CHEMICAL MICROMETHODS

in Clinical Medicine

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FOREWORD

It is a great pleasure to introduce this book by my colleague Dr. R. H. Wilkinson. The developments in modern biochemistry supply most valuable weapons for the present-day physician in his attack upon many problems. As one who has lived through these developments, from the early 1920's when colorimetric methods first came into use to the present-day wonders of spectrophotometry, electrophoresis and chromatography I can sense a growing feeling of increasing confidence and closer collaboration between ward and laboratory. All working with children can rejoice that, as Dr. Wilkinson records, venipuncture is no longer necessary in many instances, and since 1956 has not been required for fifty types of analysis at The Hospital for Sick Children, Great Ormond Street, London. This would have greatly pleased my old chief, Sir Frederic Still, who so kindheartedly dreaded the increasing use of the needle in the vein. I think he would also have been immensely impressed not only by how much knowledge can now be obtained from so little a quantity of blood but also by the speed with which answers can be given, so that, as Dr. Wilkinson points out, it is possible to have repeated records for a child whose condition is rapidly changing — the difference between a "still" photograph and a cinematograph picture. I am sure that the methods described ought to be more widely used and this book will help to make this possible.

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PREFACE

In recent years, micromethods have made it feasible to follow serial changes in body chemistry. This has led to a greater knowledge of the changes brought about by disease. Clinicians in all departments of medicine have begun to ask for these estimations, by micromethods on a routine basis, both to investigate disease and to provide a day-to-day guide for therapy. The experience gained in providing such a service over a period of years is offered in the hope that the clinical worker will appreciate the advantages of using these methods routinely, and that the laboratory worker will know the requirements and problems of providing such a micro chemical service.

I wish to thank the Hospital for Sick Children, Great Ormond Street, London, for permission to publish this description of the work of the Department of Chemical Pathology. I should like to thank very warmly all the past and present members of the Staff of the Department for their loyal support, particularly when new methods were being introduced, and especially to thank Dr. W. W. Payne. I am grateful to Dr. C. O. Carter for statistical advice, to Mr. D. Martin, Department of Medical Illustration, and to Mr. G. Lyth, Medical Artist, for Figures 1, 4, 5, 6, 7, 8, 9, 13, 14, 16 and 17. I thank Messrs. Evans Electroselenium Ltd., for Figures 2, 10 and 15; Messrs. Elliotts Liverpool Ltd., for Figure 18; Messrs. A. Gallenkamp Ltd., for Figures 11 and 12; Messrs. Hilger and Watts Ltd., for Figure 3; the *Great Ormond Street Journal* for Figures 8 and 9; the *Journal of Clinical Pathology* for Figure 16, and part of the text for pages 84 - 89; Dr.

M. C. Sanz and *Clinical Chemistry* for Figure 17. Dr. J. C. W. MacFarlane and Mr. D. J. Jenkins have read the whole of the text and given valuable advice and Mrs. P. Tizard's secretarial help has been much appreciated.

Finally, this short monograph should be regarded only as an example of the many alternative ways in which a micro chemical service can be provided for a hospital. I am convinced that whichever methods are evolved, the use of smaller amounts of blood for chemical analyses is of great importance in clinical medicine.

R. H. W.

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CHEMICAL MICROMETHODS IN CLINICAL MEDICINE

Chapter I

INTRODUCTION

MANY of the analyses performed by chemical pathology laboratories are on serum or plasma. The analytical techniques employed depend on the amount of sample taken. This is usually a measured volume and the methods fall into three groups. Volumes of 1.0 ml. or more, require macromethods. Volumes of 0.1 to 0.5 ml. require micromethods. Volumes less than 0.05 ml. (or 50 μl.) require ultra micromethods. The three groups differ in the types of pipettes used to measure the samples and in many other ways. Since the beginning of the 19th century, when blood was being examined with increasing frequency by chemical methods, the volumes necessary for the analyses have been progressively reduced. Thus 500 ml. of blood was available when venisection was practised, and occasionally was analysed for urea by the curious physician.

