

The new discoveries have, therefore, the widest implications, and for specialized matters no less than for those of a more general nature. In many cases the relationships seem more than mere analogies, and strongly suggest an underlying unity of principle in the growth and differentiation of organisms of the most highly diverse kinds. They also testify to the particular value, notwithstanding its recognized limitations, of the study of variation in unicellular organisms, and sustain the belief, long held by Dobell and others, and now more widely shared, that more than one of the current conceptions in biology must undergo profound modification as a result.

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RECENT DEVELOPMENTS IN POLAROGRAPHIC ANALYSIS

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ALTHOUGH almost twenty years have passed since the polarograph was devised by Prof. J. Heyrovský and his colleagues at the Charles University in Prague, it has only been during the last two years that use of the instrument has been widely accepted by industrial laboratories in Great Britain. Nevertheless, it is now generally agreed that the instrument is of unquestionable value, and the polarograph is taking its place in the equipment of the modern laboratory along with the spectrometer and photo-electric absorptiometer.

The fundamental principles of polarographic analysis were worked out in Prof. Heyrovský's laboratory and have since been confirmed in the United States. These principles are fully described in an excellent monograph by Kolthoff and Lingane¹ which surveys the literature of polarography up to the end of 1940. Since that date, many new applications have been developed, especially in the biological and organic fields, and it is these that I propose to survey.

Polarographic analysis depends essentially on the fact that when a gradually increasing potential is applied to an electrolyte solution in a special cell consisting of a dropping mercury electrode and a second non-polarizable electrode, it is possible to determine from the resulting current-voltage curve both the nature and the concentration of the reducible or oxidizable substance or substances present. It is these current-voltage curves that are recorded by the polarograph.

A typical polarogram obtained with an air-free solution of 0.001 *M* cadmium chloride in 0.1 *N* potassium chloride is shown in Fig. 1. Under standard conditions, the limiting or diffusion current (that is, the height of the step) is proportional to the concentration of the electroreducible substance. This serves as the basis of quantitative polarography. The half-wave potential, which, as its name implies, is the value of the potential of the dropping mercury electrode, standardized against an external reference electrode (usually the saturated calomel electrode), at that point on the current-voltage curve when the current is one-half its limiting value, is a special property of the particular electroreducible substance present and is independent of the concentration of

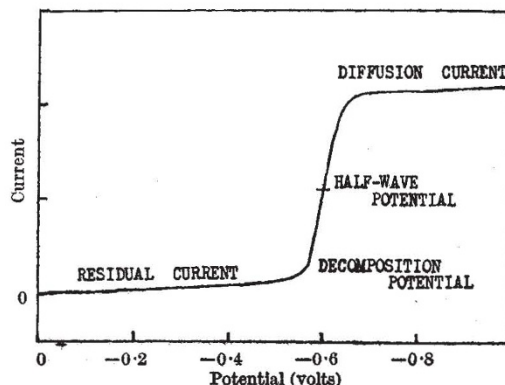


Fig. 1. CURRENT-VOLTAGE CURVE FOR SOLUTION OF 0.001 *M* CADMIUM CHLORIDE IN 0.1 *N* POTASSIUM CHLORIDE.

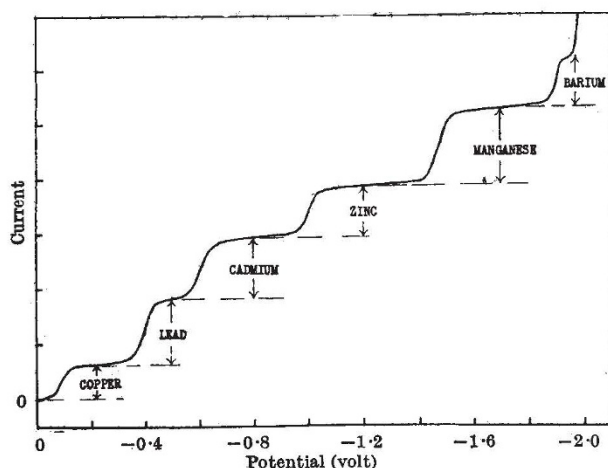


Fig. 2. CURRENT-VOLTAGE CURVE FOR SOLUTION CONTAINING COPPER, LEAD, CADMIUM, ZINC, MANGANESE AND BARIUM IN 0.1 *N* CALCIUM CHLORIDE.

the substance and of the characteristics of the electrode used. Qualitative polarography is based on this conception.

