Distribution and Excretion of Hexafluophosphate. (23276)

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(Introduced by Olaf Mickelsen)
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In a recent study of the metabolism of various complex fluorides(1), it was shown that administration of potassium hexafluophosphate to rats resulted in no increase in fluoride content of the skeletal and dental tissues, and had no effect on either incidence or severity of dental caries. These findings, together with evidence attesting to the low toxicity of hexafluophosphate in both the frog and rabbit(2), indicate that this anion is relatively, if not completely, inert. While at least one other metabolically inactive complex inorganic fluoride (KBF_4) has been reported(3), the physiological properties of PF_6^- are of some theoretical interest in that it represents, to our knowledge, the only phosphorus-containing anion* which is not readily metabolized. It was decided, therefore, to extend information relative to its metabolism by investigating the occurrence of PF_6^- in tissues other than those of bone and teeth, and to determine the mode of its excretion.

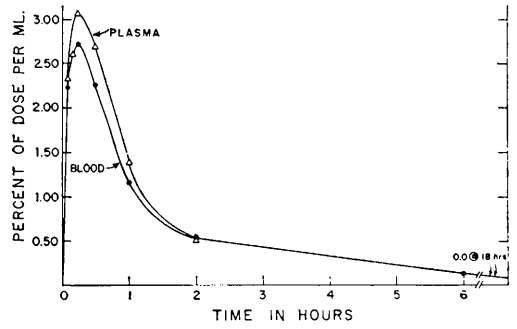
Experimental. Preparation of labelled hexafluophosphate†. $KP^{32}F_6$ was synthesized according to Woyski(5) using PCl_5 prepared from radioactive phosphorus(6,7):

$KCl + P^{32}Cl_5 + 6HF \rightarrow KP^{32}F_6 + 6HCl$ (in liquid HF). After recrystallization from hot water, the percentage composition of the product was: P, 16.75; F, 61.57; free F ion, 0.18. Theoretical for KPF_6: P, 16.83; F, 61.93. Radioactivity was determined by conventional procedures using an end-window counter, and the values obtained were corrected for self absorption. Fluoride was determined according to Willard and Winter (8) as modified by McClure(9). *Collection of excreta.* Metabolism cages were constructed to permit separate collection of urine and feces. A series of holes 5½″ in diameter were cut in a wood board, and upholsterers' tacks inserted on ½″ centers around the perimeter of each. Using the tacks as a frame, nylon cord was strung to form a screen floor beneath each opening.‡ An inverted 2 liter beaker provided with a 3/8″ hole to admit a drinking tube served as a cage. For stability, scotch tape was placed across the top of the water bottle and fastened to the board on either side. A strip of filter paper was attached to the back of the board, angulated approximately 45° to horizontal, and fastened to the edge of the work bench. The free edge was folded back to form a collection trough for the feces. Urine was trapped by absorption on the paper. Female Sprague-Dawley rats weighing 100-120 g were employed throughout. Twenty-seven rats were injected intraperitoneally with 1 mg of $KP^{32}F_6$ in 1 ml of distilled water, and placed in separate metabolism cages for 1, 3, 6, 12, or 24 hours. At the end of the collection periods each rat was induced to empty its bladder by digital pressure applied at the base of the tail. The filter paper was placed under ultraviolet light and the strongly fluorescing urine spots cut out. These were combined with the nylon cord and both extracted with hot distilled water. Recovery of labelled phosphorus added as PF_6^- to 3 control urine papers was 97.0 ± 1.3% (mean ± S.E.). An additional 42 rats were injected with 1 mg of $KP^{32}F_6$ as before and sacrificed by decapitation at the end of 5, 10, 15, and 30 minutes, and 1, 2, 6, and 18 hours. The animals were bled individually into oxalated beakers and the whole blood and plasma analyzed for

* PF_6^- is also unique in that it is the only compound of pentavalent phosphorus known to exist as the free anion(4).

† Neutron irradiation of the commercially available ammonium salt (Ozark-Mahoning Co., Tulsa, Okla.) may offer a more feasible method of preparing radiophosphorus-tagged PF_6^-.

‡ Rats have been retained up to one week in this type of cage. For retention over longer periods of time, stainless-steel wire may be substituted for the nylon cord.



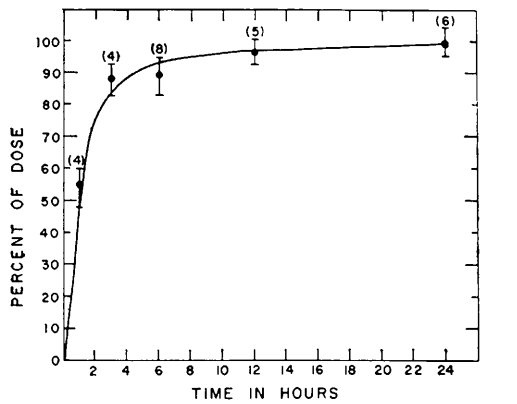


FIG. 1 (top). Conc. of radiophosphorus in blood and plasma following intraper. inj. of $KP^{32}F_6$.

FIG. 2 (bottom). Excretion of radiophosphorus in urine following intraper. inj. of $KP^{32}F_6$.

radiophosphorus. Two rats were injected intravenously with 1 mg of $KP^{32}F_6$ dissolved in 0.2 ml of physiological saline, and sacrificed by decapitation at the end of 10 minutes. The heart, liver, lungs, kidneys, spleen, and a portion of thigh muscle were excised, blotted, and weighed. The samples were ignited for 3 hours at 550°C, dissolved in 1N HCl, and analyzed for radiophosphorus. Recovery of labelled phosphorus added as PF_6^- to 6 control tissue samples was 95.9 ± 1.6% (mean ± S.E.).

