Advances in CLINICAL CHEMISTRY

Edited by

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FOREWORD TO THE SERIES

A historian of science in years to come may well be astonished at the explosive burst of scientific activity round about the middle of the twentieth century of our era. He will be puzzled by the interrelationship between the growth of population and the rise of the standard of living; he will be interested in the increased percentage of scientists among the population, their greater specialization and the resulting fragmentation of science; he will analyze the economic and the psychological motivation of scientists; he will compare the progress of knowledge with the broadness of the current of scientific publication.

Living as we do in the midst of these events, we are hardly aware of their relatively rapid rate. What we notice is a doubling of the scientific output every ten years, regardless of contemporary political events. It is this climate which has engendered the appearance of series of reviews in dozens of disciplines. It may be with yearning or with a feeling of superiority, that we look back at such annual compendia as "Maly's Jahresberichte der Thierchemie" of one hundred years ago, which encompassed the annual progress in the zoological half of biochemistry within 300 to 400 pages.

Nowadays, that number of pages would not suffice to record the complete annual increment of knowledge in a single specialized division of the subject such as Clinical Chemistry. Media already existing furnish a comprehensive list of publications and an encyclopedic summarization of their contents; the present series of "Advances in Clinical Chemistry"—like other "Advances" series—attempts something different. Its aim is to provide a readable account of selected important developments, of their roots in the allied fundamental disciplines, and of their impact upon the progress of medical science. The articles will be written by experts who are actually working in the field which they describe; they will be objectively critical discussions and not mere annotated bibliographies; and the presentation of the subjects will be unbiased as the utterances of scientists are expected to be—*sine ira et studio.*

The bibliography appended to each chapter will not only serve to document the author's statements, it will lead the reader to those original publications in which techniques are described in full detail or in which viewpoints and opinions are expressed at greater length than is possible in the text.

The selection of the subjects in the present and in future volumes will include discussion of methods and of their rationale, critical and com-

parative evaluation of techniques, automation in Clinical Chemistry, and microanalytical procedures; the contents will comprise those borderline subjects, such as blood coagulation or complement chemistry, which are becoming more chemical with increasing knowledge of the underlying reactions; in some instances the discussion of a subject will center around a metabolic mechanism or even around a disease entity.

While recognizing that the elaboration and testing of methods is of the greatest importance in a subject, part of whose function is to provide reliable, accurate diagnostic and prognostic procedures, the new series will take cognizance of the fact that Clinical Chemistry plays an essential part in the progress of medical science in general by assisting in elucidating the fundamental biochemical abnormalities which underlie disease. The Editors hope that this program will stimulate the thinking of Clinical Chemists and of workers in related fields.

HARRY SOBOTKA
C. P. STEWART

PREFACE TO VOLUME 4

Volume 4 of this series covers again aspects of Clinical Chemistry ranging from discussions of analytical methods to reviews on the biochemistry of disease, centering around the physiology and pathology, for instance, of a hormone or of a vitamin, and including the pertinent chemical procedures. Recent developments in immunoelectrophoresis, microliter analysis, and flame photometry are treated with a view to their concrete applications.

We wish to express our gratitude again to the authors and to the publisher, and we thank our colleagues for suggestions and criticisms, past and future.

November, 1961

HARRY SOBOTKA
C. P. STEWART

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FLAME PHOTOMETRY

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1. Introduction

It is the object of this review to provide a critical account of modern flame photometry in the light of the basic principles of the method. No attempt has been made to provide a catalog of the innumerable articles appearing on the subject, but it is hoped that this chapter will help clinical chemists to decide whether flame photometry is applicable to a particular problem and perhaps to suggest ways of overcoming difficulties with the method.

1.1. Historical; Background

Herschel (H1) studied the emission of salts introduced into a flame; he recorded [quoted by Mavrodineanu (M2)] that:

"Salts of soda give a copious and purely homogeneous yellow.

Salts of potash give a beautiful pale violet.

Salts of lime give a brick red, in whose spectrum a yellow and bright green line are seen.

Salts of strontia give a magnificent crimson. If analyzed by the prism two definite yellows are seen, one of which verges strongly to orange.

Salts of magnesia give no colour.

Salts of lithia give a red (on the authority of Dr. Turner's experiment with the blow-pipe).

Salts of baryta give a fine pale apple-green. This contrast between the flames of baryta and strontia is extremely remarkable.

Salts of copper give a superb green, or blue green.

Salts of iron (protoxide) gave white, where the sulphate was used . . . .