Theoretically, every substance can be analysed polarographically if it is electro-reducible or -oxidizable within the potential range of the electrode. The maximum range of the dropping mercury electrode is from +0.6 to -2.6 V. *v.* the saturated calomel electrode; but for most solutions it is much smaller. If there are several electro-active substances present in the solution, they can all be estimated, provided that their half-wave potentials are at least 0.2 V. apart. Fig. 2 shows a polarogram for a solution containing traces of copper, lead, cadmium, zinc, manganese and barium in 0.1 *N* calcium chloride. A further separation of steps can sometimes be achieved by altering the *pH* of the solution or by changing the reactants into complexes, from which they are deposited at potentials far enough apart for each step to be measured separately.

The diffusion current is governed by Ilkovič's equation,

$$i_d = knD^{1/2} Cm^{2/3} t^{1/6},$$

in which i_d is the diffusion current in microamperes, n is the number of faradays of electricity required per molar unit of the electrode reaction, D is the diffusion coefficient of the reducible or oxidizable substance in sq. cm. per second, C is its concentration in millimoles per litre, m is the weight of mercury in mgm. flowing out of the capillary per second, and t is the drop time in seconds.

It has been deduced from theoretical considerations that the constant k should be equal to 605 at temperatures between 15° and 40° C. The approximate correctness of this value has been verified experimentally in the few cases where sufficiently accurate values of diffusion coefficients are available to permit a comparison. In most instances, however, this theoretical constant cannot be used to calculate diffusion current constants with any degree of accuracy, since diffusion coefficient data for the conditions existing in polarographic measurements are not to be found in the literature. These constants must therefore be determined experimentally.

The other factors to be considered in quantitative polarography have been discussed by Kolthoff², who has also directed attention to an anomalous 'water wave', which occurs in solutions containing a rela-

tively high concentration of a supporting electrolyte. This step usually starts at about -0.9 V. and reaches a maximum at -1.3 V.; but fortunately it can readily be eliminated by adding a trace of gelatin to the solution.

Several investigators have tried to develop schemes of polarographic analysis that would permit the systematic qualitative and quantitative analysis of any mixture of the common metallic elements, but before such a scheme can be developed it is necessary to obtain detailed information about the behaviour of each of the elements under different conditions. Recently, Lingane³ has published such data for arsenic, antimony, bismuth, tin, lead, cadmium, zinc and copper in various supporting electrolytes, and has pointed out that if the diffusion current constant of each dropping mercury electrode with known concentrations of the metal, provided that the characteristics of the particular electrode are also known.

The polarographic method is widely used in metallurgical analysis for determining trace metals in alloys; but it may equally be applied to the inorganic constituents of a host of other materials ranging from tap-water to various biological products. Since many organic substances are themselves reducible at the dropping-mercury electrode, it is frequently necessary to prepare the biological samples for analysis by special pre-treatments. Such methods have been described for the determination of lead⁴, arsenic⁵, and vanadium⁶.

