Techniques of Orthomolecular Diagnosis

Arthur B. Robinson and Linus Pauling

Orthomolecular diagnosis is the process of determining and evaluating the concentrations of the substances normally present in the human body. This paper describes the general method that we are using for orthomolecular diagnosis.

Additional Keyphrases: pattern recognition • diagnostic aid • normal values • variation, sources of • screening

We believe that a significant improvement in health and a decrease in the age-specific morbidity and mortality from various diseases can be achieved by varying the concentrations in the body of the molecules that are normally present, many of which are required for life (1). The corresponding field is described as “orthomolecular medicine.” An important part of orthomolecular medicine, orthomolecular diagnosis, is the process of determining the concentrations of various substances in the human body (2). We have studied some of the techniques for determining the amounts of different substances in body fluids. Most of our experiments on orthomolecular diagnosis have involved the use of gas–liquid chromatography, ion-exchange chromatography, or mass spectrometry. In this paper we discuss the approach to this problem that we have developed during the past five years and discuss examples taken from the chromatographic experiments.

In general, we have not required that our experiments be based on any particular biochemical mechanisms related to particular diseases. We have not actively searched for qualitative or quantitative changes in single substances that are characteristic of single diseases. Instead, for each disease we have carried out careful quantitative analysis for as many substances as we are able to measure at low cost. We are particularly interested in preventive medicine. Therefore, we have restricted these measurements in the effort to achieve the goal that the entire procedure can be offered to apparently well persons at a cost of $10 or less if it is found to be useful.

After quantitative analysis has been completed, we carry out simplified pattern recognition calculations in a computer in which we use simultaneously the amounts of all of the substances we have measured in an effort to establish a pattern for each group of samples under study. These groups are formed from people with various human diseases and also people who apparently are well but who represent differences in age, sex, diet, physical activity, and other factors.

When patterns are found, we carry out a calculation in which each subject is classified according to correlation with the different patterns. We evaluate the correctness of these classifications and calculate a “diagnostic power” for the procedure (3). This diagnostic power is used to determine the value of the general procedure for medical diagnosis and to compare the values of different methods of quantitative analysis, different methods of calculation, and other alternative parts of the general procedure.

We have already reported some of our early work on urine, in which we used conventional amino acid analysis by automated ion-exchange chromatography (4) to detect patterns for diurnal variation in healthy individuals (2), individuality of well persons (2), dietary control (2), mental retardation (2), and multiple sclerosis (3, 5), and used gas–liquid chromatography of derivatized urine constituents on packed columns (6, 7) to detect patterns for diurnal variation and mental retardation (8). We have also reported development of an analytical procedure for normally volatile compounds (9–11).

We have carried out analytical work on urine, breath, blood, and saliva. In the course of this work, about 15 000 urinary chromatograms have been made and, thereby, more than one million quantitative analyses performed. Only a small part of this work has been reported. We have asked, “Is this new approach to diagnosis generally useful for the diagnosis and monitoring of many different diseases and is it technologically ready to be applied in preventive medicine?” We have not found these questions to be easy to answer. At present, we have demonstrated that there are diagnostically strong patterns present in most of the groups of urine samples that we have

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collected. These include groups with diseases—multiple sclerosis, breast cancer, Duchenne (muscular) dystrophy, olivopontocerebellar degeneration, Huntington's disease, or mental retardation—and groups of persons who are apparently well but exhibit differences attributable to sex, diet, birth-control pills, and time of day. Patterns for normal individuals under strict dietary control are so distinctive as to uniquely characterize each person according to his biochemical individuality (2). Figures 1 through 13 illustrate the current status of our work with some of these groups.

