

Quantitative Analysis of Urine Vapor and Breath by Gas-Liquid Partition Chromatography

(orthomolecular medicine/vitamins/controlled diet)

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ABSTRACT When a human being is placed for several days on a completely defined diet, consisting almost entirely of small molecules that are absorbed from the stomach into the blood, intestinal flora disappear because of lack of nutrition. By this technique, the composition of body fluids can be made constant (standard deviation about 10%) after a few days, permitting significant quantitative analyses to be performed. A method of temperature-programmed gas-liquid partition chromatography has been developed for this purpose. It permits the quantitative determination of about 250 substances in a sample of breath, and of about 280 substances in a sample of urine vapor. The technique should be useful in the application of the principles of orthomolecular medicine.

Orthomolecular medicine is defined as the preservation of good health and the treatment of disease by variation of the concentrations in the human body of substances that are normally present in the body and are required for health (1). Death by starvation, kwashiorkor, beri-beri, scurvy, or other deficiency disease can be averted by the provision of an adequate daily intake of carbohydrates, essential fats, proteins (including the essential amino acids), essential minerals, and vitamins. To achieve the best of health, the rate of intake of essential foods should be such as to establish and maintain the optimum concentrations of essential molecules. It has been pointed out by Williams (2, 3) that there is great individual variation in the optimum rate of intake of essential substances. There is evidence that for many people the optimum rate of intake of ascorbic acid is much greater than the usually recommended daily allowance (4), and it is not unlikely that rates of intake of some other vitamins considerably greater than the usually recommended daily allowances are valuable for many people. The use of large daily intakes of nicotinic acid or nicotinamide, as well as of ascorbic acid and pyridoxine, in the treatment and control of schizophrenia is an example.

The practice of orthomolecular medicine, and of much of medicine in general, is a trial-and-error procedure. This procedure is so time-consuming and expensive that it is usually practiced only on persons who are already seriously ill. If information could be obtained about the rates of intake of essential substances that would lead to optimum health for a person, it would be possible to make decisions about the regime required to preserve health and to treat disease. Information about the genetic nature of an individual human being, as reflected in the rates of various chemical reactions

that take place in his body, usually catalyzed by enzymes, could be obtained by the thorough quantitative analysis of body fluids. Moreover, the thorough quantitative analysis of body fluids might permit differential diagnosis of many diseases in a more effective way than is possible at the present time.

During the past 3 years we have been engaged in developing instrumental techniques for this purpose. The problem is to make a quantitative determination of the amounts of each of several hundred substances present in a sample of urine, blood, spinal fluid, breath, saliva, or tissue. In order that the results of the analysis be significantly representative of the person, it is essential that he be standardized with respect to diet and other environmental influences, such as the nature of the intestinal flora. This standardization is achieved by placing the subject for several days on a completely defined chemical diet (Vivonex-100)‡, consisting almost entirely of small molecules, such that the foods are absorbed into the blood stream in the stomach and the intestinal flora disappear because of lack of nourishment.

The development of computer technology during the last decade has made it possible to handle large amounts of quantitative information in a fast and economical way. We have studied several procedures for fast and economical analysis of samples, with the minimum amount of human labor required in handling, fractionating, and preparing the samples, and have reached the conclusion that at the present time, high-resolution gas-liquid partition chromatography of urine and breath, without preliminary fractionation or other treatment, is the fastest and most economical method for obtaining extensive information about the molecular composition characteristic of individual human beings.

In Fig. 1 is shown a gas-liquid chromatogram of vapor from a sample of human urine. In this chromatographic study, 280 substances are separated and quantitated. Similarly, in Fig. 2 is shown a gas-liquid chromatogram of human breath, showing 250 substances. The quantitation is achieved by a computer program that integrates the signals for the successive peaks, the integration being performed from minimum to minimum. For a person on the standard diet, the standard deviation for individual peaks on successive days, after the fourth day on the diet, is about 10%, whereas on an ordinary diet the fluctuations are several times larger.

‡ Vivonex, Inc., Mountain View, Calif. 94040.

