A 126 DETERMINATION OF URIC ACID IN BIOLOGICAL SAMPLES.
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We have developed a rate assay for the determination of uric acid in biological fluids. The assay has been designed for use on the COBAS BIO centrifugal analyzer and is a kinetic modification of the uricase method first reported by Bulger and Johns[1] and later modified by Kalckar.[2] The procedure measures the rate of decreasing absorbance of uric acid as it is oxidized by the enzyme uricase (urate: oxygen oxidoreductase E.C. 1.7.3.3) to allantoin. The reaction is monitored at 292 nm where the absorbance of uric acid is maximal and allantoin does not absorb. The method, which uses only 10 ul of sample and 200 ul of reagent, is linear to 20 mg/dl and has a sensitivity of 0.018 ΔA/min per mg/dl. The assay is performed at 37°C over a period of one minute in which 7 absorbance readings are taken at 10 second intervals. A line of best fit is then determined for the data points by an instrument programmed linear regression analysis mode and the sample concentration is then calculated by comparison of the ΔA/min of sample with that of the standards. The use of this rate method also obviates the need for sample blanking.

In order to achieve high linearity with maximum sensitivity it was necessary to select a uricase enzyme with a suitably high Km, one which would allow a sufficient substrate absorbance and still maintain first order kinetics. We have determined the following Km values of microbial uricase from four different sources, including two separate strains of Candida utilis:

Source	Km
1. Candida utilis A	1.5×10^{-5}
2. Candida utilis B	1.25×10^{-4}
3. Bacillus fastidiosus	1.0×10^{-3}
4. Aspergillus flavus	5.6×10^{-5}

Using Michaelis-Menton Kinetics, the suitability of the Bacillus fastidiosus uricase for the uric acid rate assay will be demonstrated and discussed. Reagent performance and correlation data will also be presented for discussion.

References:

1. Bulger HA and Johns HE. J Bio Chem 140:427, 1941.

2. Kalckar HM. J Bio Chem 167:429, 1947.

A 127 A SIMPLIFIED METHOD FOR MEASUREMENT OF G6PD ACTIVITY.
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Assays of glucose-6-phosphate dehydrogenase (G6PD) activity provide useful information in several clinical settings, but the commonly used qualitative screening tests do not clearly distinguish intermediate reactors (heterozygous females) from either normal or deficient persons. The automated enzyme assay developed by Catalano, et. al. (Clin Chem 21:134, 1975) permits rapid quantitation and precise separation of the three classes of patients, but proved difficult to use routinely. We have adapted their assay for use with the Cobas centrifugal analyzer, and utilized frozen (-70°C) blood samples for quality control, with considerable improvement in the efficiency of testing. The analyzer is programmed to monitor the absorbance of NADPH and Hb and to calculate the activity of G6PD and grams of Hb per liter of whole blood. The assay requires 0.1 ml of a whole blood specimen, 0.315 ml of reagent and an incubation period of eight minutes.

Precision of G6PD Measurements

(I.U. Per Gram Hemoglobin)

	Within Run (n=20)			Between Run (n=5)		
	Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
Control #1	8.7	0.1	1.4	8.9	0.2	1.7
Control #2	13.3	0.2	1.6	13.4	0.3	2.4

The simplified procedure and the procedure used at JHMI for the past six years were compared, using 20 consective patient specimens; results of the two assays were virtually identical (r=0.994). For equal precision and accuracy, this modification is more economical than the earlier method, primarily because fewer runs need to be repeated and because the cost of the control materials is negligible.

A 128 DETERMINATION OF PSEUDOCHOLINESTERASE ACTIVITY BY AN ENZYME COUPLING METHOD IN THE SERUM OF PATIENTS WITH LIVER DISEASE.
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Serum pseudocholinesterase (PChE; EC 3.1.1.8) activity was determined at 37°C and pH 8.2 using the stable substrate o-hydroxybenzoylcholine as the starter, and o-hydroxybenzoate hydroxylase (EC 1.14.13.2) as the coupling enzyme. The reaction was measured kinetically by monitoring the decrease of NADPH at 340 nm. The within-run and run-to-run precision (CV) of the test were 0.4-2.6% and 1.2-1.9% respectively. The test was linear to 400 U/L (r = 0.9995), and had the sensitivity of 0.001 ΔA/min at 9.7 U/L. The serum PChE reference range for normal Chinese was estimated to be 156-386 U/L (95% range; mean = 223 U/L; N = 120 healthy adults), with no significant difference in normal levels between males and females.

