CORRECTION OF SOURCES OF ERROR IN THE ESTIMATION OF SODIUM, POTASSIUM AND CALCIUM IN BIOLOGICAL FLUIDS AND TISSUES BY FLAME SPECTROPHOTOMETRY

by

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Using capillary aspiration via a spray chamber into a burner and a filter photometer for emission measurements, SMIT and his co-workers[1,2] have elucidated various fundamentals of flame photometry. Since 1949 the writer has had experience with a flame spectrophotometer, employing a gravity feed atomiser spraying into a horizontal chamber. The vapour is filtered into a propane burner, which could be used with acetylene by changing the grid and inserting a venturi tube into the burner barrel. The light emitted is dispersed by a prism monochromator. The required emissions, isolated by a moving slit (15–24 mμ band width), fall on red or blue sensitive phototubes, with an electronic amplification circuit to measure light intensities. The spectrum also passes through a slit fixed at 671 mμ onto a second red sensitive phototube, so that a constant lithium concentration added to solutions, enabled potentiometric measurements of light intensity ratios to be obtained[3,4]. This made feasible the comparison of the two techniques under the same instrumental conditions.

The delivery of a constant amount of element to the burner in unit time, the correction of interferences produced in the flame and the accurate isolation of emissions for measurement, was found to control exact analysis.

The minute volume of fluid atomised followed POISEUILLE's law with some modification, depending on atomisation pressure, fluid capillary radius, and solution viscosity and temperature. Air pressure and viscosity variations had little effect on ratio measurements in contrast to direct emissions, confirming the observations of BERRY *et al.*[5] With fluid diameters from 0.3–0.5 mm and 0.7 kg/sq.cm air pressure, atomisation ranged from 5–36 ml/min, producing progressive increases up to 700 % and 4 % for direct and ratio measurements. In finer capillaries, the change in atomisation rate (and direct emission) was more marked.

The amount of vapour (and kation) entering the flame was dependent on the number of stable spray particles formed on atomisation. Reducing surface tension from 72–37 dynes per cm with alcohols or bile salts increased the vapour volume and direct emissions up to 70 %, and 5 % for ratio emissions.

Solution temperatures (5–37° C), affecting fluid viscosities, size of atomiser capillary and the spray process, produced no significant alteration in internal standard measurements.

The delivery of the vapour in constant amount to the burner, therefore, necessitated considerable precautions to enable direct emissions being read from calibration curves. Determination of light intensity ratios compensated adequately for slight variations in these factors.

Flame interferences produced by self-interference (*i.e.* light emission of element in high concentration not linear to its concentration, due to self-absorption and altered dissociation) and radiation interferences (due to ionised particles of elements interfering with the atomic emission of the element being estimated) were reduced by operating at high dilution (1/100–1/200 for Na, 1/25–1/50 for K in serum). Judged by the error of replicate estimations within various standardisation ranges, such dilutions fell within the maximum sensitivity range of the instrument.

The characteristics of lithium (excitation and ionisation potentials and the influence of inorganic and organic substances on its emission in the propane and acetylene flames) indicated that it behaved identically with sodium, with slight variation in respect of potassium and some deviation for calcium. However, the flame error due to the incorporation of lithium as an internal standard was $\sim$ 0.5 % for K and $\sim$ 1.2 % for Ca, and was less than the compounding of atomisation and flame errors in the direct technique.

Spectral interference only occurred with sodium in high concentration at the 554 mμ calcium oxide band in contrast to filter photometers[6,7].

Standard solutions gave linear readings in all calibration ranges between 0–8 mg % for Na, 0–5 mg % for K and 0–10 mg % for Ca (Ca gave satisfactory emission intensities only with the acetylene flame). The standard deviation of repeated estimations did not exceed 0.9, 1.4 and 2.5 % for Na, K and Ca, respectively, until one operated at concentrations less than 0.5 mg %.

