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Enzymes as reagents in clinical chemistry

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Clinical chemistry is concerned with the measurement of substances in biological matter, predominantly blood, serum or plasma. Significant, though small, changes may take place as a prelude to a life-threatening situation. Therefore analytical techniques in clinical chemistry must be sensitive, specific and rapid.

Many features of an enzyme-catalysed reaction are incorporated in the design of diagnostic reagents. The specificity of an enzyme may be employed to measure a substrate, or to remove interferents in another reaction. The measurement of substances that act as cofactors, inhibitors or activators can be achieved by the use of the appropriate enzyme. Finally, the enzyme, as a catalyst, can be used as a label in various immunoassay techniques.

Clinical chemistry tests are carried out in a wide variety of environments, from the large laboratory undertaking many hundreds of analyses down to a clinic performing only a few tests. Enzymes are therefore employed in analytical systems based on widely differing presentations. Thus enzymes may be employed in a solution medium, immobilized on a surface of the reaction vessel or in a reagent strip. The requirements imposed on the reagent enzyme may be different in all of these situations.

Introduction

Clinical chemistry is concerned with the detection, diagnosis and management of disease by the analysis of substances in extracellular fluid. Most of these measurements are made on samples of blood serum or plasma. Measurements are also made on samples of urine, faeces and sweat.

The methods used in clinical chemistry must therefore be designed to cope with the measurement of an analyte in the presence of numerous other substances; consequently the methods must demonstrate a high degree of specificity for the analyte. Diagnostic information is obtained from a change in the concentration of the analyte, this change being brought about by a change in metabolic rate, enhanced synthesis or degradation combined with secretion, excretion or leakage from tissue of the affected organs. In many instances these changes are quite small and therefore method sensitivity must be taken into account when reviewing a method.

The changes in concentration of an analyte in the blood may occur rapidly and may result in a life-threatening crisis. In this case a diagnostic test must be rapid in its completion to be of any value. Furthermore, where it is necessary to undertake the measurement away from the strict controls of a laboratory, both the method and its reagents must be robust. Thus, diagnostic tests must be specific, sensitive and robust.

In a typical laboratory of a district general hospital at least ten of the more common tests will employ reagents that include enzymes. The amount of reagent employed in each test may vary from about 150 µl to 3 ml. The workload of that laboratory is probably in the region of one million analyses per year, with about 15% of these analyses requiring an enzyme as a

reagent. Thus enzymes are important analytical tools for the clinical biochemist. Enzymes are employed as reagents in three ways: (1) to assist in the generation of a signal; (2) in the recognition of an analyte; (3) as a label in a variety of immunoassay techniques.

Analytical techniques in clinical biochemistry must be designed not only to measure the analyte accurately, but also with regard to the clinical need. Thus the presentation of the method must be given careful consideration in the light of the total number of requests made and the speed with which the result is needed. Methods may be employed on multichannel analysers responsible for the analysis of several hundred specimens a day, or on a simple instrument performing only a few estimations a day. Furthermore, we are seeing today an increasing use of analytical systems outside the laboratory. Enzymes may form part of a reagent system to cater for any of these environments. An example of this diversity of method presentations is in the measurement of glucose where the laboratory may be required to analyse several hundred samples during the day on one highly automated instrument, and only a few at night on another instrument. In addition, the measurement of glucose may be undertaken in the clinic or the health centre.

Enzymes can therefore be employed as reagents in many different ways to meet these needs: (*a*) in a solution chemistry, (*b*) in an immobilized form, (*c*) in a reagent strip, and (*d*) in a thin-film chemistry system.

Signal generation

The most common enzymes employed in signal-generation systems in clinical chemistry are the dehydrogenases and the oxidases. These reactions can be linked directly or indirectly to reactions that produce NAD^+ or NADH, and hydrogen peroxide respectively. The reactions can be monitored by using absorbance, fluorescence or luminescence characteristics. For NADH, luminescence detection with the use of bacterial luciferase allows measurement down to levels in the picomolar range, which is superior in sensitivity to both fluorescence and absorbance techniques (Campbell & Simpson 1979). The majority of NADH-linked methods employed in clinical chemistry employ absorbance measurement.