The serum PChE activity of patients with liver diseases was significantly lower than that of normal control. Its activities were determined in 18 hepatitis patients, 126.2 ± 58.3 U/L ($\bar{x}$ ± SD; Range 29.2-206.7 U/L), in 24 cirrhosis patients, 49.6 ± 37.9 U/L (10.4-182.5), and in 19 primary hepatoma patients, 83.9 ± 46.8 U/L (14.6-220.0 U/L). In the cirrhosis and hepatoma patients, 95% had PChE levels lower than the normal control range. It is apparent that this stable enzyme coupling method for the determination of serum PChE activity may be used to study the pathophysiology of liver diseases and as a diagnostic aid as well.

A 129 A NEW TEST FOR THE DETERMINATION OF THE ALPHA-AMYLASE ACTIVITY WITH INCREASED SENSITIVITY.
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Methods for the determination of a-amylase activity using oligosaccharides or 4-nitrophenol-substituted oligosaccharides are most common in the clinical chemical laboratories. The advantages of the new methods include better precision and accuracy, shorter reaction time and easy adaptation to automated analytical instruments. Several drawbacks to these methods were discovered, such as the influence of glucose on the lag phase, interference of bilirubin and the higher affinity of the salivary isoenzyme to the oligosaccharides as substrates.

We introduce a new substrate with a better indicator system and with no interference from glucose, bilirubin, ascorbic acid, hemoglobin, pyrovate and fructose. The test has a short lag time of about 5 minutes, an excellent linearity and an increased sensitivity. The p- and s-type alpha-amylase isoenzymes show an identical substrate affinity with this modification. The identification and determination of the reaction products formed by a-amylase with the new substrate elucidates the background for the increased sensitivity of the test system. The data for precision and accuracy, as well as a comparison of the results obtained with the new technique and with other methods will be demonstrated. The reference values for serum and urine are established.

A 130 DEVELOPMENT AND VALIDATION OF A KINETIC FLUORIMETRIC ASSAY FOR THE MEASUREMENT OF ANGIOTENSIN CONVERTING ENZYME.
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The measurement of angiotensin-1-coverting enzyme (ACE, E.C.3.4.15.1) is useful for the diagnosis and management of patients suffering from sarcoidosis. Most methods of ACE measurement require the use of a variety of synthetic peptide substrates and, in general, are "fixed-time interval procedures."

We describe the development and validation of a reaction rate method for the measurement of ACE using the substrate o-aminobenzoylglycyl-p nitro-L-phenylalanyl-L-proline on a microcentrifugal analyzer. Optimum reaction conditions have been determined using a commercial, purified preparation of ACE and the enzymes present in serum samples. Characteristics of the enzyme have been investigated, including reaction pH, Michaelis constant, metal ion dependency and the effect of so called specific ACE inhibitors, Teprotide and Captopril.

This method has been compared to the fixed interval procedure of Cushman and Cheung which used the substrate Hip-L-His-L-Leu. A reference range is reported for the proposed kinetic method.

A 131 CONDUCTIMETRIC MEASUREMENTS OF ENZYME CATALYZED REACTIONS.
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Conductimetric measurements may be useful for studying enzymatic reactions since different anions and cations are formed during the reaction. This allows wide use of this technique, the only limitation being the sensitivity of the conductance bridge, as compared to the relative changes in conductance.

For many years, we have made such measurements with a highly sensitive apparatus. Relative variations of conductance of 10^{-4} may be measured.[1-3]

Reactions which have been studied recently are:

a) Urease/urea: This well-known system, first studied in 1971 by Hanss[4] has been reviewed and technical improvements allow a higher sensitivity.

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