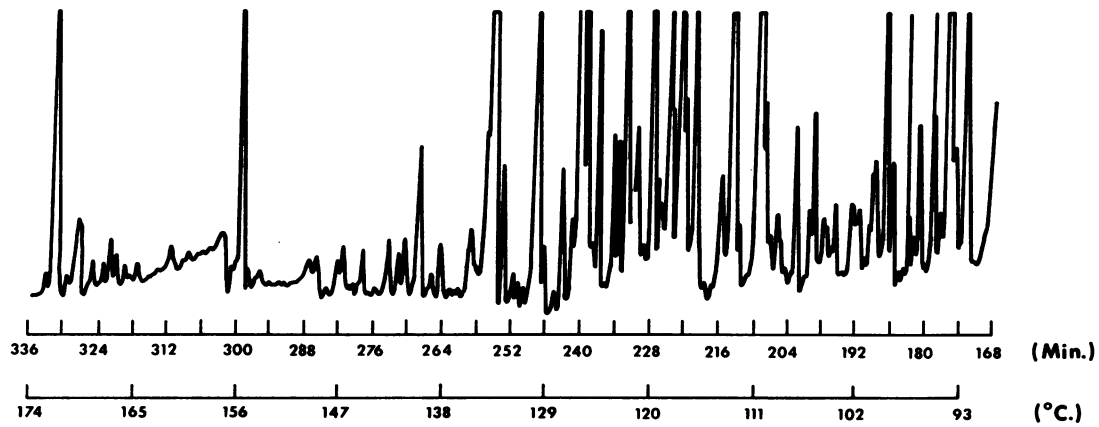
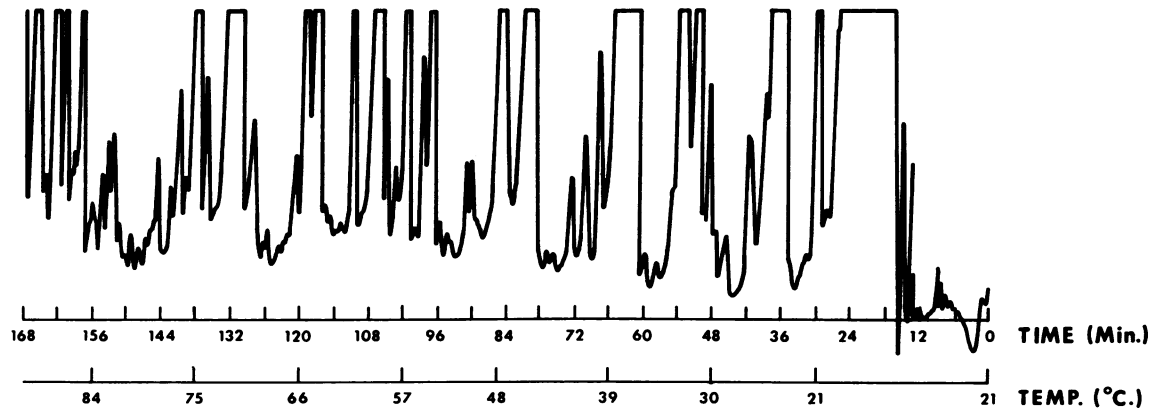


FIG. 1. Chromatogram of urine vapor.

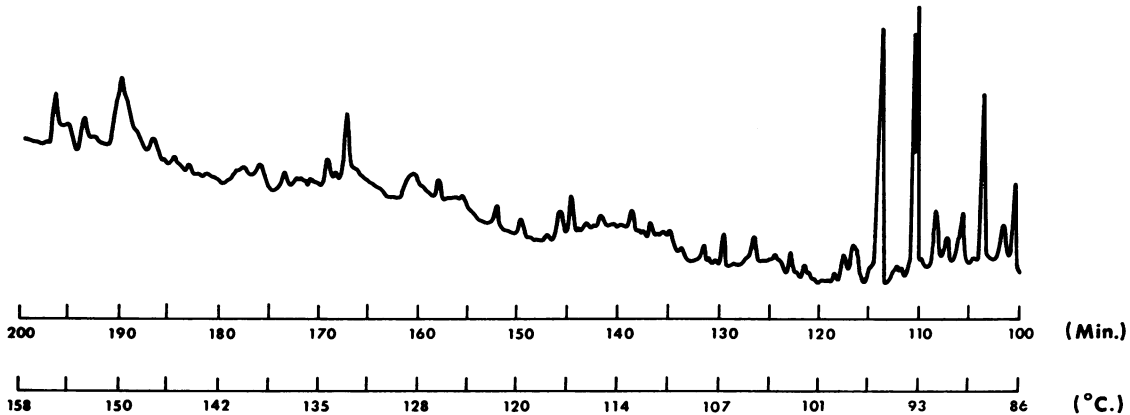
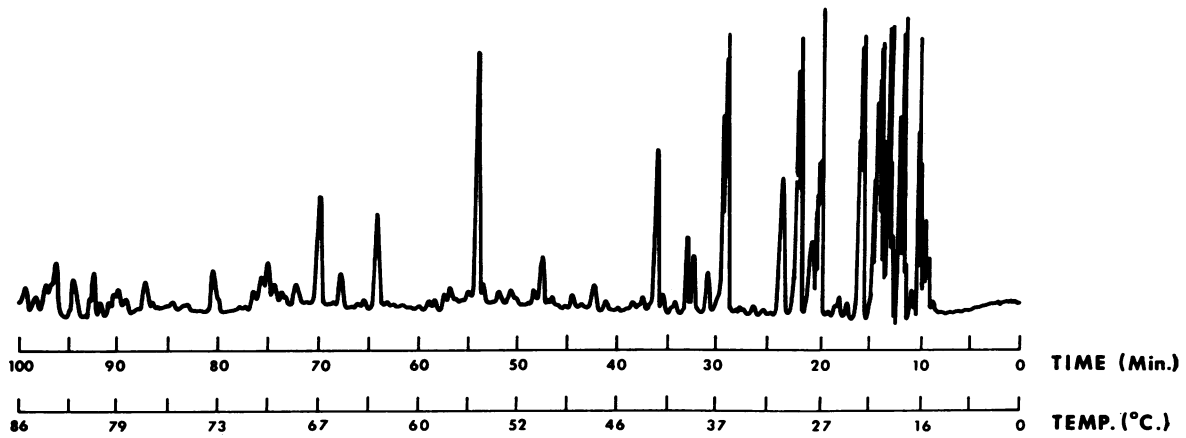


