This year marks the 50th anniversary of the American Association for Clinical Chemistry. In tribute to this semicentenary, this journal will reflect on its published achievements over the past five decades. Our Editor has recently enumerated some of the publications that have had an impact on the field, as demonstrated by a solid history of citation. This editorial, the first in a projected series of four to appear this year, will reflect on those papers that have advanced the area of enzymes and protein markers—now a regular feature in these pages.

Of the papers that have achieved citation fame, approximately 20% deal with clinical enzymology. Yet another 20% focus on the use of enzymes as analytical tools, e.g., those listed in references 8–10. In the first volume of this journal (1954), enzyme measurements as markers of human disease were the focal points of only two papers. The topics were amylase and arginase. None characterized the use of enzymes as analytical reagents, reflecting clinical laboratory practice of the day. Today, in most laboratories, of the two dozen “routine” chemistry analytes, nearly two-thirds are often determined by enzymatic analyses. This is largely the result of the explosion in the number of studies of enzymes as catalysts, markers of disease, and tools for the laboratorian over the 1960s and the subsequent two decades.

This growth phase in enzymology was concurrent with the growth of the practice of clinical chemistry and of the AACC. Without these advances, modern clinical laboratory analyses would not be possible. The detection of proteins in serum by their catalytic activity as a reporter of tissue damage is a cornerstone of medical laboratory analyses. The uses of a wide variety of enzymes from a still wider variety of sources are now firmly entrenched in the arsenal of the laboratorian. The breadth of analysis, from a humble but often critical glucose measurement to the large number of enzymes used in molecular biology, is astonishing. If I may be allowed a degree of nostalgia, this author, who (within acceptable round-off error) completes his 5th decade concurrently with the Association, recalls an analytical chemistry professor chiding him as an undergraduate for considering a career in clinical chemistry or biochemistry. “That’s not real chemistry” he asserted, with an almost Nixonian emphasis on the word real, “they use enzymes for everything!” His disdain failed to dissuade me, but his words—unlike most else from that era, these were, after all, the 1960s—are often recalled with an appropriate sense of irony.

A paper that revolutionized the assay of what was, at that time, an obscure enzyme, γ-glutamyltransferase (EC 2.3.2.2, Fig. 1), is among the 10 most cited from these pages (810 citations). It describes the use of γ-glutamyl-4-nitroanilide as a substrate that is cleaved to form the easily measured 4-nitroaniline. Until this publication, assay procedures were not only more cumbersome to perform, they did not allow monitoring of the reaction

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**A Kinetic Photometric Method for Serum γ-Glutamyl Transpeptidase**

**Gabor Szasz***

A kinetic method is described for the determination of γ-glutamyl transpeptidase activity in serum. Optimal conditions for the reaction were ascertained, normal values in milliunits per milliliter at 25° established, and the reliability of the method examined. γ-Glutamyl-p-nitroanilide in ammediol-HCl buffer at pH 8.2 is employed as substrate. The method requires only 0.1 ml. of serum and, using an automatic cuvet positioner and recorder as well as an automatic diluter, more than 60 determinations can be carried out in 1 hr. On the basis of a large number of determinations, the method was shown to be technically excellent and clinically valuable.

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*This journal followed the founding of the AACC by some six years.*

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Fig. 1. Szasz’ original paper for measurement of γ-glutamyltransferase, *Clinical Chemistry* 1969;15:124–36.
rate. Szasz’ (2) method almost instantly was adopted by clinical laboratories. It was simple, well-characterized, and for the first time allowed facile measurement of a marker of enzyme induction and cholestasis. It was widely used as a subsequent action to the finding of an increased activity of alkaline phosphatase in serum. The method is still in use some 30 years later, and the name of Szasz is indelibly linked to the measurement of this enzyme. He and coworkers improved the method by describing the use of more soluble, derivatized substrates (11). However, this later method is firmly based on the original work, and one of the more soluble substrates, γ-glutamyl-3-carboxy-4-nitroanilide, was chosen so that reference values would be comparable with those originally described (2).

