

## THE DETECTION OF YELLOW PHOSPHORUS AND PHOSPHIDES IN BIOLOGICAL MATERIAL

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Received July 10, 1958

The micro test to detect poisoning by yellow phosphorus or zinc phosphide has been modified and improved so that increased specificity is obtained. This is achieved by paper electrophoresis. Chlorine is used in the place of solid calcium hypochlorite to oxidise silver phosphide to silver phosphate.

YELLOW phosphorus is readily obtainable without restriction in the form of rat poison in Great Britain. The many cases of murder and suicide that have occurred even within the last five years in which death has resulted from the ingestion of this element show that it is still of great toxicological importance.

Death from phosphorus poisoning usually occurs in one of two ways—as a result of shock within 24–36 hours after ingestion, or as a result of liver failure several days later. In this latter type of case it is rare for a positive Mitscherlich phosphorescence to be obtained when the intestine contents are distilled and proof of the ingestion of yellow phosphorus was, until three years ago, rarely possible. In 1955, however, the introduction of a micro test<sup>1</sup> showed that in such cases yellow phosphorus could be detected in as little as 20 g. samples of liver.

The basis of the test is the micro distillation of the liver and passage of the resultant steam and phosphorus through a filter paper soaked in silver nitrate. Any silver phosphide shows itself as a black stain which can be oxidised to silver phosphate with bleaching powder and hence to a blue reduced phosphomolybdate.

There are three other compounds which could conceivably also react in this test. The first is phosphine which may be present either as a product of putrefaction or following ingestion of the rat poison zinc phosphide, zinc phosphide itself, and arsine. It is possible to differentiate phosphine and arsine from zinc phosphide in that the gases are liberated from neutral solution, even at room temperature. If zinc phosphide is present phosphine is liberated at room temperature only after acidification of the sample under test. Yellow phosphorus is unique in that it distils readily only after the sample has been heated.

It is the purpose of this paper to describe some modifications of the test which have resulted in an increased specificity and which enable a permanent record of the result of the test to be obtained.

There are four stages in the analysis which is now recommended. These are:—

(1) Passage of a stream of carbon dioxide through the liver sample and formation of insoluble silver phosphide in a Gutzeit type paper holder.

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Carbon dioxide is preferable theoretically to the stream of air recommended in the original paper because of the ready oxidation of phosphorus.

Carbon dioxide is, for the reasons described above, passed in three stages: (a) through the cold, neutral sample, (b) through the cold, acidified sample, (c) through the acidified sample heated to 100°.

(2) Oxidation of the silver phosphide to silver phosphate by the use of chlorine gas and removal of excess chlorine by aeration.

(3) Examination of the silver phosphate by electrophoresis: in this way phosphate is separated from any arsenate which might have interfered with the interpretation of the result and from the silver ion in the presence of which Hanes and Isherwood's spray for phosphates<sup>2</sup> cannot be used.

(4) The development, after electrophoresis of the paper strip with Hanes and Isherwood's reagent. This gives a record which can be photographed.

#### EXPERIMENTAL

The sample of liver, kidney or intestine contents suspended in water is put into a flask or tube fitted with the head illustrated in Figure 1. The space in the head is packed with glass wool impregnated with basic lead acetate.

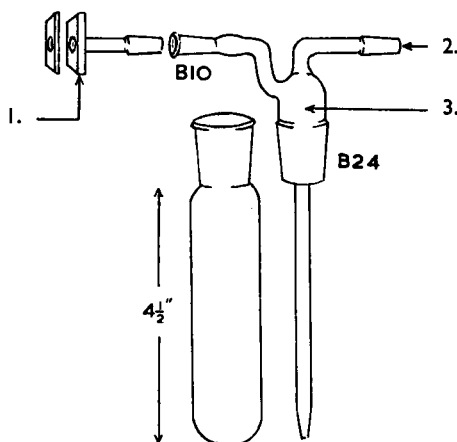


FIG. 1. Diagram of apparatus.

1. Perspex holder.
2. Carbon dioxide input.
3. Glass wool/basic lead acetate.

A strip of Whatman No. 1 filter paper (4 in.  $\times$  15 in.) is clamped between the flat faces of the perspex end. The small area of filter paper, approximately 0.4 cm. diameter, through which the distilling gases will pass is wetted by means of a capillary tube with a saturated solution of silver nitrate in methanol. The position of this area is chosen so that it lies between exactly similar spots prepared from phosphine and arsine controls, at right angles to the length of the paper, in a position suitable for subsequent examination in a paper electrophoresis apparatus. Carbon dioxide is passed into the apparatus at a slow rate (2-3 bubbles a second through a bubble counter) for 1½ hours. If no stain appears the sample is acidified and the passage of carbon dioxide continued. If still no stain appears the flask or tube is heated to 100° when any yellow phosphorus will distil. We have found it most convenient to use electric heating jackets fitting the tube or flask in use. Small tubes are used for 20 g. samples, flasks for larger quantities.

When distillation is complete the end holder is removed and placed on the B 10 socket fitted to a chlorine generator. The silver phosphide or

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arsenide spots are rapidly decolourised and excess chlorine is removed by blowing the paper with a stream of cold air from an electric hair-drier for 10 minutes. The control spots are similarly treated. We have found this method of oxidation to be vastly superior to that described in the original method.

The strip of paper is then placed in a horizontal paper electrophoresis apparatus using 5 per cent aqueous acetic acid as the electrolyte. The paper is very carefully sprayed with the electrolyte, the area about the spots being sprayed last. The current (about 2 mA at 250 v.) is passed for approximately 3 hours giving a movement of  $5\frac{1}{2}$  in. for arsenate and  $6\frac{1}{2}$  in. for phosphate.

After completion the paper is dried at  $100^{\circ}$  and sprayed with the phosphate reagent. This is prepared by mixing 62.5 ml. of 4 per cent w/v ammonium molybdate with 25 ml. N hydrochloric acid, 12.5 ml. 60 per cent perchloric acid and then making up to 250 ml.

After heating at  $85^{\circ}$  for 7 minutes the paper is exposed to steam for a few seconds and immersed in a jar of hydrogen sulphide. The blue spots on a brown background appear immediately. We have found that the sensitivity of this reagent is approximately the same as that of molybdate-benzidene<sup>3</sup>.

Comparison of the intensity of the silver phosphide or arsenide stains with the final blue colour is also possible.

We attempted in many experiments to separate phosphate and arsenate spots by paper chromatography but found that in the acid solvents which it was necessary to use because of the presence of the silver ion that a separation could not be achieved. These experiments, coupled with those using paper electrophoresis, showed that oxidation to phosphate and arsenate using chlorine was proceeding rapidly. Further experiments<sup>4</sup> showed that arc spectrographic analysis of the silver spots could also reveal the presence of arsenic provided more than one microgram of arsenic was present on the spot.

### REFERENCES

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