MICRODIFFUSION ANALYSIS AND VOLUMETRIC ERROR

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BY

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PREFACE TO FIFTH EDITION

THE use of microdiffusion analysis for various micro determinations being now widespread, it is considered sufficient in this preface to draw attention only to some new applications and developments of interest.

With regard to new methods and applications, and taking such in the order in which they appear in the present volume, one may note the following :

(*a*) The blood ammonium method has been much used of late in the study of hepatic disease. Among the many publications one may instance the paper of McDermott and Adams (1954), of Riddell and McDermott (1954), of Singh, Punjab, Barclay and Cooke (1954), of Sherlock, Summerskill, White and Phear (1954), of Traeger, Gabuzda, Ballou and Davidson (1954) and of White *et al* (1954).

As a result blood ammonium determinations are now being conducted in other laboratories as a routine procedure, using the microdiffusion technique, whereas before its introduction such measurements were regarded as an analytical refinement necessitating long experience and elaborate precautions, and unsuitable for the easy or rapid accumulation of exact data. In this connection, a recent publication of Hulme and Cooper (1956) has the title ' Blood ammonium estimation as a routine laboratory procedure '. Reference may be also made to a paper by A. E. Reif on ' The Ammonia Content of Blood and Plasma ' (1960).

Besides a description of the blood ammonium method there is included in the present volume a brief review of results obtained in the study of hepatic disease (Chapter XIII, pp. 124–128).

(*b*) In connection with total nitrogen determinations the procedure of Weil-Malherbe and Green (1955) for total nitrogen determination in brain slices is incorporated (Chapter XIV, p. 138), the interest here being the use of the microdiffusion procedure in conjunction with a mercury catalyst in the micro-Kjeldahl digest.

(*c*) A method for the determination of glutamine is described in Chapter XXI, p. 187. This method is essentially that of Krebs

(1948), adapted for microdiffusion. Glutamic acid may also be determined simultaneously with glutamine.

(*d*) The determination of monoamine oxidase and of histaminase by Cotzias and Dole (1951) using a microdiffusion procedure is described in Chapter XXII, p. 190, with the distribution of histaminase in animal tissues.

With these enzyme determinations may also be grouped that of acetylcholinesterase by Serlin and Cotzias (1955) as described in Chapter XXIX, p. 239.

(*e*) A group of microdiffusion determinations by Feldstein and Klendshoj (1954) including cyanide (even in normal blood) sulphide, phenols, methanol and isopropanol is described in Chapters XXX and XXXI, pp. 241–247 ; also their system for the determination of volatile poisons of toxicological interest in Chapter L, p. 335.

In this connection there may be mentioned the determination of azide by Brady and O'Callaghan (1955) (Chapter XXX, p. 242) and of urethane by Boyland and Rhoden (1949), the latter depending on the liberation of ethanol by alkali as described in Chapter XXXIII, p. 256.

(*f*) The application to the determination of formaldehyde, using the chromotropic acid reaction has led to the determination of the formaldehydogenic steroids by groups of workers. Bassil and Hain (1950) first reported the use of the microdiffusion procedure for absorbing the formaldehyde produced by periodic acid oxidation, the procedure being improved by Hollander, DiMauro and Pearson (1951), also by Wilson and Hildegarde (1953) as described in Chapters XXXV and XXXVI, pp. 262–268.

Brooks and Norymberski (1953) and Edwards and Kellie (1954) employed a similar technique after oxidation by sodium bismuthate instead of periodic acid in the outer chamber. Their procedures are given in Chapter XXXVI, p. 266. In this connection also, one may note the determination of the amino acid, glycine, by Schwartz, Robertson and Holmes (1955), depending on the conversion of glycine to formaldehyde by the action of ninhydrin (Chapter XXXVII, p. 269).

(*g*) The application of the microdiffusion technique by Burbridge, Hine and Schick (1950) to the determination of acetalde-

hyde, using semicarbazide as the absorbent, is described in Chapter XXXVIII (p. 273). In this connection reference may also be made to the more recent work of Berka on the estimation of carbon monoxide in blood (1955). He uses the microdiffusion procedure and in one variant shows how a semi-quantitative estimation of carbon monoxide in 0·2 ml. of a blood sample can be completed in five minutes.