FIG. 2. Chromatogram of breath.

EXPERIMENTAL METHODS

The gas-chromatograph oven and temperature controls used were those of the Varian Aerograph model 2100, equipped with dual hydrogen-flame-ionization detectors made in our laboratory (5), differential solid-state electrometer (6), and dual 1000-foot 0.03-inch (0.076 cm internal diameter) stainless-steel open tubular columns (7), coated with methyl-silicone oil SF 96(50). Gases were purified (8) and flows were regulated for optimum stability. Columns were programmed for an increase in temperature of 1/2°C per min from about 20 to 180°C.

Human-urine volatiles were analyzed directly by passing helium at 15 ml per min over a 200-ml urine sample, which was magnetically stirred and maintained at 80°C. The volatiles were collected over a 1-hr period in a trap cooled with liquid nitrogen, by the techniques used in fruit-volatiles studies (9). The volatiles were flashed into the column with a heat gun after the collection was completed.

Human-breath volatiles, 10-15 exhalations per sample, were first trapped in a coiled 5-foot by 0.20-inch (internal

diameter) stainless-steel tube cooled in an isopropyl alcohol-dry ice bath. The volatiles were then transferred to the gas chromatograph as described above.

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Correction. In the article "Repression of the Overproduction of Porphyrin Precursors in Acute Intermittent Porphyria by Intravenous Infusions of Hematin," by Bonkowsky, H. L., Tschudy, D. P., Collins, A., Doherty, J., Bossenmaier, I., Cardinal, R. & Watson, C. J., which appeared in the November 1971 issue of *Proc. Nat. Acad. Sci. USA* **68**, 2725-2729, the sentence beginning on line 20 (from the top), right-hand column of page 2728, should read "Thus, while PBG appears not to penetrate certain cells or membranes as well as ALA (21-23), . . ."

Correction. In the article "Quantitative Analysis of Urine Vapor and Breath by Gas-Liquid Partition Chromatography," by Pauling, L., Robinson, A. B., Teranishi, R. & Cary, P., which appeared in the October 1971 issue of *Proc. Nat. Acad. Sci. USA* **68**, 2374-2376, the first sentence in the **Abstract** "When a human being is placed for several days on a completely defined diet, consisting almost en-

tirely of small molecules that are absorbed from the stomach into the blood, intestinal flora disappear because of lack of nutrition" is wrong. The absorption into the blood takes place not from the stomach, but from the small intestine. The diet of Vivonex-100 used by us consists of small molecules except for an oligosaccharide, averaging about 5 glucose units per molecule. Winitz, M., Adams, R. F., Seedman, D. A., Davis, P. N., Jayko, L. G. & Hamilton, J. A. (1970) *Amer. J. Clin. Nutrition* **23**, 546, have recently pointed out that the number of bacteria in the feces is greatly decreased when this glucose-based diet is ingested, but the intestinal flora do not completely disappear. They found a decrease in the total microbial population after 3 or 4 days on the glucose-based diet to 10^{-3} times the value before beginning the diet in two subjects, 10^{-4} in four subjects, and 10^{-5} in one subject. These comments also apply to the statement "... such that the foods are absorbed into the blood stream in the stomach and the intestinal flora disappear because of lack of nourishment." on the same page.