A special application⁷ of the technique has been used for the measurement of ter- and quinque-valent antimony in blood and urine when studying the metabolism of the therapeutic antimony compounds employed in the treatment of bilharziasis and kala-azar. Using ordinary chemical methods, it is difficult to distinguish between ter- and quinque-valent antimony in biological material. However, ter- but not quinque-valent antimony in normal hydrochloric acid solution forms a good polarographic step with a half-wave potential *v.* the saturated calomel electrode at -0.15 V. (cf. Fig. 3) and consequently can readily be determined in the presence of the quinque-valent form. The latter can be determined after reduction with sodium sulphite. The procedure proved to be surprisingly rapid as well as accurate, so that it was possible to make a large number of measurements, which would have been impracticable by earlier methods. Samples of blood needed relatively little pre-treatment, and urine could be examined directly. Since the half-wave potential for ter-valent antimony is relatively low at -0.15 V. *v.* the saturated calomel electrode, its characteristic step appears before those due to the other reducible substances in urine. The steps formed by the latter substances would completely mask those produced by small quantities of a substance less readily reducible than ter-valent antimony. A similar procedure may be adopted for the determination of bismuth.

Oxygen dissolved in electrolyte solutions is reduced at the dropping-mercury electrode and yields two distinct steps, the first step being due to the reduction of oxygen to hydrogen peroxide and the second to the reduction of hydrogen peroxide, either to water or hydroxyl ion. The second step coincides with that obtained for the electrolysis of an air-free solution of hydrogen peroxide. These oxygen steps have been extensively used for measuring the oxygen content of a wide range of materials, including body fluids⁸,

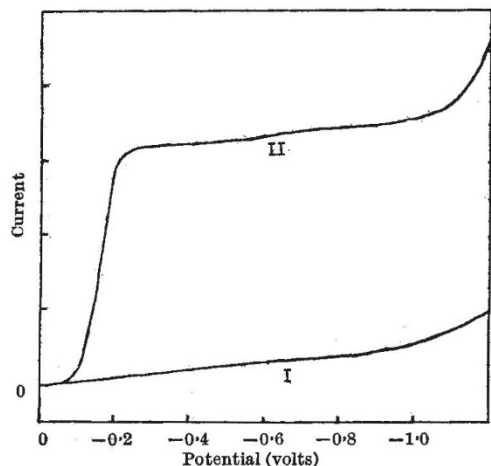


Fig. 3. CURRENT-VOLTAGE CURVE FOR ANTIMONY. I, 0.0001 M SODIUM QUINQUEVALENT ANTIMONY GLUCONATE; II, 0.0001 M SODIUM TRIVALENT ANTIMONY GLUCONATE.

technical gases, activated sludge and lake water, and for studying the photosynthesis- and respiration-rates of micro-organisms.

The polarograph is of considerable theoretical importance for the study of oxidation-reduction systems. An ideal example of a reversible reduction at the dropping-mercury electrode is given by quinhydrone and its components. In a well-buffered solution, the step due to the reduction of benzoquinone has the same characteristic half-wave potential as the step due to the oxidation of hydroquinone. If quinhydrone is examined, one half of the curve represents an oxidation of hydroquinone at the mercury anode, while the other half represents a reduction of benzoquinone at the mercury cathode. An oxidation-reduction system is thermodynamically reversible if identical polarographic half-wave potentials are obtained for the reduction of the oxidant and oxidation of the reductant. As would be expected, values for the oxidation-reduction potentials^{9,10} of such systems determined polarographically are in good agreement with those obtained by the classical methods.

The electrolytic examination of most organic substances involves reactions which are not thermodynamically reversible, since products are formed which cannot be oxidized or reduced to give the starting material at the same electrode potential. The reductions of aldehydes, ketones, unsaturated acids and nitro-compounds belong to this group.

