The advantages of our technique are as follows: (1) The volume of plasma required is small, the details of the test are simple and conveniently carried out. (2) The pH of the plasma-veronal-glycerophosphate mixture remains practically constant at about 8.6. (3) The hydrolysis of the glycerophosphate is about twice as rapid as in Kay's procedure. (4) The reaction is retarded so little during the first 2 hours that the *initial* velocity may be calculated in terms of mg. of phosphorus liberated per cc. per hour without substantial error. (5) The phosphorus liberated by 1 cc. in 2 hours is in all cases ample for several aliquot determinations. (6) Plasma phosphatase tests of the same sample or of samples secured from the same subject 24 hours apart check very well; samples obtained at greater intervals may show differences, probably attributable to physiological variability.

With the above technique as a basis, empirical factors may be determined which allow the use of 0.2 cc. of plasma for a phosphatase determination with an accuracy of ±10% when larger quantities of plasma are not available.

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Deviation from the "Beer Law" in the Kuttner-Cohen Method for Determination of Phosphorus.

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Recently, when determinations of inorganic phosphate in mixtures containing sodium glycerophosphate (used as a substrate in plasma phosphatase determinations) were required, the use of heat involved in the Benedict-Theis method rendered it obviously unsuitable. The many advantages of the Kuttner-Cohen method,[1] with the modifications later suggested by Kuttner and Lichtenstein[2] and Raymond and Levene,[3] recommended themselves to us. We found this method capable of yielding very accurate results (within ±2% of the known quantities), after correction for the considerable deviations from the "Beer law". These deviations were determined by a large number of analyses, charts were constructed and tables compiled for more convenient reading.

[1] Kuttner, T., and Cohen, H. R., *J. Biol. Chem.*, 1927, **75**, 517.

[2] Kuttner, T., and Lichtenstein, L., *J. Biol. Chem.*, 1930, **86**, 671.

[3] Raymond, A. L., and Levene, P. A., *J. Biol. Chem.*, 1928, **79**, 628.

We use 1 cc. of plasma when it is expected to contain 5 mg. of phosphorus per 100 cc. or more, and 2 cc. of plasma when it is expected to contain less. Ten cc. of water are added and 5 cc. of 10% trichloracetic acid. About 14 cc. of filtrate are available. Four cc. of the acid-molybdate mixture is added to an aliquot diluted to 5 cc., the tube is mixed by tapping, and 1 cc. of stannous chloride solution* is added. Several standards of different concentrations may be employed in a series of tests.

We found it convenient to employ a single standard containing 0.02 mg. per 5 cc. We usually include in one series about 18 tests, triplicate 0.02 mg. standards, and, as a check upon all foreseen and unforeseen sources of error, several solutions of known concentration covering approximately the same range as the unknowns. After calculation of the results the correction is applied.

When the solution compared with the standard is of a greatly different concentration, the less concentrated solution has a grayish tinge that may introduce some uncertainty into the comparison and for that reason we accept our corrected values only when the aliquot compared against the 0.02 mg. standard contains between .0150 and .0300 mg. of phosphorus. After a test of one aliquot enough filtrate is available for duplicate or triplicate determinations on aliquots chosen in accordance with the results of the first test.

An abbreviated table of corrections for use with a standard containing 0.0200 mg. follows:

TABLE I.

Calculated mg. P.	Correction mg. P.	Calculated mg. P.	Correction mg. P.	Calculated mg. P.	Correction mg. P.
.0100	—.0013	.0220	+.0003	.0340	+.0030
.0120	—.0011	.0240	+.0008	.0360	+.0034
.0140	—.0008	.0260	+.0012	.0380	+.0038
.0160	—.0006	.0280	+.0016	.0400	+.0043
.0180	—.0003	.0300	+.0021	.0420	+.0047
.0200	.0000	.0320	+.0026	.0440	+.0052

* We prefer the more concentrated solution of stannous chloride used by Raymond and Levene.[3]