TABLE I.

Compound	Highest dilution showing no growth after 10 minutes' exposure			Toxicity-index = A/B	
	Staph. aureus = B	*E. typhosa* = B	Tissue = A	*Staph. aureus*	*E. typhosa*
Hexylresorcinol	1:1620	1:1770	1:1450	0.9	0.8
p-hydroxyphenyl-n-amyl sulfide	1:2700	1:2700	1:2800	1.0	1.0
o-n-hexylphenol	1:2900	1:2800	1:3125	1.1	1.1
p-hydroxydiphenyl sulfide	1:2500	1:3125	1:3125	1.3	1.0
Phenol	1:110	1:186	1:224	2.0	1.2

agents. Also, they exhibit about the same degree of germicidal efficiency against both *Staph. aureus* (gram +) and *E. typhosa* (gram —) making them valuable germicides for general use. With the exception of iodine the non-phenolic compounds which have been tested[1] show great differences in their action on *Staph. aureus* and *E. typhosa*.

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Relationship of Pyruvic Acid to the Bisulphite Binding Substances of the Blood.

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Increases in the bisulphite binding substances (B.B.S.) of the blood have been related to increases in pyruvic acid, both in beriberi[1] and following exhaustive exercise.[2] The B.B.S. has been shown to be elevated in beriberi[1] and other disease states,[3] and also following the injection of sodium pyruvate.[4] The present communication gives a brief résumé of the relationship of the B.B.S. to the blood pyruvic acid.

Pyruvic acid was estimated in blood by Peters and Thompson's

[1] Platt, B. S., and Lu, G. D., *Proc. Chinese Physiol. Soc.*, Third General Conference, Chinese M. A., 1935, p. 16; *Ibid., Quart. J. Med.*, 1936, n.s., **5**, 355.

[2] Johnson, R. E., and Edwards, H. T., *J. Biol. Chem.*, 1937, **118**, 427.

[3] Taylor, F. H. L., Weiss, Soma, and Wilkins, R. W., *J. Clin. Investigation*, 1937, **16**, 833.

[4] Wilkins, R. W., Weiss, Soma, and Taylor, F. H. L., *Ann. Int. Med.*, in press.

modification of the Neuberg-Case method,[5] B.B.S. by a modification[3] of the Clift and Cook procedure.[6] The methods used for selection of clinical material[3] and for administering sodium pyruvate[4] are given elsewhere.

From pure aqueous solutions of lithium pyruvate an average of 85.7% of the theoretical amount of pyruvic acid as B.B.S. and 85.6% as pyruvic acid by the hydrazone method were recovered. The recovery of lithium pyruvate from blood, when added in amounts ranging between 10 and 20 mg. per 100 ml. of blood, averaged 90.4% of theoretical by the B.B.S. method and 79.9% by the hydrazone method. These values were obtained when the blood proteins were precipitated immediately after the addition of the pyruvate.

Thirteen subjects having a B.B.S. in the range previously accepted as normal[3] had blood pyruvic acid values between 0.19 and 0.79 mg. per 100 ml. Three patients with nutritional deficiency and with B.B.S. values of 6.6, 9.6, and 13.8, calculated as pyruvic acid in milligrams of pyruvic acid per 100 ml. of blood, had pyruvic acid values by the hydrazone method of 0.73, 0.46, and 0.72 mg. per 100 ml., respectively. In both normal subjects and those with elevated B.B.S. there was a much greater increase in B.B.S. than could be accounted for on the basis of the amount of pyruvic acid present.

B.B.S. and pyruvic acid were determined on the blood of 10 subjects before, and from one to 5 minutes after the slow intravenous injection of 5 gm. (100 cc. of a 5% solution) of pyruvic acid as sodium pyruvate. In each instance there was an increase in the circulating pyruvic acid and substances which bind bisulphite. From an average initial value of 0.37 mg., the pyruvic acid by direct hydrazone method increased to 4.48 mg., while the pyruvic acid by B.B.S. increased from an average value of 4.88 to 14.13 mg. per 100 ml. of blood. The average increase in pyruvic acid accounted for only 44.5% of the increase in B.B.S. expressed as pyruvic acid.

The foregoing observations were repeated on 5 individuals following the oral administration of 25 gm. of sodium pyruvate, with similar results when measurements were made before and from one to 2 hours following the administration of the sodium pyruvate. The essential difference was that the increase in pyruvic acid and B.B.S. in the blood was less than when given by vein. In these observations the increase in pyruvic acid in the blood accounted for

[5] Peters, R. A., and Thompson, R. H. S., *Biochem. J.*, 1934, **28**, 916.

[6] Clift, F. P., and Cook, R. P., *Biochem. J.*, 1932, **26**, 1788.

54% of the increase in B.B.S. Following both oral and parenteral administration of sodium pyruvate positive tests for pyruvic acid were obtained in the urine for from 7 to 10 hours, and, in some instances, longer.

When blood to which lithium pyruvate had been added was permitted to stand at laboratory conditions for several hours, marked diminution in the recovery of pyruvic acid occurred. Under such circumstances the recovery of pyruvic acid by the B.B.S. method was only slightly reduced, while that estimated by the direct method was reduced to a much greater extent, 60% or less of added pyruvic acid being recovered by the direct method as compared with 80% when precipitation was carried out immediately.

A consideration of the above data suggests that pyruvic acid was converted to other substances of a ketonic nature by blood, both *in vitro* and *in vivo*. Additional *in vitro* experiments add some weight to this concept. Following the addition of 10 mg. of lithium pyruvate to 2 samples of the same blood, immediate precipitation was carried out in one, and the other incubated at 37.5°C. for 2 hours before precipitation. Recovery of 97.8% of the added pyruvate by B.B.S. and 86% by the hydrazone method was obtained from the non-incubated sample, while the incubated sample yielded 28.6% as B.B.S. and 18% as pyruvic acid after 2 hours. The experiment was repeated with the same results. When 0.5 ml. of 5% potassium cyanide solution was added to the blood, however, the B.B.S. fell from an initial recovery of 93.1% to 69.9%, while recovery by the hydrazone method fell from 90% to 10.3% in the 2 hours of incubation.

These experiments indicate that pyruvic acid is destroyed rapidly in blood, both *in vivo* and after withdrawal from the vein. Evidence is given that pyruvic acid as such may be converted to other ketone forms which, in turn, are more slowly metabolized. Inhibition by cyanide appears to effect the oxidation of these secondary ketonic bodies rather than the conversion of pyruvic acid into them. No attempt has been made in these studies to identify ketone substances in addition to pyruvic acid. The data further indicate that determinations of pyruvic acid on blood by the hydrazone method must be carried out immediately after the sample is drawn.