The polarographic behaviour of the various vitamins which contain reducible groupings has been studied extensively. Aneurin (thiamin; vitamin B₁), riboflavin, nicotinic acid, pantothenic acid, ascorbic acid (vitamin C), α -tocopherol (vitamin E) and vitamin K can be determined in pure solution, but further work is required before they can be estimated in the presence of other reducible substances. Cholesterol¹¹ and other constituents of fish liver oils interfere with the polarographic determination of α -tocopherol. The mechanism of the riboflavin step has been investigated further by Brdička and Knoblock¹²

The dropping-mercury electrode can be used for the determination of certain sex hormones¹³: α : β -

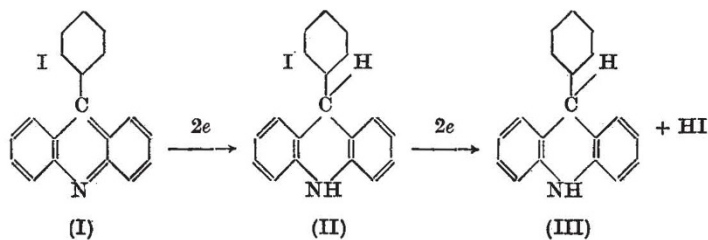
unsaturated keto-steroids (for example, testosterone, progesterone, corticosterone, and desoxycorticosterone) are reducible, while the 17-ketosteroids (for example, androsterone and *iso*androsterone) are not. Nevertheless, the latter may be condensed with excess Girard reagent T (trimethyl acetylhydrazide ammonium chloride) to yield derivatives which have an electro-active molecule. The steps are well defined and can be used for the determination of the different ketosteroids in urine extracts; but unfortunately the method cannot be applied to mixtures of ketosteroids as the individual steps are too close together.

Kolthoff and his colleagues¹⁴ have started a polarographic investigation of the sulphonamides with the view of testing theories as to their mode of action. Oxidation products of sulphonamides¹ such as *p*-hydroxylaminobenzenesulphonamide, *p*:*p'*-azoxybenzenesulphonamide and *p*-nitrobenzenesulphonamide are electro-reducible at the dropping-mercury electrode.

In addition to reversible and irreversible direct reductions, catalytic reductions may also occur at the dropping-mercury electrode. Brdička¹⁵ observed that if sulphur-containing proteins were reduced in a buffered cobalt or nickel solution, a large double step formed. Cystine and cysteine behaved in the same way, but only gave a single large step. Since these special steps were not obtained in the absence of cobalt and nickel, Brdička concluded that the hydrogen evolution from the sulphhydryl groups is catalysed by the metals.

The curious behaviour of these catalytic steps attracted considerable attention. When blood proteins from different individuals were examined, it was noticed that serum from cancer patients gave a much smaller protein double step than that resulting from the serum of normal patients. This phenomena appeared to offer great possibilities for the diagnosis of cancer, but unfortunately it has since been found¹⁶ that sera from patients suffering from pneumonia and arthritis show a similar effect.

The polarographic method may be used in synthetic organic chemistry to select the best conditions for carrying out electrolytic preparations at controlled potentials. Lingane, Swain and Fields¹⁷ found that the reduction of 5-(*o*-iodophenyl)-acridine (I) proceeds in two stages, first to 5-(*o*-iodophenyl)-dihydroacridine (II) and then to 5-phenyl-dihydroacridine (III) with elimination of iodine, the reduction potentials at the mercury cathode of the two stages being separated by about 0.3 V.



By careful control of the potential of the cathode, it was possible to prepare either compound (II) or (III) in a high state of purity and in almost quantitative yield. The method should be of particular value whenever the selective oxidation or reduction of only one out of two almost equally reactive groupings in a molecule is required.

There is no room in this short account of recent research with the polarograph to deal with the rapidly growing field of amperometric titrations, which has been developed so extensively by Kolthoff and his collaborators at the University of Minnesota. Nevertheless, it is hoped that sufficient material has been incorporated to indicate the versatility of the polarograph and the general usefulness of polarography.

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DIETARY STUDIES IN GREAT BRITAIN

THE lecture theatre at the London School of Hygiene and Tropical Medicine was well filled for the second part of the conference arranged by the Nutrition Society on "Budgetary and Dietary Surveys of Families and Individuals" held on May 20; and it remained full, practically to capacity, until the end. That there is an extraordinary and intense interest in the problems of nutrition, awakened largely as the result of the War, but drawing on the experience of those trained in nutritional problems during the War of 1914-18 and since, was manifest. Every paper was followed with close attention, though humour, conscious or unconscious—one member spoke of his "cooked figures"—received its reward.

