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A NON-INVASIVE MEASURE OF MINERALS AND ELECTROLYTES IN TISSURE

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INTRODUCTION

In clinical practice, metabolic disturbances in patients are monitored routinely by measurements of electrolytes and minerals in blood and urine. This method was used during the Skylab missions to detect potential metabolic problems in astronauts. Blood samples obtained before, during, and after the 28, 59 and 84 day flights revealed alterations in the concentration of calcium, phosphorus, sodium and potassium (1). Although these findings did not seem to present a problem to the space travelers, further monitoring is indicated because deviations from pre-flight probably represent part of man's adaptation to microgravity, a poorly understood process. A means for monitoring changes in electrolytes and minerals without venipuncture and the collection of blood samples would be ideally suited to this purpose.

DESCRIPTION OF METHOD

Dr. Silver of IntraCellular Diagnostics, formerly Spectroscan, Inc., devised a system (patented) for collecting epithelial cells from the oral mucosa for the determination of ion concentration. A number of characteristics of these cells influenced the choice for clinical testing. They are non-cornified epithelial cells located on the inferior aspect of the tongue and therefore, well protected from trauma. They have the capability of reflecting relatively recent physiologic changes since they are renewed every 3 days and have aerobic metabolism. Most importantly, they are easily accessible and can be removed by a wooden applicator stick with minimum discomfort. Smears of cells removed in this manner show predominantly individual cells rather than sheets of contiguous cells. This facilitates the visual isolation of single cells with the electron microscope for their analysis. Only intact undamaged cells, approximately 800 cubic microns, are analyzed.

The technique for obtaining the cells and preparing smears is illustrated on Figure la. The mucosa under the tongue is rinsed with distilled water three times. The cells are removed by fairly vigorous scraping of the sublingual area in the groove between the tongue and jawbone, using the pointed end of a wooden curettage stick. The cells are applied to a special slide, and fixed immediately with standard cytology fixative (2.5% carbowax) in 95% ethanol). Smears made in this manner contain cells, dehydrated by the fixative, that can be stored indefinitely at ambient temperatures.

The development of the electron microscope and instruments to control and focus electron beams to irradiate very small areas in cells and subcellular particles gave rise to the technique of x-ray microanalysis that has been applied to the measurement of ions in a number of tissues (2). X-ray emissions that are produced when atoms in a specimen are bombarded by electrons are characteristic of each ion since the charge and energies associated with orbiting electrons are unique for each element. The emission spectra of the elements exposed to preselected energy bands, in the range 500-5000 KeV, are quantified. The intensity of the fluorescence within each energy band is proportional to the intracellular concentration of the ion. Detection of the fluorescence is accomplished with commercially available silicon-lithium detector tubes that can adequately discriminate the energy bands of interest. Six of the nine elements detectable by x-ray microanalysis of sublingual cell are measured in studies of normal physiology: calcium, phosphorus, magnesium, sodium, potassium and chloride. The average concentration of a minimum of 3 cells is the basis for the result in one specimen since the cells in one individual show little variation.

One of the major problems in quantifying the element in a tissue is the background non-specific radiation

spectrum produced by x-ray photons generated by incident electron interactions with chemical elements in the analyzed field. To reduce this background, Dr. Silver developed slides or viewing substrates composed of conductive high purity carbon. The fairly constant background emitted by this material is useful in the quantification of the x-ray data that is expressed as a peak to background ratio. Method Summary is illustrated on Figure lb.

APPLICATION OF THE METHOD BY NASA

NASA's principle effort in the development of a test to measure the ion concentration in sublingual cells has been research by the biomedical program carried out by scientists with expertise in skeletal metabolism. These efforts have been directed toward determining the biological meaning and deviations in intracellular ions in non-human primates and in male volunteers for experiments in a model for weightlessness. A brief summary of the experiments and results follows.