The colors thus communicated by the different bases to flames afford in many cases a ready and neat way of detecting extremely minute quantities of them; . . ."

Talbot (T1) had previously studied the flame spectra of lithium and strontium, but the full potentialities of Herschel's observations for qualitative analysis were first realized in the work of Kirchhoff and Bunsen (K1). These authors discovered cesium in 1860 and rubidium in the following year by observation of their flame spectra.

Janssen (J1) suggested that spectral analysis, until then used only for qualitative observations, was suitable also for quantitative work. He felt that such a development would be particularly advantageous in the case of elements like sodium which were difficult to determine by classic procedures. His suggestions bore fruit 3 years later when Champion *et al.* (C1) constructed an instrument for the determination of sodium in plant ash. A solution of plant ash was introduced into the flame by means of a platinum wire and the emission intensity measured by comparing it by means of a visual photometric attachment with light from a reference constant-intensity sodium flame. This "spectronatromètre" was the first flame photometer; and when one considers that it was capable of an accuracy of between 2 and 5 %, it is interesting that it was not for more than 70 years that the method was applied to clinical problems.

However, the principles of flame photometry were not fully developed until the brilliant work of Lundegårdh (L1, L2). This worker devised for the first time a satisfactory method of introducing solutions into the

flame at a constant rate. This was by means of a concentric atomizer which dispersed the solution into droplets which were then led through a spray chamber to remove the larger particles before passing into the flame. Instead of the Bunsen flame used by the earlier workers, Lundegårdh substituted an air-acetylene flame and photographed the emission on a photographic plate after it had been dispersed by means of a spectograph. He also developed procedures in which the intensity of an emission line was recorded directly by a galvanometer connected to the amplified output from a photocell which had been placed so as to receive an appropriate portion of the flame spectrum dispersed by a prism. Lundegårdh was able to measure almost half of the elements in the periodic table, and his work is the foundation of all modern work on flame photometry. It is only in the last few years that substantial advances have been made beyond the techniques employed by Lundegårdh.

Schuhknecht (S1) produced a greatly simplified instrument by substituting a filter for the monochromator used by Lundegårdh, and this type of apparatus is probably the most widely used today. But for the outbreak of war in 1939 this simple method would certainly have been rapidly adopted by clinical chemists, but it was not until the work of Barnes and associates (B1) and Domingo and Klyne (D2) after earlier work by Ells and Marshall (E2), Ells (E1), Griggs *et al.* (G2), and Cholak and Hubbard (C2) that the method became widely adopted.

In modern laboratories sodium and potassium are almost exclusively determined by flame photometry and it seems likely that the same will shortly become true of calcium and magnesium and possibly of iron, copper, chromium, manganese, cobalt, lead, and zinc.

2. The Flame Photometer

The flame photometer consists essentially of an atomizer, a burner, some means of isolating the desired part of the spectrum, a photosensitive detector, sometimes an amplifier and, finally, a method of presenting the desired emission, whether by galvanometer, null meter, or chart recorder.

2.1. The Atomizer

Two main types are in common use: (*a*) an atomizer which produces an aerosol which passes through a spray chamber before reaching the flame (Fig. 1); (*b*) an atomizer which sprays directly into the flame. This type is sometimes an integral part of the burner, as with the Beckman or Zeiss atomizer-burner (Fig. 2). Atomizers of type (*a*) are usually

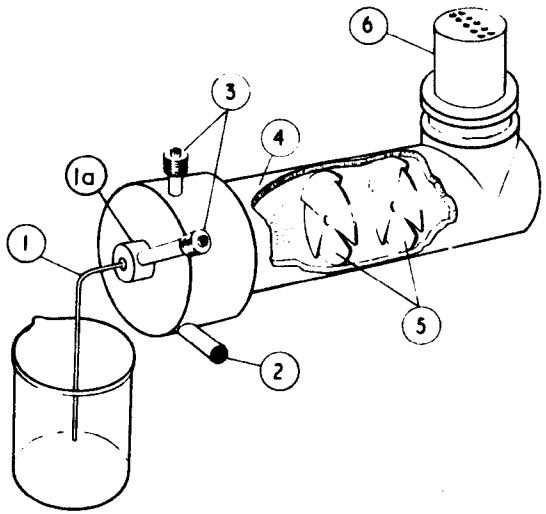

FIG. 1. An atomizer of the aerosol-producing type; aerosol passes through a spray chamber before reaching the flame. (1) Inlet capillary; (1a) metal atomizer; (2) drainpipe; (3) fuel and air inlets; (4) spray chamber; (5) baffles; (6) burner. (By permission of Evans Electroselenium Ltd., Halstead, Essex, England.)