Gabor Szasz was a European, but he had strong links to AACC, and he chose this journal for much of his work, foreshadowing a wave of manuscripts in subsequent years from outside the US. He is but one of two names that appear more than once in the list of highly cited papers. He alone appears as first author on two publications (2, 5). Seeing his name twice on this list gives us pause to reflect about contributions that might have come from his laboratory had the field not lost one of the pioneers of its growth phase. Upon Szasz’ premature death early in 1979, Albert Dietz, then president of the AACC, reflected upon Szasz’ collaboration with his American colleagues and recognized his “important contributions to enzymology and clinical chemistry”.

I remember Gabor’s kindness, wit, and insight. Once, on a visit to my laboratory, the conversation turned to publications and research. I asked if he had read the latest work of so-and-so on some topic of mutual interest. “No!” he replied immediately, in his unique Hungarian-German accent. “One only has time to read or write, not both. I write”. I only recently came to know what he meant.

Late in his short life, Szasz turned his attention to creatine kinase (CK, EC 2.7.3.2), which along with its isoenzyme forms, was at that time the single most important laboratory marker for myocardial infarction. His thorough investigation of the conditions for measurement with Wolfgang Gruber and Erich Bernt (reference 5; Fig. 2) established the foundation for today’s measurement of this enzyme. Five subsequent papers (12–16)– also appearing in these pages–would round out these comprehensive efforts and form the basis for standardization of measurement of this enzyme.

CK isoenzyme measurements helped clinician discriminate between nonmyocardial and myocardial sources of the enzyme. Before the development of polyclonal and monoclonal antibodies for isoenzyme measurements, physical separation was the only option. Donald Mercer (7) in his classic paper on chromatographic separation of CK isoenzyme forms (Fig. 3) provided the first rapid and quantitative technique for the estimation of this critically important analyte.

Mercer was frustrated that analysis by polyacrylamide gel electrophoresis and subsequent staining was not sufficiently quantitative for the measurement of CK-2 activity for clinical applications. Then fresh out of graduate school, he had previously used minicolumns for the purification of radiolabeled substrates and reasoned that

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**Creatine Kinase in Serum: 1. Determination of Optimum Reaction Conditions**

Gabor Szasz,¹ Wolfgang Gruber,² and Erich Bernt²

To establish optimum conditions for creatine kinase (EC 2.7.3.2) activity measurement with the creatine phosphate → creatine reaction, we re-examined all kinetic factors relevant to an optimal and standardized enzyme assay at 30 and 25 °C. We determined the pH optimum in various buffers, considering the effect of the type and concentration of the buffer, as well as the influence of various buffer anions on the activity. The relation between activity and substrate concentration was shown and the apparent Michaelis constants of creatine kinase for creatine phosphate and ADP were evaluated. We tested the effect on creatine kinase measurement of the concentration of substrates (glucose and NADP⁺) in the auxiliary and indicator reactions, especially the influence of the added auxiliary (hexokinase) and indicator (glucose-6-phosphate dehydrogenase) enzymes on the lag phase, at different temperatures. The NADP⁺ concentration proved to be the factor limiting the duration of constant reaction rate. We studied the inhibition of creatine kinase and adenylate kinase by AMP and established a convenient AMP concentration. For reactivation of creatine kinase, N-acetyl cysteine as sulphydryl compound was introduced. Finally, we examined the relationship between activity and temperature.

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Fig. 2. The first in a series of six papers describing the optimal conditions for measurement of creatine kinase in serum, *Clinical Chemistry* 1976;22:650–6
Separation of Tissue and Serum Creatine Kinase Isoenzymes by Ion-Exchange Column Chromatography

Donald W. Mercer

I describe a simple, rapid anion-exchange column chromatographic technique for separating the creatine kinase (CK) isoenzymes in human serum and tissue. Extracts of CK-rich tissues (skeletal muscle, cardiac muscle, and brain) were used to determine optimum conditions for separating CK isoenzymes MM, MB, and BB. Samples, layered on mini-columns (0.5 × 6.0 cm) of DEAE-Sephadex A-50, were eluted stepwise with Tris-buffered sodium chloride (100, 200, and 300 mmol/liter). Column effluents were assayed by the Rosalki CK method. Distribution of total activity among the eluted fractions was tissue-specific and reproducible. Evaluation of sera from 71 patients with myocardial infarction and other diseases associated with elevated CK activity revealed isoenzyme patterns that resembled those of either cardiac muscle or skeletal muscle. Cardiac pattern (presence of MB isoenzyme) and clinical documentation of myocardial infarction were 100% correlated in the 35 patients so studied.

