7442 C

Comparison of Jenner-Kay and Bodansky Methods for Determining Phosphatase in Plasma and Serum.*

L. S. PALMER AND J. W. NELSON.

From the Division of Agricultural Biochemistry, University of Minnesota, University Farm, St. Paul.

Since the autumn of 1932 we have made phosphatase determinations in several hundred samples of plasma from cows' blood by the clinical method of Jenner and Kay[1]. This method as well as that of Kay[2], of which it is a modification, has been criticized by Bodansky[3, 4], because of unnecessarily large experimental errors; the method developed by Bodansky[3, 4] is more rapid and is believed by him to eliminate the errors inherent in the Kay and Jenner-Kay technics.

Before we began to employ the Jenner-Kay method in the routine analysis of bovine plasma we had satisfied ourselves that sufficiently uniform results could be secured when it was applied to the type of plasma samples employed by us for other plasma analyses, namely, total calcium and inorganic phosphorus. These samples are a composite of equal parts of plasma centrifuged at once from citrated blood obtained on 3 successive days. Preliminary data obtained by the Jenner-Kay method from normal heifers whose plasma showed widely different levels of phosphatase showed for example that samples containing 31.46, 36.16 and 36.92 units per 100 cc. on successive days showed 34.70 units in the 3-day composite against a calculated value of 34.85 units. The same composite sample analyzed 38.72 units phosphatase after 6 days' storage. Another animal with plasma phosphate values of 16.50, 16.28 and 16.76 units per 100 cc. on successive days showed 16.70 units for the composite against 16.51 units calculated from the individual samples. A third animal with plasma phosphatase of 6.18, 6.98 and 6.96 units per 100 cc. on successive days showed 7.84 units in the composite against a calculated value of 6.71. These data, which were subsequently verified by a very large amount of data which will not

*Published with the approval of the Director as Paper 1258 Journal Series, Minnesota Agricultural Experiment Station.

1 Jenner, H. D., and Kay, H. D., *Brit. J. Exp. Path.*, 1932, **13**, 22.

2 Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 235.

3 Bodansky, A., PROC. SOC. EXP. BIOL. AND MED., 1931, **7**, 760.

4 Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.

be reported at this time, suggest that the phosphatase content of the blood plasma in dairy cattle is to a considerable extent an individual characteristic. Furthermore, it seemed apparent that the Jenner-Kay technic is applicable to the determination of plasma phosphatase in composite samples within reasonable limits of accuracy. The results also suggest that greater errors may be expected when the phosphatase content is low and that some inaccuracy may result if the composite sample is not analyzed promptly.

After the publication by Bodansky[4] of his study of errors involved in a phosphatase estimation we became interested in a comparison of his and the Jenner-Kay technic to samples of plasma and particularly to definitely proportional mixtures of plasma samples having widely different concentrations of phosphatase. The latter type of study has long been regarded as a satisfactory test of the reliability of an analytical procedure.

In a first comparison of the methods plasma from citrated and oxalated blood as well as the serum from 2 normal heifers, one with high and the other with low blood phosphatase content, as shown by previous analyses were analyzed simultaneously by the Jenner-Kay and Bodansky technics when freshly drawn and after 2 and 4 days. Bodansky[4] prefers serum to oxalated plasma, but has not reported data on citrated plasma which we have employed throughout; Kay[2] and Jenner-Kay[1] use oxalated plasma. For a number of years we have used the Fiske-Subbarow[5] method for inorganic phosphate and therefore employed it for the Jenner-Kay phosphatase determination. We found that the Fiske-Subbarow reagents give a precipitate with the Bodansky phosphatase substrate, making it necessary to employ the Kuttner-Cohen[6] method for inorganic phosphate with the Bodansky phosphatase method, which Bodansky recommends. Therefore the comparison of the methods for phosphatase likewise included a comparison of the results of phosphate by the Fiske-Subbarow and Kuttner-Cohen methods. All analyses were made in triplicate. Incubation was for 3 hours at 38° in the Jenner-Kay method and for one hour at 37°C. in the Bodansky method. Preserved samples were kept in the refrigerator.

It was found that the initial inorganic phosphate in the samples as determined by the Kuttner-Cohen method showed greater irregularity than by the Fiske-Subbarow method when the fresh, 2-day and 4-day old samples were compared. The average variations were 0.56 mg. % in the former and 0.26 mg. % in the latter. This

[5] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

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

may have been due in part to a lack of previous experience with the former method. Oxalated blood plasma sometimes showed higher inorganic phosphorus than citrated plasma by both methods, amounting on the average to 1.25 mg. %. No explanation suggests itself for this inasmuch as the samples represented the same bleeding. Regarding the enzyme units there was no greater uniformity among comparable samples from the same animal by the Bodansky method than by the Jenner-Kay method. Some uniformity was found in the ratio of the results by the two methods for the same animal but the ratio was clearly different for different animals, the J.K. units ranging from 1.8 to 3.3 times the Bodansky units in the cases studied. An increase in phosphatase activity was not noted in preserved samples.