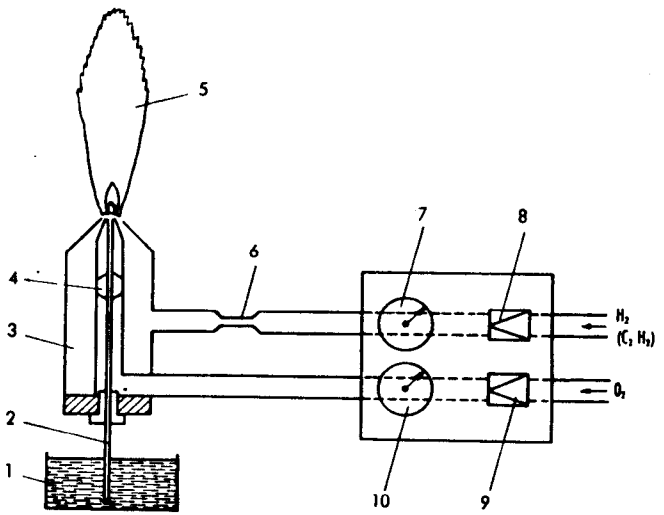

FIG. 2. Schematic diagram of burner of Zeiss flame spectrophotometer. (1) Sample; (2) cannula; (3) fuel gas inlet pipe; (4) guide piece for cannula; (5) flame; (6) throttling device for regulation of pressure indicator; (7) pressure gage; (8 and 9) pressure regulators; (10) pressure gage. (By permission Carl Zeiss, Germany, through Degenhardt & Co. Ltd., London, W.1.)

concentric, as shown in Fig. 1, but are sometimes constructed of two capillaries at right angles (Fig. 3). The spray chamber-atomizer is generally employed with simpler instruments and cooler flames, while the integral atomizer-burner is usually used when hotter flames such as oxyacetylene are burned.

In the author's opinion the integral type is much superior for most purposes. Unlike the spray chamber atomizer, the integral type reaches equilibrium almost instantly when solutions are sprayed and, if correctly designed, is extremely robust in use. A further important distinction is

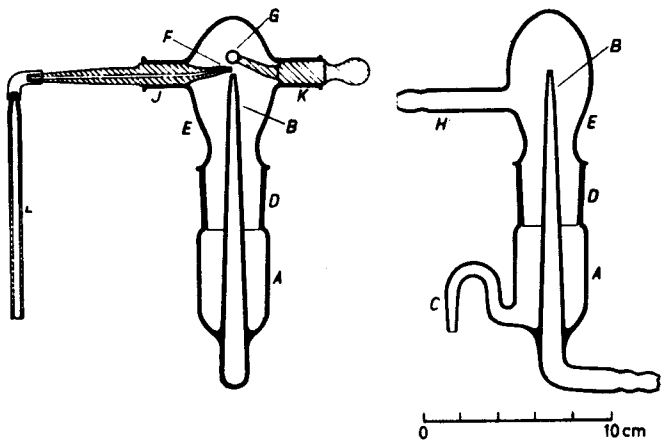

FIG. 3. Atomizer constructed from two capillaries at right angles. Atomizer (two vertical cross sections at right angles): (*A*) lower part carrying air inlet (*B*) and drain for waste (*C*). (*A*) is joined by the ground glass joint (*D*) to the upper part (*E*), which carries the solution inlet (*F*), the baffle plate (*G*), and the outlet to the burner (*H*). (*F*) and (*G*) are mounted on ground glass joints (*J*, *K*). (*F*) is joined by a piece of narrow rubber tubing to the vertical tube (*L*) which dips into the solution to be analyzed. (By permission of Domingo and Klyne (D2).)

that organic solvents can be used only in the integral atomizer without difficulty and without producing an unstable flame. On the other hand, flames cooler than air-hydrogen are not usable with type (*b*), while the spray chamber-atomizer can be used with any flame.

2.2. The Burner

As will have been gathered from the preceding description, two main types of burners are employed. The Meker type burner is most often used for cooler flames. In this type the flame gases are mixed inside the burner tube and are prevented from striking back by a grid at the mouth of the tube. Different grids are employed for different gas mixtures, but

the orifices in the grid for the hotter flames are generally inconveniently small so that frequent cleaning may be necessary; the integral atomizer-burner has already been mentioned.