From 1900 to 1940, macromethods were used in the majority of laboratories. These are described for example, by Peters and Van Slyke (1932). Early in the century Bang had founded a system of micromethods, based on weighing blood absorbed on paper, by means of a torsion balance (Bang 1922). From then on, an increasing variety of micromethods have been described. Micromethods became much more widely used when photoelectric absorptiometers became available generally, in the period 1935 to 1945 (King 1946). The precision of the micromethods has been increased since then, by the use of spectrophotometers. Ultra micromethods have been used

in biological science, for example by Linderstrøm-Lang and his school, in beautifully precise researches. Similarly the success of microgram analyses in the Manhattan project for nuclear chemistry, has been described by Kirk (1950). The principles of these methods have been applied to the analysis of blood by Natelson (1951), Sobel and Hanok (1952), and Caraway and Fanger (1955).

Before 1950, the Department of Chemical Pathology of The Hospital for Sick Children, Great Ormond Street, London, used micromethods for serum chloride, proteins, blood urea, and blood sugar. The blood analyses required for the U.S.A./U.K. project on the cortisone treatment of rheumatic diseases accentuated the demand for more micromethods. These had already become necessary for the control of many metabolic diseases of children. The estimation of plasma bicarbonate was completed first, with its attendant problem of obtaining true plasma from capillary blood without loss of carbon dioxide. A flame photometer was constructed for the estimation of sodium and potassium. The treatment of severe burns required a capillary tube method for the packed cell volume (or haematocrit) together with plasma protein and haemoglobin estimations. There followed a series of different micromethods, each adapted to the particular needs of the children and of the laboratory. Since 1956, venipunctures have been practically unnecessary for any of the fifty types of blood analyses required in this large children's hospital.

There have been several reasons for the reduction in the volumes of blood used for analyses. The removal of a large volume of blood required for a single analysis harmed the patient. Later, only 10 to 20 ml. volumes were removed, and these were sufficient for a limited number of analyses. It was realized that groups of analyses gave greater clinical insight into the patient's disease, and

that related estimations could not be inferred from a single analysis. A serum chloride level was of great help, but in many cases, sodium and bicarbonate levels would vary independently of the chloride. Similarly, bilirubin levels were no substitute for the five or six liver functions measured routinely today. Using macromethods, the average number of analyses done today on one specimen would require 100 to 150 ml. of venous blood. It was realized that in difficult problems a single estimation done once a week bore the same relation to more frequent estimations as did "still" to cinema photography. This was found to be particularly true of the rapidly changing pattern of plasma electrolytes seen in paediatric surgery. A report on plasma electrolytes given to the paediatrician 24 hours later was useless, because it reflected little of the current situation.

The frequent removal of blood by venipuncture from adults was found to be unpleasant and capillary blood obtained from a finger or ear, after puncturing the skin with a lancet, was used. This coincided with the greater use of micromethods, photoelectric absorptiometers and flame photometers. Although micromethods allowed sufficient analyses to be made on venous blood, they restricted the number done on capillary blood. A finger prick could yield 0.5 ml. of plasma, sufficient for five micro analyses. Using ultra micromethods, some twenty analyses would be possible, and even when the haematocrit was 90% about five quantities could be estimated. Pathologists working amongst children have seen the obvious advantages of obtaining capillary blood from finger or heel puncture. The children react emotionally far less to a skin puncture than to a veni-puncture, especially of the internal jugular or femoral vein, and the blood is obtained with much greater certainty.

The choice before the pathologist lies between micro and ultra micromethods. The present situation is that

micromethods are readily available and suitable for a busy routine hospital laboratory serving any number of adults or children. The laboratory staff need little additional training, new equipment or accommodation, when the change is made from macro to micromethods. On the other hand, ultra micromethods are suitable for single or small groups of workers in quiet surroundings set apart from the general laboratory. Special training and special equipment are required. The methods are as accurate and as dependable as their larger counterparts.

In this hospital, the main laboratory uses micromethods entirely, to serve the needs of a children's hospital of 300 beds. A separate unit is coming into use for ultra micromethods for specific projects. This monograph is concerned with micromethods. Although the methods were designed for use with capillary blood, they can be used perfectly well with venous samples sent to the laboratory in the usual manner. The apparatus which is described should be taken as an indication of what is required. Alternative apparatus is usually available in any particular country.