Results. Concentration of radioactivity in whole blood and plasma reached a maximum at 15 minutes, then fell precipitously (Fig. 1). No detectable activity was found in the blood 18 hours after injection. Concentration values were consistently higher in plasma than in blood, and may reflect differences in water content. The findings are consistent with the belief that blood cells are freely permeable to the PF_6^- ion.

Ten minutes after intravenous injection the mean concentration of radiophosphorus in the tissues, expressed as percent of dose per gram of fresh weight, was: liver, 0.57; spleen, 0.55; heart, 0.51; lungs, 0.97; kidney, 1.67; and muscle, 0.29.

The high radiophosphorus content of kidney seems in accord with the role of this organ in the excretion of the label. Thus 55% of the dose had been cleared at the end of one hour, and 88% by the end of 3 hours (Fig. 2). The 24-hour urine contained all of the injected radiophosphorus. The rapidity of clearance suggests that tubular reabsorption was minimal, if not absent.

In 8 urine samples selected at random, the ratio of calculated-to-found fluoride was 0.98 ± 0.03 (mean ± S.E.). This finding substantiates the physiological stability of PF_6^-. Similarly, the quantitative recovery of injected radiophosphorus in urine can only be explained by the inability of the rat to hydrolyze PF_6^- to $PO_4^{\equiv}$ and F^-.

The physiological stability of this ion, coupled with its quantitative excretion in the urine, suggests consideration of radiophosphorus-tagged hexafluophosphate as a possible tool in renal function studies.

Summary. 1. Distribution and excretion of radiophosphorus labelled hexafluophosphate ion has been investigated in the rat. 2. The concentration of PF_6^- in whole blood and plasma reached a maximum at 15 minutes, fell precipitously, and none was found 18 hours after its injection. 3. Evidence is presented that PF_6^- is not hydrolyzed by the rat. 4. Injected PF_6^- is rapidly and quantitatively excreted in the urine.

1. Zipkin, I., and McClure, F. J., *U. S. Pub. Hlth. Rep.*, 1951, v66, 1523.

2. Sidgwick, N. V., *Chemical Elements and Their Compounds*, 1950, vI, p757, Oxford Univ. Press, London.

3. Largent, E. J., and Heyroth, F. F., *J. Ind. Hyg. and Toxicol.*, 1949, v31, 134.

4. Sidgwick, N. V., *Chemical Elements and Their Compounds*, 1950, vI, p756, Oxford Univ. Press, London.

5. Woyski, M. M., in *Inorganic Syntheses* (L. F.

Audrieth, Ed.), 1950, vIII, p3, McGraw-Hill Book Co., Inc., New York.

6. Forbes, M. C., Roswell, C. A., and Maxson, R N., *ibid.*, p145.

7. Maxson, R. N., *ibid.*, p99.

8. Willard, H. H., and Winter, O. B., *Ind. Eng. Chem.*, Anal. Ed., 1933, v5, 7.

9. McClure, F. J., *Ind. Eng. Chem.*, Anal. Ed., 1939, v11, 171.

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Pharmacodynamics of Chlorothiazide (Diuril), An Orally Effective Non-Mercurial Diuretic Agent.* (23277)

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The following report is concerned with chlorothiazide (Diuril)† a diuretic agent of low toxicity, which is well absorbed by the oral route(1) and is equally as potent as meralluride (given parenterally).

Methods and materials. The acute renal hemodynamic responses to doses up to 10 mg/kg of chlorothiazide (Diuril) given intravenously were observed in anesthetized dogs employing methods and technics described previously(2). The acute effect on water and electrolyte excretion was also observed in unhydrated dogs(3).

The clinical studies were done on 10 male patients who had been in mild heart failure but who at the time of the study were free of edema. The technic for evaluation of diuretic agents has been described previously(4,5). The subjects of the study were ambulatory, hospitalized patients maintained on a metabolic ward, eating a diet containing 50 mEq of sodium per day, and drinking 3,000 ml of distilled water per day. After a suitable period of adjustment to the diet the patient's urinary sodium was approximately 90% of the dietary intake, *i.e.*, about 45 milliequivalents per 24 hours. When this point was reached, the drug was given as a single dose at 6 a. m. and observations were made of subjective and objective signs of toxicity as well as of the urinary sodium excretion, analyzed with the use of a Beckman flame photometer. After another suitable interval (5 to 7 days) for equilibration, the next dose was given and so forth. The drug was administered 10 times at each dose and the results subjected to appropriate statistical analysis as justified by previous studies(4).

Results. Following intravenous administration of chlorothiazide (Diuril) to dogs there was a significant increase in water excretion associated with an increase in sodium, potassium, and chloride excretion which was generally proportional to the dose (Fig. 1A and 1B). The diuretic threshold dose was less than 0.5 mg/kg body weight. The diuretic effect was not due to an increase in glomerular filtration rate since renal blood flow and glomerular filtration rate were not increased (Fig. 2). This leads to the conclusion that the increase in water and electrolyte excretion is due to a direct tubular effect of the chlorothiazide which blocks the reabsorption of these substances. Mean blood pressure was not altered.

The particular electrolyte excretion pattern associated with administration of a single 2000 mg oral dose of chlorothiazide to a typical patient is presented in Fig. 3. The greatest increase in cations was in the excretion of sodium but potassium excretion was also increased temporarily. There was a moderate decrease in ammonia excretion following administration of the drug. Among the anions observed, the greatest increase in excretion rate was in chloride with a significant but temporary increase in bicarbonate. Total in-

* Supported in part by Houston Heart Assn.

† 6-chloro-7-sulfamyl-1,2,4 - benzothiadiazine-1, 1-dioxide. Supplied as Diuril by Merck, Sharp & Dohme, West Point, Pa.