the vacuum chamber.

The results support the hypothesis outlined above. When argon was used, lines originating in the ^7P and

[1] *Phys. Rev.* 29, 914, 1927; *Nature*, May 21, 1927.
[2] *Phys. Rev.* 29, 925, 1927.
[3] *Nature*, Jan. 15, 1927; *Phys. Rev.* 29, 830, 1927; *ibid.*, 30, 26, 1927.