A Continuous Spectrophotometric Method for Measuring the Activity of Serum Alkaline Phosphatase

George N. Bowers, Jr., and Robert B. McComb

A continuous spectrophotometric method for measuring serum alkaline phosphatase activity is described. The effects of temperature, pH, substrate concentration, type and molarity of the buffer, sample size, cofactors, and inhibitors on the enzymatic hydrolysis of p-nitrophenyl phosphate were studied. The optimal conditions for assay of serum alkaline phosphatase at 30° were found to be 0.75 M 2-amino-2-methyl-1-propanol buffer, pH 10.5, 4 mmole substrate, and 100 μl or less sample size. Studies of the factors affecting analytical precision—i.e., control of reaction temperature, of reagent manufacture, and of standardization—are discussed. The precision of this method was 2.3% (relative standard deviation) on 10 within-day replicates and 5.0% on day-to-day replicates spread over a 5-week period. The range of activity for 258 apparently healthy adult blood donors was 6–110 mU/ml. (International milliunits per milliliter), with a mean of 49 and a standard deviation of 14.
for the decades when the diagnostic application of this test was secured.

Alkaline phosphatases have been nearly a lifelong labor for George Bowers and Robert McComb. It would not be hyperbole to state that they wrote the book on this topic (20). However, their development of a continuous monitoring technique for measuring alkaline phosphatase (reference 4, Fig. 4) was their very first collaboration. Until that point, Bob McComb’s interest in the enzyme was as a basic researcher investigating its activity in tumor cells. George Bowers’ interest was as clinician, but he is more of a chemist than many trained in that discipline. One of the first lectures on clinical enzymology that I heard was one presented by George Bowers. His incredible enthusiasm was infectious.

The synergistic cooperation of these two workers gave rise to many fruitful collaborations. Many readers will be familiar with papers dealing with enzyme measurements and their standardization from the Hartford team over three decades. It is, therefore, not surprising that their careful description of the conditions for measuring alkaline phosphatase formed the basis for the most popular technique for measuring its activity and the continued citation of this paper over the past 30 years.

As might be expected, the topics of the most highly cited papers (1) correspond well with the workload of most clinical laboratories. An exception is the description of an assay for dopamine-β-hydroxylase (3), which ranks 12th on the list of citations. This method was developed for clinical applications and allowed investigation of the role of this enzyme in neurological dysfunction. As with the case of CK-2, immunoassay and immunolocalization of this enzyme have had an increased role in studies of neurochemical function.

A common thread of these papers is the thorough investigation of the problem at hand, an attention to the details of measurement, and the primary objective of developing techniques suitable for the clinical application at hand. It is noteworthy that three of these papers (2, 4, 5) have served to great extent as the foundations of the International Federation of Clinical Chemistry (IFCC) reference methods for the three enzymes studied: γ-glutamyltransferase (21), creatine kinase (22), and alkaline phosphatase (23). Those who were active over the late 1960s through the early 1980s may recall the plethora of techniques available for enzyme activity, each claiming some degree of superiority over the others. That was the milieu when these papers were written. Not unlike today’s computer industry, the benefits of some degree of standardization have allowed significant progress and comparability of results. The basic work exemplified by these three papers (2, 4, 5) has measurably contributed to this accomplishment.

As we look towards the start of the new millennium and the 50th anniversary of this journal a few years later, it appears likely that we will continue to use enzymes for everything. These papers, and their new companions published each year in these pages, will continue to provide the useful tools in the field and exert an influence on still future work.

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