This paper presents a simplified procedure for the identification and determination of the keto acids, namely pyruvic acid, α-ketoglutaric acid, and oxaloacetic acid. It is an extraction method specific for pyruvic acid with one extraction of the hydrazone and one reextraction with carbonate. Total pyruvic acid can be measured by the proper choice of solvent and one reextraction with carbonate. The method for the determination of pyruvic acid involves the reaction with nitrophenylhydrazines and simplifies the technique of Lu.1 Just one extraction of the hydrazones is sufficient and accurate. It can also be used for the differential extraction of specific keto acids with a choice of a specific solvent for each. Xylene, toluol, and benzeno are recommended for the determination of pyruvic acid. Ethyl acetate or other nonspecific solvents such as ethyl ether and caprylic alcohol are useful for the determination of total keto acids. Subsequently, the acid hydrazones are reextracted only once with sodium carbonate. The differences in the absorption of light by various hydrazones may be measured by the ratio of photometric densities, preferably at two wavelengths of light.

The method for the determination of pyruvic acid involves the reaction with nitrophenylhydrazines and simplifies the technique of Lu.1 Just one extraction of the hydrazones is sufficient and accurate. It can also be used for the differential extraction of specific keto acids with a choice of a specific solvent for each. Xylene, toluol, and benzeno are recommended for the determination of pyruvic acid. Ethyl acetate or other nonspecific solvents such as ethyl ether and caprylic alcohol are useful for the determination of total keto acids. Subsequently, the acid hydrazones are reextracted only once with sodium carbonate. The differences in the absorption of light by various hydrazones may be measured by the ratio of photometric densities, preferably at two wavelengths of light.

There was never any lack of participants in our experimental work. Office workers, laboratory workers, and medical students were always willing to serve as subjects, sometimes for remuneration and often as justification of a social visit to the laboratory personnel. Perhaps the reasons for citation are the simplicity, accuracy, and exhaustive details of the method for pyruvic acid, which holds a significant role in carbohydrate metabolism. With a relatively simple and reliable basic method, other aspects of pyruvic acid metabolism were suggested and investigated. We later studied the effects of anoxia at high altitudes, various foods, and various degrees of muscular activity.2 Related work that cites the pyruvic acid method includes studies of pyruvate kinase,3 pyruvate and acetate metabolism,4 and formation of serine from pyruvate.5

Friedemann, the senior author (who died in 1983), made significant contributions in his career of more than 50 years in the fields of biochemistry, bacteriology, physiology, and nutrition, with publications on antiketogenesis, lactic and pyruvic acids, metabolism and methods for the determination of alcohol, oxidation of sugars and acetone bodies, human carbohydrate and fat metabolism, and utilization and methods for the determination of vitamins. He has been listed in American Men and Women of Science.

As a graduate student at Northwestern University Medical School, I had the privilege of working under T.E. Friedemann in a new, well-equipped research laboratory, when pioneer work in the identification and physiology of vitamins and methods for the determination of vitamins in biological materials was in progress. It was known that human pyruvate deficiency could be diagnosed with a method for the measurement of the concentration of either thiamine, or cocarboxylase—the keystone of carbohydrate metabolism in the tri-carboxylic acid cycle. Thiamine plays an important role in the metabolism of carbohydrates. Cocarboxylase, or diphosphothiamine, catalyzes the aerobic decomposition of pyruvic acid to acetaldehyde and carbon dioxide.

A collaborating physician had hospitalized two patients in the hope of reducing their weights from over 300 pounds to approximately 150. Their food intake had to be closely supervised. Of concern to the physicians was a careful record of vitamin B1 levels in blood during the weight loss. We were motivated to complete a reliable method for measuring vitamin B1 levels in blood and urine and to correlate these with pyruvic acid levels. The dietary history of the two patients indicated a daily intake of less than 500 mg of thiamine over a period of several months prior to their admission. After admission, they were given a reducing diet for a relatively short period of time that was deficient in the vitamin B complex. In thiamine deficiency, both pyruvic acid and lactic acid accumulate during increased breakdown of carbohydrates. It was later shown that the ratio of lactic acid to pyruvic acid in the blood proved to be more sensitive for detecting thiamine deficiencies than the blood levels of the patients at rest. The patients accomplished their goals, and the laboratory staff did Not.

3 Washo S, Mano T & Shiodzono N. Purification and assay method of pyruvate kinase from baker's yeast / J Biochem 46 1661-3, 1959
4 Foster J M & Villee C A. Pyruvate and acetate metabolism in the isolated rat diaphragm / J Biol Chem 211 797-809, 1954

©1985 by ISI® CURRENT CONTENTS®