Reactions that yield NADH may also be monitored by using diaphorase and a tetrazolium dye, reduction producing a coloured formazan product. This method may offer up to a three-fold increase in sensitivity compared with the monitoring of absorbance at 340 nm. However, there are disadvantages to this approach in that the working reagents are not particularly stable; furthermore, reducing agents that may be present in serum may lead to the non-enzymatic formation of formazans. It may then be necessary to run a sample blank (Mollering *et al.* 1978).

Reactions that generate hydrogen peroxide may also be monitored by absorbance, fluorescence or luminescence characteristics. The most common approach is that in which the peroxide is broken down by peroxidase with oxidation of an acceptor molecule to give a coloured product (Trinder 1969; Putter 1974). One suggested reaction mechanism with a reagent containing 4-aminophenazone and phenol involves the oxidation of aminophenazone to give an electrophilic intermediate, which then reacts with the phenol to give a coloured quinoneimine dye (Witte *et al.* 1978). The relative sensitivities of some of the common monitoring reactions are shown in figure 1. The data were obtained by using methods for measuring urate on a centrifugal analyser. The enzyme uricase produces hydrogen peroxide, which can be measured by production of a quinoneimine dye or NADH.

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Despite the high potential specificity available when employing an enzyme as a reagent, problems may arise from interference in some other component of the reactions involved. In reactions employing NAD^+ or NADH, problems may arise when measuring absorbances at 340 nm due to the presence of light-scattering species in the sample or reagent; this is particularly true when using a centrifugal analyser where light-scattering particles may spin through the light path during a measuring period.

In the hydrogen peroxide-generating reactions there are several reports of substances that interfere with some of the detector reactions (Blaedel & Uhl 1975). This is particularly true for bilirubin and reactions involving the production of a coloured quinoneimine dye (Witte *et al.* 1978). In an attempt to overcome this problem a method has been devised for measuring hydrogen peroxide based on the use of catalase, ethanol and aldehyde dehydrogenase (Haeckel 1976). However, although this procedure appears to overcome the problem of interference from substances such as bilirubin it is less sensitive than the quinoneimine dye method.

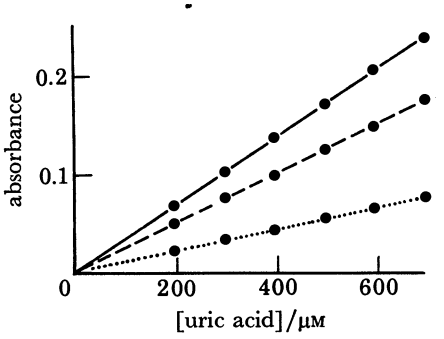

FIGURE 1. Calibration curves from three methods for the measurement of urate by using uricase. The hydrogen peroxide was measured with peroxidase to produce a quinoneimine dye (——), catalase, aldehyde dehydrogenase, NAD^+ and diaphorase (– –), and catalase, aldehyde dehydrogenase and NAD^+ (·····).

There are several reactions in which enzymes may be used to facilitate a monitoring reaction. Thus in the measurement of creatine kinase activity the ATP produced by the action of the kinase on creatine phosphate is measured by linking the glucose, hexokinase, glucose 6-phosphate dehydrogenase and NAD^+. Where enzymes are used in this way it is important that the linking enzymes are present in excess. A further example of this use of enzymes is in the measurement of aspartate aminotransferase where a reagent enzyme, malate dehydrogenase, in the presence of NADH can be used to measure a product of the reaction of interest, namely oxaloacetate. This particular method serves to illustrate a potential pitfall in the use of dehydrogenase reactions with biological samples; in this case a serum sample will contain pyruvate and the enzyme lactate dehydrogenase. The addition of NADH will allow the conversion of pyruvate to lactate in addition to the aspartate aminotransferase activity using NADH, until all of the pyruvate is converted to lactate. Thus the initial rate of reaction will be greater than that due to the aminotransferase. This problem can only be overcome by the addition of a vast excess of lactate dehydrogenase to the reagent, converting the endogenous pyruvate to lactate as quickly as possible (Rogerson & Osberg 1974).