Methods of estimation in all biological fluids and tissues have been developed. The internal standard procedure yielded more accurate results and recoveries in plasma, urine, gastro-intestinal secretions and tissue analyses than direct flame photometry or chemical precedures. The standard

deviation for duplicates and recoveries by this method in all fluids and tissues was 1.3, 1.9 and 2.9% (confidence limits for 96 of 100 analyses) for Na, K and Ca. The internal standard method was found particularly advantageous for estimations in a. bile—containing specimens (bile, intestinal juice), where surface tension affected direct emissions; b. tissue ashes, where iron and phosphates depressed direct intensity measurements; and c. urines containing high protein, glucose or phosphate concentrations.

The detailed investigations will be published.

REFERENCES

1 J. SMIT, C. T. J. ALKEMADE, AND J. C. M. VERSCHURE, *Biochim. Biophys. Acta*, 6 (1951) 508.
2 C. T. J. ALKEMADE, J. SMIT, AND J. C. M. VERSCHURE, *Biochim. Biophys. Acta*, 8 (1952) 562.
3 R. E. BERNSTEIN, *S. Afr. J. Med. Sci.*, 14 (1949) 163.
4 R. E. BERNSTEIN, *Nature*, 165 (1950) 649.
5 J. W. BERRY, D. G. CHAPPELL, AND R. B. BARNES, *Ind. Eng. Chem., Anal. Ed.*, 18 (1946) 19.
6 V. LEPPÄNEN AND O. FORSANDER, *Scand. J. Clin. Lab. Invest.*, 3 (1951) 33.
7 J. U. WHITE, *Anal. Chem.*, 24 (1952) 394.

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SPECTROPHOTOMETRIC MEASUREMENTS OF THE METABOLIC FORMATION AND DEGRADATION OF THIOL ESTERS AND ENEDIOL COMPOUNDS

by

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The role of thiol esters of coenzyme A and of glutathione as intermediates in the oxidative metabolism of aldehydes and keto acids has been recently elucidated[1]. The enzymic oxido-reduction of methylglyoxal to lactic acid consists of 2 steps catalyzed by glyoxalase I and II[2]. In the first step a condensation of glutathione with methylglyoxal gives rise to a thiol ester (lactyl glutathione), which is hydrolyzed in the second step to lactic acid and glutathione. It has now been found that in the presence of glyoxalase I and glutathione, other ketoaldehydes (glyoxal, phenylglyoxal and hydroxypyruvic aldehyde) also give rise to the corresponding thiol esters. These esters all show a high light absorption in the low ultraviolet region and give a characteristic color reaction with hydroxylamine and ferric chloride similar to that given by acyl phosphates[3]. The formation and hydrolysis of the thiol esters was therefore followed spectrophotometrically as well as colorimetrically. A partially purified preparation of glyoxalase II catalyzed the hydrolysis of the lactyl-, glycolyl-, mandelyl-, and glyceryl-esters of glutathione. It also split acetyl glutathione slowly but was apparently inactive with acetyl thioglycolate as substrate. The latter compound as well as acetyl glutathione recently have been shown to be hydrolyzed by large amounts of crystalline glyceraldehyde-3-phosphate dehydrogenase[4]. A non-enzymic ammonolysis of acetyl thioglycolate to acetamide was also observed.

A diphosphopyridine nucleotide linked enzyme is present in yeast which catalyzes the oxidation of lactyl and glycolyl glutathione[2]. Since these thiol esters are presumed to be in equilibrium with their enediol forms, the possibility that they may act as substrates for enzymes which oxidize some enediol compounds was investigated. It was found however that neither ascorbic acid oxidase nor peroxidase catalyzed the oxidation of lactyl glutathione nor did the yeast enzyme which oxidizes the thiol esters have any effect on ascorbic acid or dihydroxymaleic acid.

In the course of these investigations methods were developed to follow the oxidation of enediol compounds spectrophotometrically. At the appropriate wavelengths the enediols absorb light very strongly while the oxidation products do not. Thus the oxidation of ascorbic acid by ascorbic acid

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