

Effect of Addition of KCN to Whole Blood on Indophenol-reducing Power of Plasma.

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(Introduced by Elaine P. Ralli.)

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Pi Joan, Townsend and Wilson¹ noted a considerable loss of vitamin C in blood serum done by the Farmer and Abt² method. Pi Joan and Klemperer³ reported that this loss could be prevented by the immediate addition of M/50 KCN to the blood. In order to determine the difference in the concentration of vitamin C in blood with and without KCN, the following experiments were done. Forty cc. of venous blood were drawn and placed immediately in an Erlenmeyer flask containing 8 drops of a 20% solution of potassium oxalate. The specimen was shaken gently and divided into two equal fractions, to one of which (B) were added 8 drops of 10% KCN resulting in an M/26 solution of KCN. Both fractions (A and B) were then immediately centrifuged and the plasma drawn off. The separated plasma from each fraction was divided into four 2 cc. portions. One of each of these samples (A₀ and B₀) was precipitated immediately, using 2 cc. of plasma, 4 cc. of redistilled water and 6 cc. of a 10% solution of metaphosphoric acid. These samples were then centrifuged for 5 minutes and the filtrate decanted off. Three cc. samples of the filtrate were used for the titrations, which were done by the Farmer and Abt modification of the Tillman's method.⁴ The remaining 6 plasma specimens (3 with KCN and 3 without KCN) were placed in the icebox and determinations of the vitamin C content were done after intervals of approximately 30, 90 and 160 minutes as shown in the chart. The first determinations, in this experiment, were done 45 minutes after the blood was drawn. This represents the maximum time required for preparing the filtrate; the average time taken in other experiments was 25 minutes.

Immediately after the first specimen of blood had been taken from the subject, 1000 mg. of vitamin C (Merck) were injected intra-

¹ Pi Joan, M., Townsend, S. R., and Wilson, A., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **35**, 224.

² Farmer, C. J., and Abt, A. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 146.

³ Pi Joan, M., and Klemperer, F., *J. Clin. Invest.*, 1937, **16**, 443.

⁴ Tillmans, J., Hirsch, P., and Hirsch, W., *Z. für Untersuch d. Lebensmitt.*, 1932, **63**, 1.

venously and after a period of 20 minutes a second 40 cc. sample of blood was drawn. In this way, it was possible to study the effect of KCN on blood from the same individual at high and low concentrations of the vitamin. This blood sample was also divided into 2 equal fractions (C and D) of 20 cc. each, to one of which (D) KCN was added in the same concentration as above. The plasma samples were treated in the same manner as specimens A and B and titrated at the same intervals of time. All plasma protein precipitations were done just prior to titration.

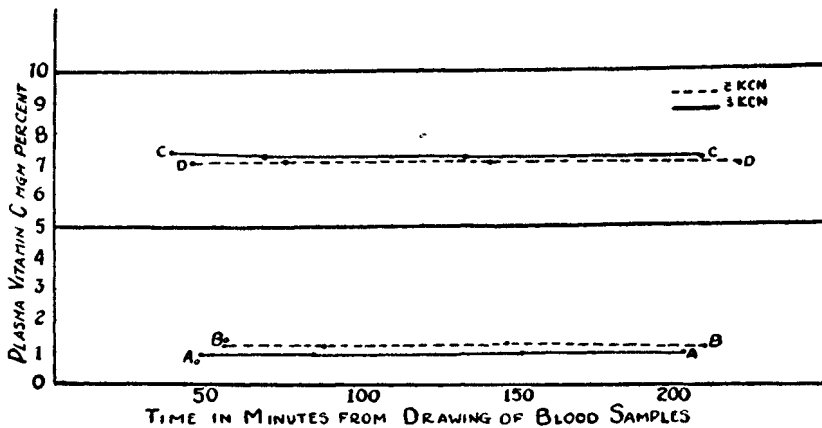


FIG. 1.

The results are shown graphically in the chart. At the low concentration of vitamin C, in the plasma to which KCN had been added (B), the indophenol-reducing power was equivalent to 1.13 mg. % of vitamin C. The sample to which KCN had not been added (A) had a concentration of 0.89 mg. %. Over the period of time observed (160 minutes) these concentrations showed little variation, remaining always within the experimental error of the method. At the higher concentrations (C and D), the dye reduction was equivalent to 7.39 mg. % in the specimen *without* KCN and 7.03 mg. % in the specimen to which KCN had been added. Again over the period of time observed (160 minutes) there was little change in the concentration of vitamin C at this level.

In a second experiment the effect of KCN (M/54) on the plasma vitamin C of another subject was determined at 4 different plasma levels, following the injection of crystalline vitamin C (Merck).

The results were as follows (Table I).

This showed again the greater reduction of the dye by the plasma without cyanide at the higher vitamin C concentrations. The dif-

TABLE I.
Concentrations of Vitamin C mg. %.

Blood without KCN	1.42	2.92	5.40	8.16
Blood with KCN	1.88	2.88	5.14	7.54

ferences at the higher concentrations are consistent and are not due to experimental error.

These observations on the rate of destruction of the indophenol-reducing power of plasma at normal vitamin C levels do not agree with the findings of Pijoan, *et al.*,^{1, 3, 5} who found an average half-life of about 60 minutes for vitamin C in separated plasma.

There is no evidence that the differences in reducing power observed in bloods at normal vitamin C levels are due to an inhibition of the oxidation of vitamin C. It may be that the greater reduction of the dye is due to the activation of some other redox system by the cyanide. It seems to us, on the basis of these observations, that there is no reason for the addition of KCN to blood for the determination of plasma vitamin C.

We are unable to explain the fact that at the higher levels of vitamin C KCN added to the blood actually resulted in a lessened reduction of the dye, an observation that we have confirmed repeatedly. Experiments bearing on this point are in progress.

9854

Longevity of Erythrocyte and Reticulocyte in Normal and Splenectomized Guinea Pigs.

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We have employed the method of Escobar and Baldwin¹ to determine the longevity of the erythrocyte of the normal and splenectomized guinea pig. This consists in subjecting the animals to pressures sufficiently low to result in a significant increase in red cell count, and then recording the time necessary for the count to reach its original level. Use of this method has also been made for estimating the longevity of the circulating reticulocyte in such animals.

⁵ Pijoan, M., and Eddy, E., *J. Lab. Clin. Med.*, 1937, **22**, 1227.

¹ Escobar, R. A., and Baldwin, F. M., *Am. J. Physiol.*, 1934, **107**, 249.