This example serves to highlight another use for an enzyme, to enable the removal of interferents. Further examples where enzymes can be used to remove interferents are in the use of ascorbate oxidase to remove the interference of ascorbic acid in some methods for the measurement of urate. Glucose oxidase or hexokinase may be used to removed glucose from serum so

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that xylose may be measured by using a reductiometric technique or by the use of glucose dehydrogenase and NAD^+ (Borner & Danz 1981).

Enzymes may also be used to break down molecules to produce a measurable species. Thus cholesterol esterase is used to release cholesterol from its esters before measurement of the total cholesterol. Lipase is used to release glycerol in the measurement of triglycerides. It is very important that an esterase or lipase employed in this way should be active against all of the complexes present in samples from patients (Bucolo & David 1973; Zak 1977). Care must be taken particularly with quality control materials, to ensure than any esters or glycerides employed to enhance the analyte concentration should be capable of complete degradation by the appropriate enzyme.

Analyte recognition

The high degree of specificity of an enzyme has been used in the analysis of many substrates, coenzymes and activators or inhibitors of the enzyme. The measurement of glucose illustrates the way in which an enzyme can be used in the recognition of a substrate. Early methods were based on the reducing properties of glucose and were consequently of only limited specificity. The introduction of glucose oxidase led to great improvements in method specificity, although some problems have been encountered in the determination of the hydrogen peroxide produced. The use of the enzyme hexokinase and a second reaction employing glucose 6-phosphate dehydrogenase is regarded by many analysts as the most specific method; however, it is a more expensive method (Passey *et al.* 1977). A third approach employing glucose dehydrogenase may in many respects be considered as the method of choice as it is a single reaction with an enzyme having a high degree of specificity for glucose and the hydrogen acceptor, NAD^+ (Banauch *et al.* 1975). There are many more examples where enzymes are used in the measurement of metabolites, and some are shown in table 1.

The point has already been made that the specificity of the enzyme reaction may be compromised by a lack of specificity shown by the indicator reaction. This is so in the measurement of urate which has shown a significant improvement over the years with the development of enzyme-based methods. The enzymic methods are based on the action of uricase leading to the production of hydrogen peroxide and allantoin. It is possible to monitor the decrease in absorbance at 293 nm due to the disappearance of urate; however, the method may suffer from interference and simple instrumentation is not always capable of measurement at this wavelength. The measurement of the hydrogen peroxide produced may be affected by the presence of drugs and some endogenous compounds depending on the colour reaction used. Measurement of the hydrogen peroxide by using catalase, ethanol and aldehyde dehydrogenase overcomes many of these problems (Haeckel 1976), but the low concentration of urate present in serum means that a small absorbance change is obtained and at 340 nm the potential interference of particles in reagent or sample must be considered. Thus although the production of a quinone-imine dye offers greater method sensitivity over a 340 nm procedure, it may offer greater problems with specificity.

Quantitation of NADH by fluorescence measurement will offer improved sensitivity over absorbance measurements. This approach has been used for the measurement of intermediary metabolites, including lactate, pyruvate, alanine, glycerol and β-hydroxybutyrate (Lloyd *et al.* 1978); however, the measurement of these metabolites is generally confined to research laboratories because there is only a limited application for these tests in routine clinical biochemistry.

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TABLE 1. COMMON ENZYME-MEDIATED REACTIONS USED IN THE ANALYSIS OF SUBSTRATES IN CLINICAL CHEMISTRY

analyte	primary enzyme	auxiliary enzymes
acetoacetate	hydroxybutyrate dehydrogenase	—
ammonia	glutamate dehydrogenase	—
cholesterol	cholesterol oxidase	cholesterol esterase, peroxidase
ethanol	alcohol dehydrogenase	—
glucose	glucose oxidase	peroxidase
	hexokinase	glucose 6-phosphate dehydrogenase
	glucose dehydrogenase	—
hydroxybutyrate	hydroxybutyrate dehydrogenase	—
lactate	lactate dehydrogenase	—
pyruvate	lactate dehydrogenase	—
triglycerides	glycerol kinase	lipase, pyruvate kinase, lactate dehydrogenase
	glycerol dehydrogenase	lipase
urea	urease	alone or with glutamate dehydrogenase
urate	uricase	peroxidase/catalase, aldehyde dehydrogenase

