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## Method for Determination of Oxygen-18 Content of Inorganic Phosphate

**Abstract.** The reaction of inorganic phosphate and mercuric cyanide at temperatures from 240° to 300°C leads to the quantitative conversion of the phosphate oxygens to carbon dioxide. This report describes the proper conditions for the advantageous use of this reaction for the determination of the oxygen isotope composition of the inorganic phosphate.

The mechanism of several enzymatic reactions has been successfully investigated through use of O<sup>18</sup> as an isotopic tracer. Various phosphorylation reactions involving the transfer of oxygen between inorganic phosphate or phosphate derivatives and organic substrates have been particularly susceptible to analysis by this technique. These analyses invariably involve the determination of the O<sup>18</sup> content of inorganic phosphate, and although the available methods for inorganic phosphate O<sup>18</sup> determination give satisfactory results, they suffer from mul-

multiple disadvantages. The dehydration method (1), based on the pyrolysis of inorganic phosphate to H<sub>2</sub>O and pyrophosphate, followed by equilibration of the H<sub>2</sub>O with CO<sub>2</sub>, is lengthy and time-consuming. Furthermore, since this method involves dilution of the phosphate oxygens, relatively large amounts of phosphate are needed for accurate determinations. A second general analytical method involves heating carbon and inorganic phosphate at 1350°C to yield CO (2). This method requires an elaborate apparatus and has the disadvantage that the product, CO, is not easily separable from air and has the same mass as N<sub>2</sub>.

A procedure has been developed in this laboratory which largely circumvents these disadvantages (3). The method is similar to that developed by Rittenberg and Ponticorvo for the determination of O<sup>18</sup> in organic compounds (4). The modified method involves heating KH<sub>2</sub>PO<sub>4</sub> and Hg(CN)<sub>2</sub> (5) in a sealed tube for 1 hour at 250°C. Under these conditions, the phosphate oxygens are converted to CO<sub>2</sub> without dilution. The CO<sub>2</sub> is collected and subsequently introduced into the mass spectrometer (6). The original Rittenberg-Ponticorvo method, as developed for organic O<sup>18</sup> determination, is not applicable to inorganic phosphate because of oxygen exchange reactions with the glass container (7).

The procedure is as follows:

1- to 50 mg samples of KH<sub>2</sub>PO<sub>4</sub> are placed in break-seal tubes, as described by Rittenberg and Ponticorvo (4). The tubes are dried at 100°C under reduced pressures. Each break-seal tube is then thickened a short distance from the open end and cooled, and 25 mg of dry Hg(CN)<sub>2</sub> is introduced. The tube is evacuated to a final pressure of 7 to 10 μ-Hg, sealed, and heated at 250°C for 1 hour.

The CO<sub>2</sub> formed in the sealed tube is collected in the apparatus shown in Fig. 1. Following the introduction of the sealed tube and the assembly of the apparatus, the whole system is evacuated to a pressure of 7 to 10 μ-Hg without liquid N<sub>2</sub> over the second U-tube trap. Following evacuation, stopcock A<sub>1</sub> is closed, and the break seal is broken with

the aid of the magnet. Stopcock C is then closed, trap 2 is immersed in liquid N<sub>2</sub>, and stopcock A is opened to allow CO<sub>2</sub> to distill into trap 2. Any HCN which is formed is frozen out in the Dry-Ice acetone trap (trap 1). After 3 or 4 minutes, stopcocks B<sub>1</sub> and B<sub>2</sub> are closed and stopcock C is opened to evacuate any gas not frozen out in liquid N<sub>2</sub>. Stopcock C is then closed, and trap 2 is removed from the apparatus and connected to the mass spectrometer.

The results of a series of determinations are given in Table 1.

In a large number of determinations, the results were extremely consistent. The O<sup>18</sup> concentration of KH<sub>2</sub>PO<sub>4</sub> over a range of from 2 to 50 mg of the salt could be determined with a deviation of ± 0.5 percent. Furthermore, it would appear that the heating conditions do not have to be reproduced to any high degree of consistency. Over a temperature range of 230° and 300°C and a time range of 40 minutes to 2 hours, identical O<sup>18</sup> values were obtained.

