

ferent techniques proposed for this purpose are based upon the formation of arsenic trisulfide (As_2S_3) which is obtained in the form of yellow-brown to greenish yellow, rounded and elongated, highly refractive crystals, which are insoluble in water, alcohol, xylol and Canada balsam, but soluble in ammonium carbonate, potassium hydroxide and ammonium hydroxide. The fundamental aspects of these methods were developed by Justus in 1905. Modifications and improvements were developed later by Osborne and Brünauer. According to recent investigations of Tannenholz and Muir, this method is not entirely specific for arsenic trisulfide, as similar crystals may be formed, apparently representing a combination of sulphur-proteins.

Osborne's Method:

- 1) Tissue is fixed in a 10 per cent formalin solution for 24 to 48 hours.
- 2) It is then washed in running tap water for at least 6 hours, better for 24 hours.
- 3) The tissue is then cut into small and thin slices, not more than 2 mm. thick.
- 4) The slices are placed into a one ounce glass stoppered bottle filled with a freshly prepared, neutral hydrogen sulfide solution. A small amount of liquid petrolatum is put on the stopper, which is then tightly inserted, and bound down by a cord. The bottle is placed into an incubator at 70°C for 4 days. Fresh hydrogen sulfide is added every day. If the slices are placed into a pyrex tube which is sealed in an oxygen flame, this procedure is not necessary. As the stainability of the tissue is greatly reduced, if they are kept at a temperature of 70°C , the container may be kept in an incubator of 56°C for 6 days instead. The surface of the tissue assumes a dirty grey to black color on account of the formation and precipitation of iron sulfide.
- 5) The tissue is then washed again in running water for 6 to 12 hours.
- 6) Dehydration is accomplished in successive steps in a 50 per cent to 100 per cent alcohol series containing an addition of 10 per cent ether.
- 7) It is then embedded in celloidin and sections five microns thick are prepared.
- 8) The cut sections are placed for 20 minutes into a 10 per cent HCl solution in 70 per cent alcohol. This procedure is essential, since all sulphides precipitated by the hydrogen sulfide in the tissues are dissolved with the exception of the insoluble arsenic trisulfide. This procedure impairs, however, the stainability of the sections.
- 9) The sections are washed in 70 per cent alcohol for 20 minutes and then stained with hematoxylin and eosin.
- 10) They are differentiated in 95 per cent alcohol.
- 11) The sections are cleared in oil-of-clove; the excess is removed with xylol.
- 12) They are finally mounted in Canada balsam.

The irregularly round to oval, bright yellow crystals of arsenic trisulfide, varying in size from 0.0005 to 0.00075 mm. in diameter have a faintly greenish tint. When they are viewed well focused under the microscope, they appear as solid crystals with a bright halo. The high refractivity and the greenish halo of the arsenic trisulfide crystals distinguishes them from similarly colored granules of non-arsenical nature (melanin-lipoid pigment, particles