Figure 1 illustrates our general procedure. After the analytical data have been collected, the chromatographic peaks on the different chromatograms are automatically integrated and matched according to relative retention time. The computer teaches itself about chromatogram-to-chromatogram retention time differences and corrects for them. A normalization procedure is carried out, to correct for variations in urine volume and other factors, as has been described previously (2, 3). A simple scan for unusual peaks characteristic of a particular group is carried out. However, the primary calculations consist of testing for the existence of a pattern among all of the peaks and testing the diagnostic power of patterns that have been found.

The test for a pattern is carried out by calculating the probability, \( P \), that each substance is systematically different in its value for two different groups. This probability is calculated by means of a two-tailed Wilcoxon test (12) of the null hypothesis that the two groups have identically distributed peak areas for the substance. The Wilcoxon test statistic is corrected for ties (13, 14) and assumed to be asymptotically normal. A continuity correction of one-half and the first term of the Fix and Hodges (15) correction are used (13). This nonparametric method is used because we have no foreknowledge of the shape of the distribution functions for the peak areas. The computer then plots the number of substances with probabilities of correlation less than or equal to each maximum probability value. Examples of these plots are shown in Figures 3, 5, 6, and 9 to 13. If the plotted values are systematically above the values expected for a random distribution, as shown by the straight lines in the figures, a pattern is present. It is possible that patterns exist that will not be demonstrated by this calculation technique. However, we know of no way in which this calculation can lead to the false conclusion that a pattern is present when it is not.

If a pattern is present, a test of the diagnostic power of that pattern is carried out in the computer.
Fig. 4. Test for the diagnostic power of the pattern in Figure 3. Other experiments are being carried out on the urine of students who were not correctly diagnosed by the procedure. (The work shown is being carried out in collaboration with Dr. Henri Dirren.)

Fig. 5. Test for the existence of a pattern correlating with the use of birth-control pills

Some of the female Stanford undergraduates whose urine was used for the experiments in Figures 3 and 4 were taking birth-control pills. This Figure demonstrates the existence of a urinary amine pattern correlating with ingestion of birth-control pills. (This work is being carried out in collaboration with Dr. Henri Dirren.)

Fig. 6. Test for the existence of a pattern correlating with grade-point average in male Stanford students

Because the experimental points lie along the line of expected values for random numbers, we conclude that no pattern has been detected in the urinary amines that correlates with grade-point average in these students. (This work is being carried out in collaboration with Dr. Henri Dirren.)

Fig. 7. Urinary-vapor-analysis chromatograph from a normal subject who has been equilibrated on the synthetic small-molecule diet, "Vivonex 100"

The baseline is computer calculated and is based on the chromatogram itself. The off-scale values are known to the computer although they are not shown on this plot.

Fig. 8. The experimental errors in the analytical procedure for: urinary amines, I; urinary vapors, including only well-resolved peaks, II; and urinary vapors, including both peaks and shoulders on peaks, III

The lines are drawn along the points of maximum number of substances with probable errors at or below those values indicated on the vertical axis.

Fig. 9. Test for the existence of a pattern in urinary-vapor analysis that is characteristic of subjects with multiple sclerosis

These female subjects were subjected to no dietary control at all before the samples were collected. (These experiments are being carried out in collaboration with Dr. F. C. Westall and Dr. G. W. Ellison.)
We carried out computer calculations to estimate the diagnostic power of the method. The calculation was based on the noncorrelation-index method that we have previously described (2, 3). An average chromatogram is constructed by averaging the matched peak areas for all of the subjects in each group of interest. The chromatogram for each individual subject is compared to the average chromatograms and a noncorrelation-index for each chromatogram with each of the average chromatograms is calculated. These indexes allow the diagnostic placement of each individual along a linear axis extending between any pair of groups. Division of this linear axis at any arbitrary point allows computation of the number of errors in diagnosis caused by division at that point, and division at several points along the axis allows construction of a diagnostic power curve (3) as illustrated in Figure 4. We take the diagnostic power of the method to be the ratio of the relative areas of area II to area I + II, as illustrated in Figure 4. This ratio is chosen so as to avoid bias by those subjects who would be correctly diagnosed on a random basis, as illustrated by the diagonal line in Figure 4. Figure 2 shows a computer plot of typical raw data from a urine analysis by the automated amino acid analyzer; Figure 3 illustrates the existence of a pattern in Stanford University students that is characteristic of sex. The corresponding diagnostic-power calculation is illustrated in Figure 4. This diagnostic-power calculation was made with

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**Fig. 10.** Test for the existence of a pattern in urinary-vapor analysis that is characteristic of subjects with Huntington's disease.