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5. We are indebted to Dr. David Samuel for suggesting the use of Hg(CN)<sub>2</sub>.
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## Nuclear Sex of Patients with Testicular Tumors

**Abstract.** In view of the occurrence of tumors of the testis of "female" nuclear sex, the cells of the hosts of such tumors have been examined. The nuclear sex of a series of 75 such patients was found to be uniformly "male."

The introduction by Barr and Bertram (1) of a reliable technique for cytological determination of the chromosomal sex of an individual has had widespread repercussions in human pathology. One of the most interesting developments has been the discovery by Hunter and Lennox (2), amply confirmed by others (3) that teratomas in male patients may sometimes be of female nuclear sex, whereas "male" tumors are not seen in females. It is claimed that testicular tumors are much more common in malde-

Table 1. Effect of varying amounts of KH<sub>2</sub>PO<sub>4</sub> on the O<sup>18</sup> concentration as measured in the CO<sub>2</sub>.

Sample	Ratio mass 46/44	Atom- percent excess
1 mg KH <sub>2</sub> PO <sub>4</sub> <sup>18</sup> *	0.026874	1.13
3 mg KH <sub>2</sub> PO <sub>4</sub> <sup>18</sup> *	0.027445	1.15
5 mg KH <sub>2</sub> PO <sub>4</sub> <sup>18</sup> *	0.027453	1.15
10 mg KH <sub>2</sub> PO <sub>4</sub> <sup>18</sup> *	0.027229	1.15
50 mg KH <sub>2</sub> PO <sub>4</sub> <sup>18</sup>	0.004072	0.0

\* The theoretical O<sup>18</sup> atom-percent excess was 1.2.

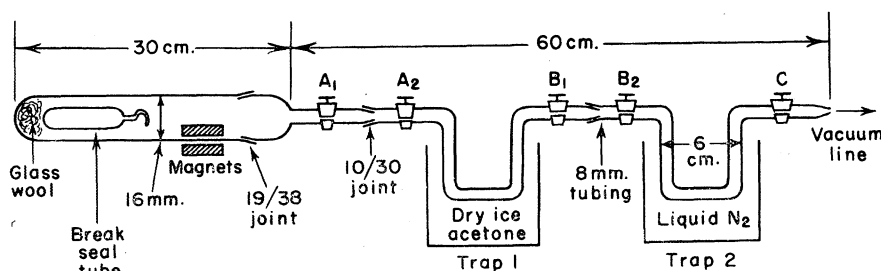


Fig. 1. Apparatus for collection of CO<sub>2</sub>.

scended testes than in scrotal testes (4, 5), and two varieties of ovarian tumor, the tubular type of arrhenoblastoma and the dysgerminoma, are often seen in association with the pseudohermaphrodite state (6). It has also been reported that the testes in which tumors develop may exhibit aberrant differentiation in the form of tubules lined by immature Sertoli cells (7).

A study was undertaken of the nuclear sex of patients in whom testicular tumors had developed. Seventy-five cases were taken from the testicular tumor registry at the Armed Forces Institute of Pathology; the criteria of selection were that normal testicular tissue must be present in the sections and that preoperative radiation therapy must not have been employed. Most of the patients were members of the U.S. Armed Forces and were accepted both at their induction physical examination and by their fellows as males; only two showed cryptorchidism. All varieties of germinal testicular tumors were represented in the group; 29 were seminomas, 14 were embryonal carcinomas, 25 were teratocarcinomas, and 7 were of mixed pattern. This distribution of histological types corresponds to that of the much larger series of Dixon and Moore (4).