The procedure, using semicarbazide absorption, has recently been employed by Ryan (1958) in this laboratory for an improved method for the determination of lactic acid in blood, and applicable also to tissues (Chapter XXXIX, p. 277). In this the lactic acid is oxidised to acetaldehyde by ceric sulphate in acid solution in the outer chamber of the microdiffusion ' unit '. With this improved method, which gives practically quantitative results over a wide range, glucose need not be removed prior to the oxidation, and a trichloroacetic acid filtrate can be used directly.

(*h*) Developments in halogen determinations may be noted with respect to the use of Fast Green as absorbent by Gordon (1952), Chapter XLIII, p. 301, and the determination of organically bound halogens by Gordon (1952) and by Pirt and Chain (1952), as described in Chapters XLIII (p. 301) and XLVI (p. 320).

(*i*) With regard to carbon monoxide determinations, a rapid clinical method by Lehmann (1944) is described in Chapter XLIX, p. 333, his procedure for plasma bicarbonate being outlined in Chapter XXIV, p. 201.

New developments in apparatus

A modification of the standard microdiffusion unit, by Öbrink (1955) is described in Chapter II, p. 13. It should prove useful in special conditions, e.g. relatively high temperatures, and in halogen determinations, at the lowest concentration levels. Another modification has been described by Berka (1959) which could be used also for relatively high temperatures.

A modification used by Edwards and Kellie (1954) which they found useful in the determination of formadehydogenic steroids is described in Chapter XXXIV, p. 259, and a ' diffusion-distillation unit ' introduced by Kirk (1950), in Chapter II, p. 14.

A rack and holder used for the handling of large numbers of

' units ' at the same time and introduced by Schwartz, Robertson and Holmes (1955) is shown in Fig. 57, p. 270.

For occasional shaking of ' units ' when this is required, a new ' vibrating table ' is described in Chapter III, p. 24. A ' vibrating table ' for shaking has been in occasional use in this laboratory since 1953 and is open to further improvement. From the results obtained therefrom, it appears that a vibrating table in one form or another is the most efficient shaker for the ' units '.

My thanks are due to the various workers mentioned above for their kind permission to include material and illustrations from their published work ; and also the editors and publishers of the *Annals of Surgery* ; *Analytical Chemistry* ; the *Analyst* ; the *Biochemical Journal* ; the *Journal of Biological Chemistry* ; the *Journal of Clinical Endocrinology* ; the *Journal of Laboratory and Clinical Medicine* ; and the *Lancet.*

While this edition was in press my attention was drawn by Professor Ishizaka of Nagoya City University (who translated this book into Japanese) to a microdiffusion unit in use in Japan. The upper edge has a rim which projects out some few millimetres, and the unit is covered by an octagonal shaped lid. A flexible and simply made metal band can be used (if desired) to fix the lid more firmly in position. Professor Ishizaka has also designed a special lid with suitable inlet tubes, which he has used successfully to determine accurately the oxygen content of blood. It is hoped to describe this work in a subsequent edition.

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CHAPTER I

INTRODUCTORY

In the development of modern biological research large numbers of observations become increasingly necessary in determining the questions at issue. It becomes essential to have analytical methods of a micro kind in which, without sacrifice of accuracy with respect to macro procedure, the labour and time expended with the single analysis are reduced to a minimum.

In the search for such a method, primarily for ammonia and urea, the microdiffusion principle of analysis was elaborated here and a special diffusion apparatus and burette designed to give it effect. It was subsequently found applicable to a variety of micro determinations by the author and other workers.

The method in general would appear to be the simplest possible consistent with the maximum attainable accuracy in the handling of micro volumes. Distillation and aeration are eliminated, the passage of ammonia or other substance taking place by diffusion from one chamber, in which it exerts a certain tension, into an absorbing fluid in another chamber in which its tension is reduced to zero. With a knowledge of the diffusion conditions controlling this passage and used in the design of the standard microdiffusion apparatus or unit, full absorption times with many substances run from about half an hour to two hours.

With serial determinations the time expended on each determination need be only a few minutes; one of the special advantages of the method being the ease with which large numbers of accurate data can be assembled.

With the standard unit, the accuracy of ammonia and consequently, of urea and other determinations, is limited only by the accuracy of delivering and titrating fluid volumes of the order of 1 ml. With the development of the titration principles described in the text, this accuracy can be brought to any desirable level in practice, so that with comparative ease the percentage error need