These subjects were subjected to strict dietary control by equilibration for 4 days on Vivonex 100 small-molecule diet. (These experiments are being carried out in collaboration with Dr. L. D. Brenneman.)

**Fig. 11.** Test for the existence of a urinary-vapor analysis pattern distinguishing fasting normal subjects from nonfasting normal subjects.

The same experimental male co-workers at the Institute of Orthomolecular Medicine and at Stanford University were used for the fasting and nonfasting samples. All samples were collected at the same time of day.

**Fig. 12.** Test for the existence of a pattern in urinary-vapor analysis that is characteristic of subjects with breast cancer.

All samples, breast cancer and control, were collected without dietary control from women about to undergo exploratory surgery for breast cancer at the Stanford Medical School Hospital. The samples were later characterized on the basis of pathology reports from the operations and grouped accordingly. (These experiments are being carried out in collaboration with Dr. C. A. Arnold, Mr. J. Cheronis, and Mr. M. Brenneman.)

**Fig. 13.** Test for the existence of a pattern in urinary-vapor analysis that is characteristic of subjects with Duchenne dystrophy.

No dietary control was used for these young male subjects. (These experiments are being carried out in collaboration with Dr. F. C. Westall and Dr. J. B. Peter.)
use of the 11 most strongly correlating peaks shown in Figure 3 while weighting the noncorrelation-index term for each of these peaks so that the strongest peak has a weight of 11, the next strongest a weight of 10, and so on to the least strongest with weight 1.

Figure 5 illustrates the existence of a urinary-amine pattern characteristic of ingestion of birth-control pills. Figure 6 shows the failure of the procedure of urinary-amine analysis to detect a pattern characteristic of grade-point average in male Stanford students.

Figure 7 shows a computer plot of a chromatogram from the procedure for urine-vapor analysis described in reference 10. This procedure was developed in the hope that the inclusion of a greater number of substances in the procedure would increase its diagnostic power. Figure 8 illustrates the greater number of substances available by this procedure.

Initial experiments have been carried out with urine-vapor analysis for comparison of several groups of subjects and appropriate control groups. Figures 9 through 13 show that the initial results of these experiments are quite promising. We are convinced that the procedure is useful, and are now proceeding with the qualitative characterization of the unidentified substances in our patterns.

One problem, however, remains unsolved. We have not proved that, for most of our sample groups, the only systematic property that contributes to the pattern for the group is that for which the group is labeled. We also have not shown how early the patterns for disease develop. We do not know whether or not the patterns are present before the disease is extensively developed, and therefore are useful for preventive medicine.

To answer these questions, the Institute of Orthomolecular Medicine has initiated a program in which urine and serum samples will eventually be collected at six-month intervals from between 10,000 and 20,000 apparently well subjects. These samples will be stored indefinitely at −76 °C. As time passes, some of the subjects in this group will become ill from various diseases. When sufficient numbers of subjects have become ill, the stored samples will allow rigorous elimination of individual variability and systematic sampling errors. It will also be possible to test properly for early patterns that indicate increased probability of sickness in well subjects.

In the meantime, we shall continue to adopt improved analytical procedures and to test these procedures with the best groups of samples that we are able to obtain, with special emphasis on the fluctuation of patterns with changes in therapy and disease severity. When the experimental programs illustrated in Figures 1 through 13 are sufficiently complete, they will be submitted for separate publication.

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References