2.3. The Optical System

Flame photometers can be divided into two groups on the basis of their optical systems. In group 1, the required part of the spectrum is selected by means of absorption or interference filters. These instruments are suitable only for the determination of sodium and potassium in biological fluids, whatever the claims of manufacturers. In group 2, emissions are isolated by means of a prism or diffraction-grating monochromator. When used with a higher temperature flame, such as oxy-acetylene, instruments of this type are capable of determining sodium, potassium, calcium, and magnesium in all biological fluids and tissues; they are also capable of determining many other elements of biological importance, such as iron, manganese, and cobalt, after a suitable preliminary extraction into organic solvents.

2.4. Photosensitive Detectors

Three types are used. (*1*) *Barrier-layer cells.* These are satisfactory only for simple filter instruments. (*2*) *Vacuum phototubes.* These tubes require an external power supply, unlike barrier-layer cells, and their output is usually amplified before measurement. (*3*) *Photomultiplier tubes* are easily the most satisfactory detectors for use in flame photometry. The photocurrent is amplified inside the tube in such a way that much lower light levels can be detected and measured accurately than is possible with vacuum phototubes with amplifiers. A stable source of high voltage up to perhaps 2000 volts is required to operate the photomultiplier tubes, but these tubes are almost universally used in high-performance instruments and are essential if the advantages of using narrow band width are to be obtained.

2.5. Measurement of Emission

The emission of the selected element may be presented in several ways. Easily the most satisfactory is a direct-reading galvanometer. While most simple instruments use this method, some of the more expensive instruments use a nullpoint method. In the author's opinion this latter method of measuring emissions is less suitable for the best flame photometric work.

For more complete and advanced studies, recording devices which

record the emission while the wavelength is continuously varied give much more information than is easily obtained by other means.

2.6 Internal Standard Instruments

Internal Standard Instruments. In some instruments a double-beam principle is employed. An internal standard element, such as lithium, is added to a constant concentration to all unknown and standard solutions. Dual optical paths are employed and the internal standard emission, after

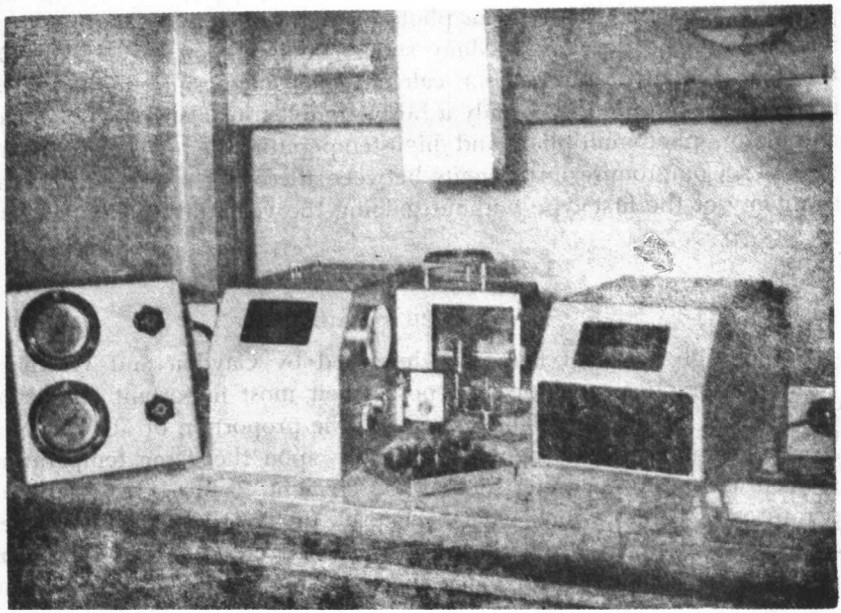

Fig. 4. A flame photometer with double monochromator. (By permission of Carl Zeiss, Germany, through Degenhardt, London, W.1.)

isolation by a suitable optical system, is focused on a photocell. The current produced from this cell is opposed to the current from a separate photocell which responds to the isolated emission from the element being measured. The opposing currents are balanced by means of an accurate potentiometer, the potentiometer readings to produce balance when known solutions are sprayed is noted, and the unknown concentrations are deduced. This method is effective in eliminating or minimizing spray interference (see below) but is incompletely effective in the other types of interference met with in biological samples. Unfortunately, it is just these types of interference which occur most often and which present