

shrinkage indicated that about three-quarters of the mitochondrial water volume was osmotically dead space.

9. It is concluded that the mitochondrial preparations are probably mixtures of two kinds of particle. One kind has a low water content, is rich in potassium and phosphate and almost impermeable to sucrose, sodium chloride and potassium chloride. The other kind has a high water content and is rather permeable to these solutes.

10. Evidence from electron microscopy suggests that the former particles are relatively undamaged mitochondria, whereas the latter have developed a vesicular appearance.

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APPENDIX

A Lithium Internal-Standard Flame Photometer

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Berry, Chappell & Barnes (1946) showed that, in estimating sodium and potassium by flame photometry, there were definite improvements in precision and accuracy when lithium was added to the samples as an internal standard (compare Spencer, 1950; Bernstein, 1952). In this Appendix a simple coal-gas/air flame photometer is described which may be used either as a direct-reading instrument or with lithium internal standard.

The internal-standard flame photometer is a modification by two of us (J.E.A. and W.C.W.) of a direct-reading flame photometer, which was developed by one of us (D.S.P.) from the apparatus described by Leyton (1951). A commercial single-

cell direct-reading flame photometer based on the design of D.S.P. is available from A. Gallenkamp and Co., London, E.C. 2. This Appendix is limited to those essential particulars in which the internal-standard flame photometer differs from the Gallenkamp instrument.

EXPERIMENTAL

Description of the flame photometer

The stainless-steel concentric atomizer is fitted with a capillary tube of smaller bore (0.33 mm.) than is used in the Gallenkamp instrument, so that the rate of sample consumption is only 0.9–1.1 ml./min. with 9 lb./in.² air pressure. The aspiration rate may be decreased by fitting a finer hypodermic needle to the atomizer. The spray chamber and burner (Fig. 1) are quite different from those used by

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Gallenkamp. The chimney is lowered, by means of a loop of wire attached to its top, down the metal flue after lighting the burner. It stabilizes the flame and prevents condensation on the filters. The coal-gas pressure (about 1 in. water) is adjusted to yield an inner cone about 2 cm. high while spraying water.

The flame chamber is water-cooled, which allows continuous running. It has three ports (in addition to the inspection port) at right angles to the flame axis, to accommodate separate filter and photocell assemblies for detecting lithium, sodium and potassium radiation of 671,

589 and 766 $m\mu$ respectively. Interference filters (2 in. in diameter) are used (Barr and Stroud, Ltd., Glasgow, W. 3), with Chance ON 20 heat filters to protect them from the flame. The filters are placed directly in front of the barrier-layer photocells (37 mm. in diameter), which are mounted 9 cm. from the flame. The 766 $m\mu$ filter is used with an infrared-sensitive photocell (Megatron Ltd., London, N. 4). The 589 and 671 $m\mu$ filters are used with EEL photocells (Evans Electro Selenium Ltd., Harlow, Essex), whose low sensitivity at 766 $m\mu$ is advantageous, on account of the slight residual transmission (0.3–0.5%) of the filters at this wavelength.

The circuit diagram is shown in Fig. 2. The galvanometer is type V.S. 6.45 (H. Tinsley and Co., London, S.E. 25). At maximum sensitivity (direct reading) a full-scale deflection of the galvanometer (0.1 μA) is obtained by spraying 0.08 mm-sodium, or 0.06 mm-potassium, or 3 mm-lithium salts.

Operation of the flame photometer

Standard solutions containing lithium sulphate equivalent to 5.0 mm-lithium and sodium chloride and potassium chloride (in equal concentrations) in the range 0–2.0 mm were prepared. Calibration curves were prepared in three concentration ranges: 0–0.2 mm, 0–0.6 mm and 0–2.0 mm. The sensitivity of the instrument is set by spraying the highest standard of the chosen range, switching S2 to 'standardize', and (switching S1 to positions 4 and 5 as appropriate) adjusting the sodium and potassium potentiometers so that the galvanometer is not deflected. S2 is then switched to 'unknown', solutions containing lower concentrations of sodium and potassium are sprayed, and the settings of the lithium potentiometer which are required to balance the galvanometer are recorded. The shapes of the calibration curves are stable indefinitely, but it is necessary to reset the sensitivity of the instrument as above, at the start and at intervals of 5–10 min. during operation. No interference was detected between sodium and potassium in the concentration range 0–2.0 mm.

The samples to be analysed were diluted with inclusion of 0.1 vol. of 0.025 M- $Li_2SO_4 \cdot H_2O$, so that the final concentration of Li^+ ions was 5.0 mm, and of Na^+ and K^+ in the