TABLE 2. EXAMPLES OF THE MEASUREMENT OF ANALYTES BASED ON ACTIVATION OR INHIBITION OF A REAGENT ENZYME

analyte	reagent enzyme
theophylline	liver alkaline phosphatase
methotrexate	dihydrofolate reductase
digoxin	cardiac ATPase
pyridoxal phosphate	aspartate aminotransferase
thiamine pyrophosphate	pyruvate decarboxylase

The ability of a substance to activate or inhibit an enzyme can be used in the measurement of the substance. Some examples of this approach are shown in table 2. It is preferable that the reagent enzyme is not present in the sample; if it is, then extraction of the analyte from the sample is necessary. It must also be remembered that other factors affecting the reagent enzyme may be present in the serum, for example metal ions such as magnesium.

LABELS IN IMMUNOASSAYS

The third major use for an enzyme as a reagent is as a label in an immunoassay. Enzymes have been employed in several different approaches to measurement of antigens and antibodies (Feldman *et al.* 1976; Wisdom 1976; Schuurs & Van Weemen 1977). The attraction of the enzyme-labelled approach lies in its ability to replace the radioisotope, which may reduce the need for dedicated equipment and specialized laboratory facilities. It has been shown that replacement of a radioisotope with an enzyme label will result in comparable analytical performance for a similar assay protocol. However, the major interest in the use of an enzyme as a label is that it is possible to produce a homogeneous immunoassay making the need to separate free and bound labelled antigen redundant (Kabakoff & Greenwood 1981). An illustrative protocol for a homogeneous enzyme immunoassay is shown in figure 2. The sample antigen reacts with the antibody and then enzyme-labelled antigen is added, thereby competing for antibody-binding sites. The system is designed such that the antibody binding the labelled antigen results in either inhibition or activation of the labelled enzyme. In the former

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case only free enzyme-labelled antigen is active; thus the more sample antigen present, the more enzyme activity there is.

Thus homogeneous enzyme immunoassay is well suited to the measurement of low molecular mass haptens and has been particularly successful in the measurement of drugs (Kabakoff & Greenwood 1981). More recently the homogeneous approach has been applied to the measurement of high molecular mass antigens such as immunoglobulin G and C-reactive protein (Gibbons *et al.* 1980).

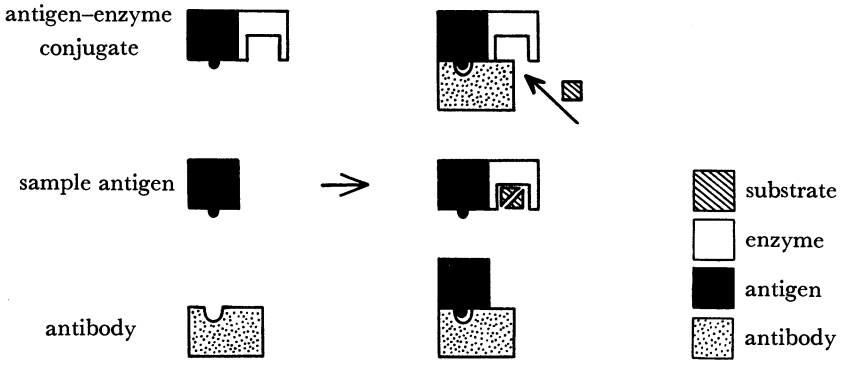

FIGURE 2. Major reactants and products in one approach to a homogeneous immunoassay with an enzyme-labelled antigen.

One of the major advantages of the homogeneous immunoassay is the reduction in the number of manipulative steps and the ease with which the method can be automated. These features have resulted in improvements in method precision. One disadvantage of the homogeneous approach is that constituents in the sample may produce an interfering side reaction. Thus in a homogeneous immunoassay with a dehydrogenase label it is possible for endogenous pyruvate and lactate dehydrogenase to combine with reagent NADH to produce an interfering reaction. It is important that the reagent includes a means whereby the side reactions can be inhibited.

Although an enzyme label led to the development of the first homogeneous immunoassay, there are now other competing options. Thus a substrate-label approach has also been developed with binding of the antibody to substrate-labelled antigen, effectively inhibiting the action of an enzyme on the reagent. This approach has been employed with an umbelliferyl galactoside label with galactosidase in the reagent (Burd *et al.* 1977). A further approach has been described with the use of an enzyme inhibitor as a label, with antibody binding to the labelled antigen modifying the action of the inhibitor toward the enzyme in the reagent (Finley *et al.* 1980).