Non-human primates were fed synthetic diets containing no vitamin D, the same amount of phosphorus (0.5%) and 2 levels of calcium (1.2 and 0.3%). The Rhesus monkey is known to be highly susceptible to vitamin D deficiency, a problem that might complicate experiments designed to evaluate the response of the skeleton to microgravity. The study in which intracellular ion analysis of sublingual cells was done, was carried out to gather information on the effect of dietary calcium on the biochemical expression of vitamin D deficiency (3). Vitamin D deficient animals showed marked increases in the intracellular concentrations of calcium and phosphorus in sublingual cells that bore no relationship to the changes in serum levels. The result of the intracellular ion assay was consistent with one of the known biologic effects of vitamin D to facilitate the transport of calcium and phosphorus out of cells (4). This experiment provided the biological verification of the test for disorders of calcium and phosphorus metabolism.

The experiments carried out in human adults were designed to establish normative data in healthy 30-55 year old men, the most common candidate for the astronaut corps, and to define the effects of a model of weightlessness, -6 degree head down tilt bed rest, on intracellular ion concentrations. Sublingual cell ion concentrations of calcium, phosphorus and potassium increased by the 3rd bed rest day and remained higher than pre-bed rest levels for the duration of a 28-day study, exceeding the percent changes in the normal variation in concentrations 10 to 50 fold (5). The changes in intracellular ions could be demonstrated before increases in circulating calcium and phosphorus that generally occur on the 6th bed rest day. In one group of subjects who performed a specific type of exercise daily as a countermeasure during a 30-day bed rest study, there were no deviations in the concentration of intracellular ions from pre-bed rest levels (6). Importantly, in these subjects, the common problem of increased urinary calcium excretion and alterations in the hormones of the calcium endocrine system did not take place. We have also obtained cross-sectional data in healthy ambulatory men that relates the levels of sublingual cell calcium, phosphorus, and potassium to the individual's relative level of daily physical activity.

The results from the above experiments provide every indication that the concentration of at least 3 of the ions in sublingual cells accurately reflect an individual's level of musculoskeletal activity. While the mechanism of the observed changes in intracellular ions is not known, the basic explanation may reside in alterations in the calcium endocrine system during musculoskeletal activity that influence the transcellular transport of the calcium, phosphorus and potassium. Future research directed toward these basic questions is needed. For the present, analysis of sublingual cell ion concentrations can be applied to monitoring the adequacy of an individual's exercise program and schedule with confidence. There is enough data to support the design and development of a small instrument for measurements of intracellular ion concentrations in space. With such a system, astronauts could monitor one objective measure of their physical well being in space and use the test results as a guide for planning their daily activity.

Potential applications of sublingual cell analysis for clinical medicine are numerous, but currently limited by the few clinical research studies in patients with well characterized problems. Current research involves analyses in patients with magnesium deficiency and cardiovascular diseases, two areas where estimations of the ion concentrations of cells as an index of mineral and electrolyte metabolism may be more useful in the

management of patients than blood tests.

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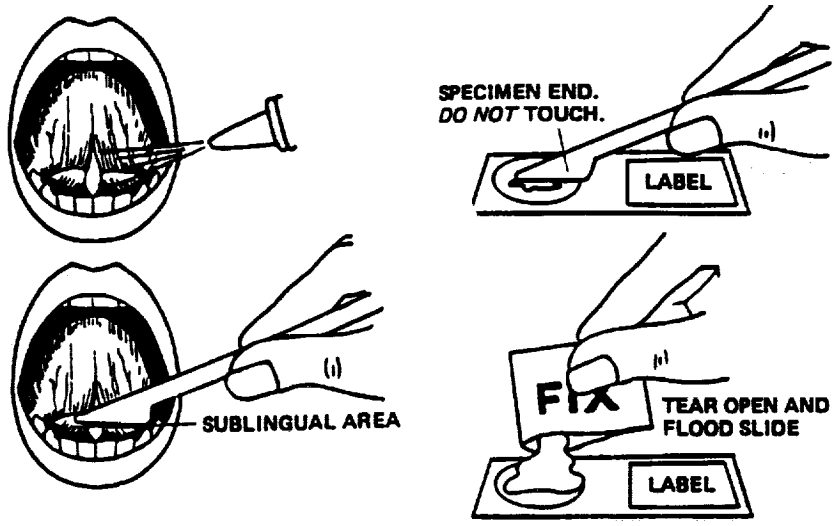
FIGURE 1.

a. SAMPLE PREPARATION



b. INTRACELLULAR ANALYSIS