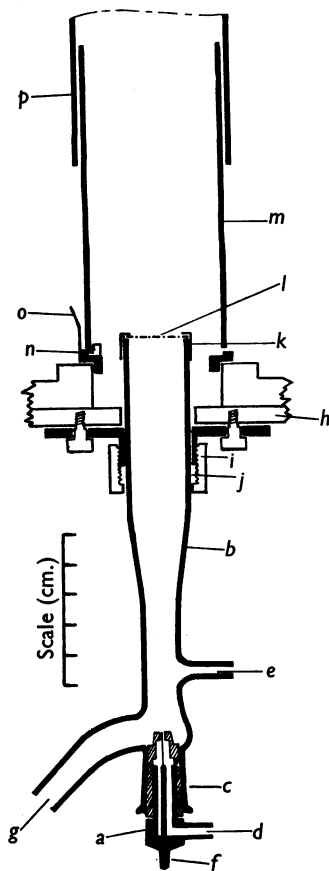


Fig. 1. Atomizer, spray chamber and burner assembly of flame photometer. The adjustable atomizer *a* is fitted into the Pyrex-glass spray chamber *b* at the standard taper joint *c* and held by two springs (not shown). The air inlet is *d*, the gas inlet *e*, and the sample is aspirated through a hypodermic needle (not shown) fitted on the taper *f*. The drain tube *g* leads to a water trap. The spray chamber is supported in the base plate *h* of the flame chamber by the aluminium collar *i* containing asbestos packing *j*. A stainless-steel collar *k* holds the disk of 20 mesh/in. nickel gauze *l* in place. The Pyrex-glass chimney *m* is supported on three distance-pieces *n* so as to leave an annular air-gap 1 mm. wide, 5 mm. below the base of the flame. Guides *o* assist in locating the chimney correctly when it is lowered down the brass flue *p*.

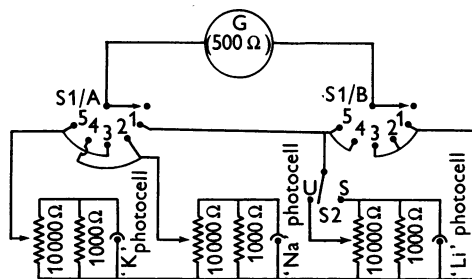


Fig. 2. Circuit diagram of lithium internal-standard flame photometer. The potentiometers (Reliance) are three-quarter-turn wire-wound, and are fitted with pointers and dials divided into 100 divisions. Switch S1/A, B: position 1, lithium; 2, sodium; 3, potassium, by direct reading; position 4, sodium; 5, potassium, by lithium internal standard. Switch S2: S, standardize; U, unknown.

range 0.02–2.0 mm. Widely differing concentrations of sodium and potassium (up to 50:1 or 1:50) were estimated in a single dilution, and in one spraying, less than 2 ml. of the diluted sample being consumed.

DISCUSSION

The mutual interference between sodium and potassium observed in the acetylene/air flame (Domingo & Klyne, 1949; Leyton, 1951; Smit, Alkemade & Verschure, 1951; Baker, 1955) is avoided by the use of the cooler illuminating-gas/air flame (Alkemade, Smit & Verschure, 1952), which simplifies the determinations. The internal-standard procedure is preferred to direct reading, because the instrumental stability and the precision of a single reading are greater (Berry *et al.* 1946; Spencer, 1950; Bernstein, 1952; Baker, 1955), which makes multiple sprayings of the same sample unnecessary. The reading is unaltered by $\pm 25\%$ variation in spraying rate, which was in practice the most difficult variable to control in direct reading (Leyton, 1951). The lithium internal standard has been reported to compensate for interferences by many substances (Berry *et al.* 1946; Spencer, 1950; Bernstein, 1952); this has

been verified for the components of our own samples.

SUMMARY

1. The design and operation of a simple lithium internal-standard flame photometer are described.

We are very grateful to Mr J. T. Cox for building the apparatus and for devising the atomizer, to Mr H. Vincent for evolving the spray chamber, and to Mr A. Renshaw and Mr D. Sproat for assistance. We also thank Dr A. Leaf, Mr M. P. Esnouf and Mr D. Exley for advice and help. One of us (W. C. W.) is a Research Fellow of the National Cancer Institute, U.S. Public Health Service.

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Studies on the Biosynthesis of Cholesterol*

6. COENZYME REQUIREMENTS OF LIVER ENZYMES FOR SYNTHESIS OF SQUALENE AND OF STEROL FROM DL-3-HYDROXY-3-METHYL-[2-¹⁴C]PENTANO-5-LACTONE

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The synthesis of cholesterol from 3-hydroxy-3-methylpentano-5-lactone (mevalonic acid) was discovered by Tavormina, Gibbs & Huff (1956) and the high yield of labelled sterol from the DL-[2-¹⁴C]-mevalonic acid incubated with liver preparations has been confirmed (Cornforth, Cornforth, Popják & Gore, 1957, 1958). It was further shown (a) that mevalonic acid is also an effective precursor of squalene, (b) that all the carbon atoms of the acid maintain their individuality during biosynthesis, and (c) that five of its carbon atoms (C-2, C-3,

3-Me, C-4 and C-5) provide the isoprenoid units in the biosynthesis of squalene and hence of sterol (Cornforth *et al.* 1957, 1958).

This paper deals with the identification of the various coenzymes required by liver-enzyme preparations for the synthesis of squalene and of sterol from mevalonic acid. A brief account of this work has already been given (Popják, Gosselin & Gore, 1958; Gould & Popják, 1957).

METHODS

The following abbreviations will be used in addition to those noted in the text: diphosphopyridine nucleotide (DPN) and its reduced form (DPNH); triphosphopyridine nucleotide (TPN) and its reduced form (TPNH); adenosine triphosphate (ATP); coenzyme A (CoA or CoA-SH); cysteine (CySH); glutathione (GSH); *p*-chloromercuribenzoate (PCMB).

* Part 5: Cornforth, Cornforth, Popják & Gore (1958)
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‡ Participated in the early part of this work during tenure of a United States Public Health Service Special Research Fellowship, and while on leave of absence from the Los Alamos Scientific Laboratory of the University of California, Los Alamos, New Mexico.