REAGENT MEDIUM

The most common presentation of a method is in solution form. Instrumentation available to the biochemist means that depending on what equipment he buys he may be considering a reagent consumption of 3 ml per test, down to 150 µl. This means that reagents may be required in bottles from 3 ml up to 2 l or more. There are two major implications as a result of this diversity of requirement. It is important that the working reagent is stable; if this is so then fewer bottle sizes are required because prepared reagent can be stored. A brief survey of the stability of some working reagents in my own laboratory indicates a wide variation in reagent stability from method to method, with very poor stability in many cases (table 3).

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When reagent stability is poor, the reagent manufacturer must consider the provision of different sizes of reagent kit. If this is necessary it is important that the reagents from these different pack sizes are capable of giving the same analytical performance. The variation in performance between kits (shown in table 4) for the measurement of creatine kinase was considered not be be acceptable. The results from 50 samples analysed by three different formulations of the same method were compared with one kit from another manufacturer. The clinical need for this assay was an attempt to detect female carriers of muscular dystrophy, where very small elevations in the serum creatine kinase levels are seen (Tippett *et al.* 1982). The lack of good comparability between the methods means that each laboratory would have to establish its own experience with normal controls and carriers and one assay kit before being able to use the test with any confidence.

TABLE 3. A BRIEF SURVEY OF THE MANUFACTURER'S QUOTED STABILITY OF SOME ROUTINE WORKING REAGENTS IN CLINICAL CHEMISTRY

assay	quoted stability (days) at 2–8 °C	25 °C
cholesterol	28	5
triglycerides	1	0.2
urate	1.5	0.3
creatine kinase	7	0.5
aspartate aminotransferase	1	0.3
alanine aminotransferase	0.4	0.2

TABLE 4. REGRESSION PARAMETERS FOR A COMPARISON OF RESULTS WITH THREE KITS OF DIFFERENT SIZES (Y_1, Y_2, Y_3) FROM ONE MANUFACTURER FOR THE MEASUREMENT OF SERUM CREATINE KINASE

(The results from 50 samples were compared with a kit (x) from another manufacturer; all the kits employed the same optimized reaction conditions.)

size	regression parameters
100 ml	$Y_1 = 0.93x + 1.4$
20 ml	$Y_2 = 1.02x + 0.2$
3 ml	$Y_3 = 0.89x + 3.0$

Kinetic assays

The advent of continuous reaction-monitoring discrete analysers has led to the development of kinetic assay procedures as an alternative to endpoint techniques (Pardue 1977). In the use of an endpoint procedure it may be necessary to include the determination of a blank; furthermore, the reaction may require a long incubation period to reach completion. The kinetic approach can overcome these problems in that by measuring the rate of reaction it is not always necessary to undertake a separate blank determination, and the incubation period can be reduced. The result is that assay precision is improved, interferences reduced and sample throughput increased (Tiffany *et al.* 1972).

The data in Table 5 show the within-batch precision for three enzyme-mediated techniques in fixed-interval and endpoint mode. The results were obtained on a Multistat III micro-centrifugal analyser (Instrumentation Laboratory, Lexington, U.S.A.).

Consideration of enzyme reaction kinetics suggests that first-order reactions are most suited to the measurement of substrates, with the change in concentration of the substrate over a

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specified time interval being proportional to the initial substrate concentration. The Michaelis–Menten theory of reaction kinetics shows that pseudo-first-order reaction kinetics apply if the substrate concentration is much lower than the Michaelis constant (K_m) of the enzyme; results suggest that the ratio of substrate concentration to K_m should be below 0.2 (Ziegenhorn 1980).

The applicability of this technique depends on the concentration of the analyte and the K_m of the enzyme. However, in some instances the K_m of the enzyme may be too low; in this situation it is possible to increase the K_m by the use of a competitive inhibitor. Fixed-interval techniques have been described for the measurement of several analytes, including glucose, triglycerides, urate and urea (Ziegenhorn 1980). It is important in this type of method that the time interval over which the reaction is monitored is strictly controlled; these techniques are therefore generally linked to instruments in which this part of the operation is controlled by the instrument rather than by the operator.

