13. F. Reindel and K. Niederländer, *Ber. deut. chem. Ges.* **68B**, 1969 (1935).
14. T. Reichstein and C. W. Shoppee, *Discussions Faraday Soc.* 7, 305 (1949).
15. E. Seebeck and T. Reichstein, *Helv. Chim. Acta* **26**, 536 (1943).
16. Microanalyses and determinations of optical rotation were carried out under the direction of Dr. W. C. Alford of our laboratory. We are grateful to him and his staff for their invaluable assistance.
17. H. Wieland and P. Weyland, *Z. physiol. Chem. Hoppe-Seyler's* 110, 123 (1920).
* Visiting scientist, National Institutes of Health.

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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^{18} 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^{18} content of inorganic phosphate, and although the available methods for inorganic phosphate O^{18} determination give satisfactory results, they suffer from multiple disadvantages. The dehydration method (*1*), based on the pyrolysis of inorganic phosphate to H_2O and pyrophosphate, followed by equilibration of the H_2O with CO_2, 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_2.

Table 1. Effect of varying amounts of KH_2PO_4 on the O^{18} concentration as measured in the CO_2.

Sample	Ratio mass 46/44	Atom-percent excess
1 mg $KH_2PO_4{}^{18}$*	0.026874	1.13
3 mg $KH_2PO_4{}^{18}$*	0.027445	1.15
5 mg $KH_2PO_4{}^{18}$*	0.027453	1.15
10 mg $KH_2PO_4{}^{18}$*	0.027229	1.15
50 mg $KH_2PO_4{}^{16}$	0.004072	0.0

* The theoretical O^{18} atom-percent excess was 1.2.

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^{18} in organic compounds (*4*). The modified method involves heating KH_2PO_4 and $Hg(CN)_2$ (*5*) in a sealed tube for 1 hour at 250°C. Under these conditions, the phosphate oxygens are converted to CO_2 without dilution. The CO_2 is collected and subsequently introduced into the mass spectrometer (*6*). The original Rittenberg-Ponticorvo method, as developed for organic O^{18} 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_2PO_4 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 $Hg(CN)_2$ 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_2 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_2 over the second U-tube trap. Following evacuation, stopcock A_1 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_2, and stopcock A is opened to allow CO_2 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_1 and B_2 are closed and stopcock C is opened to evacuate any gas not frozen out in liquid N_2. Stopcock C is then closed, and trap 2 is removed from the apparatus and connected to the mass spectrometer.

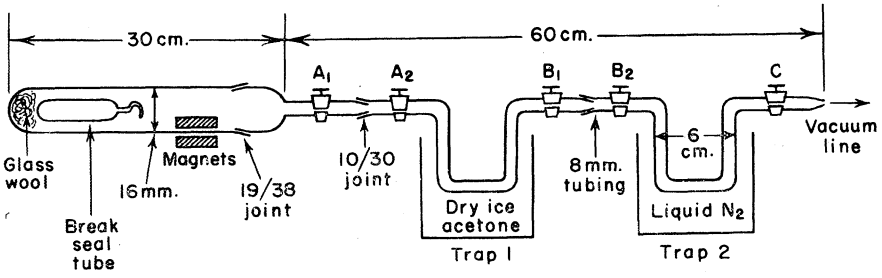

Fig. 1. Apparatus for collection of CO_2.

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^{18} concentration of KH_2PO_4 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^{18} values were obtained.

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References and Notes

1. M. Cohn, *J. Biol. Chem.* 201, 735 (1953).
2. ——— and G. B. Drysdale, *ibid.* 216, 831 (1955).
3. This work was supported by grants from the National Science Foundation and the National Institutes of Health.
4. D. Rittenberg and L. Ponticorvo, *Intern. J. Appl. Radiation and Isotopes* 1, 208 (1956).
5. We are indebted to Dr. David Samuel for suggesting the use of $Hg(CN)_2$.
6. Oxygen 18: 16 ratios were determined on CO_2 samples in the Consolidated Nier mass spectrometer.
7. D. Rittenberg, personal communication.

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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-