The first part of the conference, held on February 5 (see *Nature*, March 11, p. 306), dealt with the practical and theoretical problems involved in making dietary surveys; the accuracy and the statistical validity of such surveys and the interpretation of results. The second part of the conference dealt with the results of surveys obtained by the individual, the group, the reminiscent and housekeeping methods, though no budgetary surveys were given. It was clear that each protagonist was satisfied with the method he adopted and inclined to doubt the validity of the other methods. Also it was clear that the Nutrition Society is bowing its knee to the Dagon of statistics, possibly with too little care to see that the figures before 'manipulation' approximate to reality. However that may be, there were gathered together a large number of people willing and able to bring their scientific ability to bear on the problem of feeding the people in the optimal way.

The chairman, Dr. J. Hammond, opened with almost the fewest possible remarks, and called on

Miss E. M. Widdowson and Dr. R. A. McCance (Department of Medicine, University of Cambridge) for their paper on dietary surveys by the individual method. The paper was based by Miss Widdowson on the dietary surveys of more than a thousand middle-class children in the years before this War, on some recent surveys, and on figures already reported for 63 men and 63 women. The problem was to obtain the figures and then to 'manipulate' them. The figures were obtained by the weighing of foods at table by intelligent, though not necessarily laboratory-trained, people. In calculation, tables for cooked, not raw, foods were taken. When results from tables were compared with actual laboratory analysis, there was not much difference, except in calcium and iron. The calcium and iron were higher in analysis as the result of tap-water cooking and contamination of the foods with iron from knives, saucepans and mincing machines. A week's survey is sufficient, but there are huge daily fluctuations in intake. The results obtained from the thousand children show almost as great a deviation from the mean as those of the 63 men and 63 women. At any age, one child in that age group might be eating twice as much as another in that same group. A boy of two years might be eating more of some dietary constituent than one of seventeen; protein and vitamin B₁ increase with age, calcium and vitamin C remain constant or fall as the age increases. Boys of fourteen ate four times as much meat as those of four, but only twice as many potatoes. Family and individual dietaries should be carried out side by side. If a family is getting its 'right' quota, but father is taking 4,500 calories, then the rest of the family (say mother and son) are being underfed.

Prof. V. H. Mottram (King's College of Household and Social Science, University of London) agreed that the man-value story has proved useless and should be buried. He believed that all this work points back to the calorimeter. The daily fluctuations cancel out in a group, so that a day's intake is sufficient guide. Prof. J. R. Marrack (London Hospital Medical School) asked what was done about made dishes which might vary in cooking, and suggested that the histories of these children who over- or under-fed should be followed up.

Dr. F. C. Happold (University of Leeds) said that the determination of expenditure of energy by medical students agrees with both weighed and estimated food intakes with small standard deviation. Others asked whether the readers' figures had been analysed statistically and whether colds did not alter food intake. Mr. A. L. Bacharach (Glaxo Laboratories) pointed out that what children eat is not necessarily a measure of what they ought to eat. In reply, Miss Widdowson admitted that made dishes are the nightmare of dietary surveys, minor ailments do influence food intake, though minor ailments are part of ordinary life and that her figures had been submitted to statistical manipulation. Dr. Gertrude Wagner (Wartime Social Survey) gave the results of surveys of methods used in preparing and cooking food. These investigated the ways in which people in different parts of England buy and prepare vegetables. 2,600 interviews were made. 100 per cent of families take potatoes, 90 per cent 'greens', 80 per cent carrots but only 47 per cent leeks. Higher income classes eat more vegetables than lower, but women at work often cook as many vegetables as those with more leisure. Only the well-to-do use