TABLE 5. COMPARISON OF WITHIN-BATCH PRECISION FOR THREE ENZYME-MEDIATED TECHNIQUES WITH THE USE OF FIXED-INTERVAL AND END-POINT MODES

analyte	concentration mM	coefficient of variation (%) (n = 18) fixed-interval	end-point
cholesterol	3.3	1.09	2.92
	5.7	0.97	2.71
	7.8	0.74	1.94
triglyceride	0.9	2.80	5.24
	1.8	1.71	3.92
	3.8	1.62	3.40
urea	3.6	1.41	4.20
	12.4	0.97	2.31
	18.2	0.82	1.71

Immobilized enzymes

Although most enzymes as reagents are employed in solution chemistries, in recent years attempts have been made to immobilize the enzyme. This has led to the use of immobilized enzymes in continuous-flow analyzers (Campbell & Hornby 1977; Carr & Bowers 1980) and also to the development of so-called enzyme electrodes (Guilbault 1975). The advantage of this approach is in the reduced usage of expensive reagent enzyme, in that the enzyme can be reused, and furthermore the enzyme often demonstrates enhanced stability in the immobilized form. This is illustrated in figure 3, which shows the stability of uricase in an immobilized continuous-flow system, compared with a similar chemistry in solution form.

This approach is limited to instrumentation in which the reaction chamber is reusable and therefore is most appropriate to the continuous-flow analyser. The use of immobilized enzyme systems has been successfully applied to a continuous-flow analyser used for the monitoring of intermediary metabolites *in vivo* (Noy & Alberti 1981) and also in a prototype artificial pancreas system (Albisser *et al.* 1974).

The immobilization of a reagent enzyme has also been employed in discrete analysis systems, albeit in the more manual types of apparatus. Thus enzymes have been immobilized on to mixing devices and also on to the ion-sensitive surfaces of electrodes. This technique has been used in the Yellow Spring Instruments glucose analyser in which the hydrogen peroxide

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generated by the action of glucose oxidase is oxidized at the platinum anode (Chua & Tan 1978: the current produced is proportional to the concentration of glucose. The circuit is completed by a silver cathode. The electrode is protected from the sample and diluent by a double membrane. An outer polycarbonate membrane allows the diffusion of glucose and other small molecules toward the electrode but excludes cells and proteins. The inner cellulose acetate membrane allows hydrogen peroxide, oxygen and water to pass through but excludes glucose and interfering substances such as ascorbic acid. Between the membrane layers glucose oxidase is immobilized in a thin layer of resinous material. The system can be used for the measurement of glucose in whole blood, serum or plasma. This type of equipment is best suited for single or small-batch determinations. At present this probably accurately reflects the use of this type of immobilized enzyme system. When considering this type of equipment it is important to remember where it will be used, in the laboratory or on the ward. If this type of equipment is to be employed outside the laboratory it is important that the instrument be robust and simple to operate.

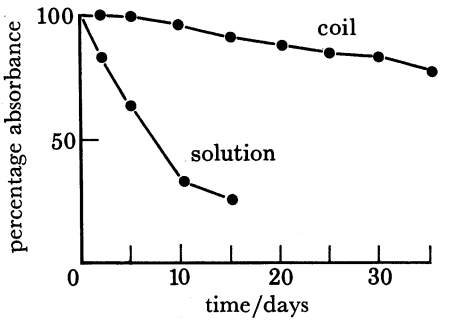

FIGURE 3. Data to illustrate the stability of uricase in immobilized and solution forms for the measurement of urate.

Reagent strip technology

One of the more recent and exciting innovations in clinical chemistry has been the development of chemistry systems immobilized on reagent strips or in thin films. Enzymes have figured in this development, and reagent strips for measuring glucose have been available for several years (Stewart 1976). In this approach a drop of blood is placed on to a reagent pad. The glucose diffuses through a semipermeable membrane into the body of the reagent pad, which contains glucose oxidase and reagents for the colorimetric measurement of the hydrogen peroxide formed. The colour produced may be matched against a colour chart to give a semiquantitative result or may be read off a simple but purpose-built reflectance meter. Evaluation of the latter approach in our own laboratory with Dextrostix and a Glucometer (Ames Division, Miles Laboratories, Stoke Poges, U.K.) with one trained operator indicated a between-batch precision coefficient of variation of 2.5 % at a sample glucose concentration of 6.2 mM. Comparison of results with an automated solution technique in the laboratory showed good agreement. It must be stressed that this performance was achieved using skilled personnel and does not necessarily reflect performance in unskilled hands.