Sections were stained by hematoxylin and eosin and by the Feulgen technique. In each instance Leydig cells, Sertoli cells, and the cells of the supporting connective tissue were examined. In all cases the nuclear sex was diagnosed as male. In the 25 teratomas, sexing of the tumor was carried out, and 7 contained female elements. These findings indicate that, in the genesis of testicular tumors, little part is played by gross error of sexual differentiation, and that the "crossed sex" teratomas cannot be explained by the thesis that "female" tumors arise in "female" patients.

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5 DECEMBER 1958

## Effects of Magnesium and Tetraethylammonium Chloride on the Hypothermic Heart

**Abstract.** Ventricular fibrillation was induced by the injection of magnesium chloride in isolated canine hearts as well as in intact animals. Ventricular magnesium tolerance was greatly reduced during hypothermia. Defibrillation was achieved by intracoronary injection of tetraethylammonium chloride and electrical shock, even at temperatures as low as 21°C, where it could not be achieved by other means.

Great advances in cardiac surgery have been achieved by the use of induced hypothermia. However, there remains the hazard of irreversible ventricular fibrillation at low temperatures.

In a series of experiments (1) on myocardial excitability in this laboratory, where both a nonfailing isolated canine heart preparation and intact animals were used (2), both at normothermic and hypothermic levels, it was found that magnesium chloride (but not sodium or potassium chloride) in amounts of 1 to 2 milliequivalents injected rapidly into the coronary system invariably produced ventricular fibrillation. The fibrillation threshold to magnesium was greatly reduced during hypothermia.

In a recent publication by Stovner (3) it was shown that a block at the neuromuscular junction produced by an excess of magnesium chloride was released by tetraethylammonium chloride and that the magnesium-induced block was temperature-dependent. This led us to examine the effects of tetraethylammonium chloride on normothermic and hypothermic hearts, with special reference to its effect on ventricular fibrillation. In 22 experiments involving 20 dogs, which included isolated heart experiments as well as single, intact animal experiments, it was found that tetraethylammonium chloride invariably allowed electrical defibrillation at temperatures as low as 21°C, and that defibrillation was achieved with great ease, whereas, without the use of tetraethylammonium chloride, defibrillation could not be achieved. Furthermore, the minimum amount of magnesium required to induce fibrillation was more than doubled after the use of tetraethylammonium chloride under these conditions.

The tetraethylammonium chloride dosages introduced into the coronary system were from 1 to 2 mg/kg of body weight. This dose is small enough to avoid those side effects, such as hypotension and gross electrocardiographic changes, which have been associated with the clinical parenteral dosage of 10 mg/kg of body weight. Other cardiovascular effects of tetraethylammonium chloride observed included decrease of coronary venous

magnesium content and increase of coronary blood flow, as well as a strong positive inotropic action; the last observation confirms previous work by Acheson and Moe (4).

Our results seem to suggest an important role for magnesium in the mechanism of ventricular fibrillation during hypothermia. Its effects might be exerted through a slowing of conduction or by interference with the effect of transmitter substances at the membrane level. Its fibrillatory action is apparently directly antagonized by tetraethylammonium chloride. In addition, tetraethylammonium chloride increases myocardial contractility, probably by increasing coronary flow as well as by acting directly on the muscle.

In four human beings undergoing cardiac surgery for correction of congenital or acquired heart defects under artificial hypothermia, electrical defibrillation of the ventricles has been achieved only after introduction of 1 to 2 mg of tetraethylammonium chloride per kilogram of body weight, by coronary perfusion (5).

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## Ion-Exchange Equilibria on Single Beads

**Abstract.** Equilibria on single ion-exchange resin beads show that large differences may exist between beads from the same batch. They may be so large that a significant contribution to deviations from ideality can be due to this heterogeneity effect. Correlation between swelling and equilibrium properties shows that bead-to-bead variations are due to differences in cross-linking.

Ion-exchange equilibria on single beads can be studied by radioactive tracer methods (1). Below are given some results for the  $\text{Ag}^+ - \text{H}^+$  system on Dowex 50 X-4 resin. The beads were taken from the 20–50 mesh sieve fraction. The tracer was  $\text{Ag}^{110}$ , obtained by neutron bombardment of silver foil in