For the chief method selected for a comparison of the 2 phosphatase procedures 4 pairs of animals were chosen having in 3 instances widely different blood phosphatase concentration and in the fourth instance nearly the same phosphatase concentration. The individual samples of each pair were analyzed and also mixtures of these pairs in ratios of 1:2 and 2:1. All analyses were made on freshly prepared citrated plasma. The Fiske-Subbarow method for inorganic phosphate was used for the Jenner-Kay procedure and the

TABLE I.
Comparison of Jenner-Kay and Bodansky Phosphatase Units in Mixtures of Plasma of Determined Phosphatase Content.

Date	Sample	Phosphatase units per 100 cc. Jenner-Kay Found	Jenner-Kay Calculated	Phosphatase units per 100 cc. Bodansky Found	Bodansky Calculated	Ratio J-K / B units
11-6-33	E150	49.25		23.76		2.07
	2E150:1E78	36.25	33.78	16.05	16.20	2.26
	1E150:2E78	17.24	18.30	8.98	8.64	1.92
	E78	2.83		1.07		2.65
1-18-34	E150	48.00		24.53		1.96
	2E150:1E78	28.39	32.92	17.40	16.65	1.63
	1E150:2E78	19.75	17.83	10.22	8.77	1.93
	E78	2.75		0.89		3.09
1-22-34	E193	13.81		7.03		1.96
	2E193:1E191	15.48	15.04	8.19	8.01	1.89
	1E193:2E191	16.38	16.25	9.00	8.98	1.82
	E191	17.48		9.96		1.75
1-24-34	E150	40.59		27.20		1.49
	2E150:1E151	28.83	27.93	17.56	18.60	1.64
	1E151:2E150	15.92	15.35	10.00	10.01	1.59
	E151	2.72		1.41		1.93
1-26-34	E184	12.70		5.62		2.26
	2E184:1E183	8.91	9.01	3.87	3.81	2.30
	1E184:2E183	5.24	5.30	2.16	2.00	2.43
	E183	1.64		0.40		4.10

Kuttner-Cohen method for this analysis in the Bodansky procedure. The incubation procedures were according to directions. All analyses were made in triplicate.

Table I shows the results of these comparisons, the inorganic phosphate determinations being omitted for lack of space. It was evident, however, that the inorganic phosphate was estimated with essentially equal accuracy by the 2 methods, as judged by a comparison of the calculated values with those determined in the various mixtures of plasma; the mean percentage error was 0.75% for the Fiske-Subbarow method and 1.23% for the Kuttner-Cohen method. The results of this study also fail to show any superiority of the Bodansky method over the Jenner-Kay method for plasma phosphatase so far as accuracy of calculated vs. determined values is concerned. The range of errors for the Bodansky method was from 0.10 to 16.53% with a mean error of 4.36%, while the range of errors for the Jenner-Kay method was 0.80 to 13.76%, with a mean of 5.04%. The choice of method seems to be one of personal preference rather than significantly greater accuracy when applied in routine analysis. When only a few samples are to be analyzed the shorter incubation period of the Bodansky method is an advantage. For a larger series the longer incubation period of the Jenner-Kay method is definitely advantageous in routine work. Inasmuch as both methods are empirical and, in our judgment, equally accurate, it is unfortunate that there does not seem to be a more uniform ratio between the units arrived at by the methods. While the ratio of Jenner-Kay to Bodansky units is roughly 2:1 in our series, the range for individual samples is from 1.49:1 to 4.10:1.

7443 C

Fading of Methylene Blue-Acetone Solutions by Ultra-Violet Light.

WILHELM STENSTROM AND CARL E. NURNBERGER.

From the Laboratory of Biophysics, University Hospital, Minneapolis, Minn.

Recently Nurnberger and Arnow[1] found that solutions of methylene blue in water irradiated under a mercury-arc therapy lamp had their absorption spectra changed quantitatively when the rays from the lamp were filtered through quartz. If, however, the rays shorter than 270 millimicrons were absorbed by a suitable filter, then prac-

[1] Nurnberger, Carl E., and Arnow, L. Earle, *J. Phys. Chem.*, 1934, **38**, 71.