Thin-film technology

The development of thin-film technology to produce analytical systems for clinical biochemistry has also included the use of enzymes (Curme *et al.* 1978). Systems have been described

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for the measurement of urea and glucose (Spayd *et al.* 1978). The general construction of a thin-film reagent system is shown in figure 4; it includes a means of excluding high molecular mass species while allowing diffusion of the sample into a reagent layer. It is also possible to use two reagents that are not entirely compatible. The product of the enzymic reaction can diffuse into a colour reagent. The colour produced is measured with a purpose-built reflectance meter.

Evaluations of this approach have appeared in the literature and, although there are certain problems that are not seen with solution chemistries, the performance appears to be acceptable (Burnett *et al.* 1982). This type of analytical system clearly requires a much tighter control of reagent preparation and gives the analyst less opportunity to exercise his skills.

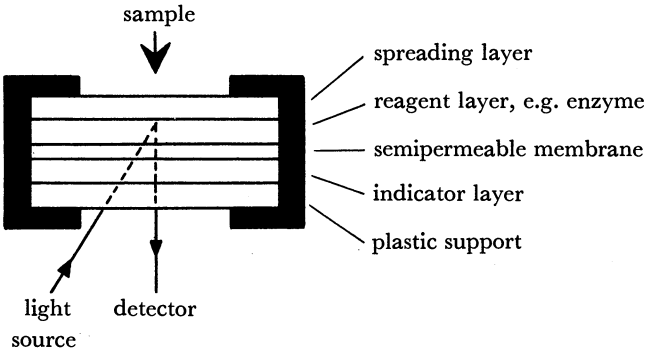

FIGURE 4. General construction of a thin-film reagent analytical system.

FUTURE POTENTIAL

Clearly enzymes play an important role in clinical chemistry and a large number of enzymes are used in a variety of ways and in different environments.

Perhaps two of the most exciting areas for future development of reagents are in the field of microbial enzyme technology. It has been shown that enzymes from thermophilic bacteria have far superior stability characteristics compared with their mesophilic counterparts. It has been shown for instance in the case of glycerol kinase that enzyme from a thermophilic bacterium has a catalytic activity half life of 310 min at 60 °C compared with less than 5 min for the enzyme from other bacteria and shows exceptional stability when stored at room temperature (Comer *et al.* 1979). Improved shelf life of reagent enzymes would be of great benefit to manufacturer and clinical biochemist alike.

Considerable interest is now being shown in the ability of bacteria to metabolize unusual substrates. Many species of bacteria produce enzymes that degrade or inactivate antibiotics. Thus it has been shown that gentamicin and chloramphenicol can be measured by the use of the respective acetyl transferase enzyme. For chloramphenicol the analytical system has been linked to a bioluminescent indicator reaction, giving good method sensitivity (Boeckx & Brett 1981).

Recently an enzyme capable of degrading paracetamol has been isolated and used in the measurement of the analgesic. As a result of the specificity of the enzyme, which produces acetate and aminophenol, together with a specific colour reaction for the aminophenol, the method is specific for the parent compound (Price *et al.* 1982). This method appears to offer all

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of the features required for an assay measuring a drug in an emergency situation, in that it is rapid and accurate with few manipulative skills required.

The development of microbial enzyme technology points to an exciting future in the field of diagnostic reagents. The stability that can be achieved with enzymes from thermophilic microorganisms will stifle one of the major criticisms of enzymes as reagents. The possibility of developing novel enzyme systems points to a significant increase in the variety of analytes that might be measured by using enzymes as reagents. It is not possible at present to predict the limitations of this approach beyond that of molecular size and specificity. However, it is clear that enzyme-mediated analytical techniques can bring a wider variety of robust and flexible methods to clinical biochemistry.

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