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THE BIOLOGICAL BEHAVIOR OF ORGANIC COMPOUNDS CONTAINING RADIOPHOSPHORUS*

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Introduction.

This study was undertaken with the objective of observing the distribution in the rat of organic compounds of phosphorus labelled with the P^{32} isotope as tracer. The fate of several of these compounds was studied in animals bearing tumors. The distribution of inorganic phosphate in animal tissues is well known but was included in this work for comparison with the organic phosphorus compounds.

Thirteen substances were synthesized and represented six different classes of organic phosphorus compounds. These were: five phosphine oxides, two phosphinic acids, one phosphonic acid, three esters and a di as well as a tri anilide of phosphoric acid. The distribution data were obtained from radioactivity measurements only and the possibility of chemical alteration of the injected compound in the animal body was not examined. A number of similar tracer studies have been carried out using radiophosphorus in various ester-type compounds. In addition, some animal distributions of non-radioactive organic phosphorus compounds have been observed.

Preparation of labelled di-isopropyl fluorophosphate is described by Witten and Miller (7) and also by Saunders and Worthy (8) while its distribution in rabbits has been studied by Jandorf and McNamara (2). The latter

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found that the concentration of radioactive fluorophosphate was highest in liver, kidney and lung. They also obtained radioactive sodium di-isopropyl phosphate and noted that in the rabbit it was excreted with little retention in any organ.

Glyceryl phosphates and phosphorylcholine have been tagged with P^{32} for studies related to phospholipid metabolism (3, 17, 40). Their conversion into phospholipid of liver and kidney was shown in surviving slices and in the intact animal. Distribution of radioactive phosphorylcholine has been studied in rats after intraperitoneal injection by Riley (10, 11) and has been shown to be only slightly different from phosphate, though excreted somewhat more slowly than the latter. This author assumes hydrolysis of the phosphorylcholine after a brief period and then distribution of the P^{32} as inorganic phosphate.

Beta and alpha glycerophosphates were made radioactive (40) and tested as precursors of phospholipid synthesis in the rat liver. The esters were apparently hydrolyzed after injection and the constituents passed into the cells before phospholipid synthesis took place.

Aminoethylphosphoric acid, which has been found to be a constituent of many organs of the rat and of human and animal tumors (1, 16) was prepared containing P^{32} by Chargaff and Keston (4). These workers studied transformation of the injected material into phosphatides in normal and tumor bearing animals. The compound was thought to have no specific function in tumor growth but to be a product of cephalin katabolism. It was not found however in fresh rat carcinoma by Le Page (32) who assumed it may be a product of autolysis.

Methods for the preparation of radioactive glucose-6-phosphate and of

propanediol-alpha-phosphate have been described by Lampson and Lardy (5, 6) starting from radiophosphoric acid. Propanediol phosphate has been found in appreciable amounts in brain tissue and also in rat liver, kidneys and carcinoma and in the eggs of some marine forms (14, 32). The ester (labelled with P^{32}) was obtained by Lindberg (14, 15) and its rapid uptake by rat liver was examined.

Blood, urine and feces values of orally administered phosphanilic acid (para-aminobenzene phosphonic acid) in mice were determined by Pendse and Bhide (9) but the compound was not made radioactive. Only small amounts were present in the blood but the substance was excreted in urine and feces in considerable amounts.

Radioactive tri-orthocresyl phosphate (tri-orthotolyl phosphate) was prepared by Hodge and Sterner (12) and its skin absorption and its excretion studied in human subjects and in dogs. In both cases absorption was rapid from the site of application. Distribution results in the dog showed high liver values and some affinity for nerve tissue but very low bone values. The ester was thought to be resistant to hydrolysis during the one day period of the test. Necrosis of rat sarcoma has been observed with this ester (37) and in 10% of the cases total regression of the tumor occurred.

Some distribution results in nerve tissues of the cat were found by Bird, Cohn and Weiss (13) of P^{32}-labelled triphenyl phosphite. This substance, which is a convulsant, was rapidly absorbed and soon hydrolyzed after intraperitoneal injection. The animals were sacrificed after 1.5 hours and the hydrolysis products (phenol and phosphorous acid) were widely distributed in the tissues.

The insecticide, octamethyl pyrophosphoramide (OMPA) has been made radioactive (18) and its uptake by plants studied. The entire plant is made

insecticidal to biting and sucking insects by this substance. The compound also has an anti-cholineesterase activity in rabbits (39).

The property of a citrus phosphotransferase in transferring the phosphate group from p-nitrophenyl phosphate to methanol was studied by Axelrod (33) using the radioactive ester. The methyl phosphate formed had the same specific activity as the p-nitrophenyl phosphate even when incubated in the presence of non-radioactive inorganic phosphate showing the latter is not involved in the reaction.

Orstrom (45) has isolated labelled phosphoglycollic acid from human erythrocytes after injection of radiophosphate.

Several esters of interest as insecticides were tagged with P^{32} and their distributions and excretion rates studied in the roach Periplaneta americana (46).

Three esters were employed in the present work, all of which are of some biological interest.

Cholesterol phosphate is of interest in that its hydrolysis products are widely distributed in the body. The structure of the compound is uncertain, however, and it may be an ester of pyrophosphoric acid (43).

Di(beta-aminoethyl) phosphoric acid was made by Jackson (36) as an intermediate for the synthesis of sulfanilamide derivatives. It is the secondary ester of ethanolamine corresponding to the primary ester (aminoethyl-phosphoric acid) referred to above.

Synkavit is the sodium salt of the diphosphate of 2-methyl-1-4-naphthohydroquinone and was synthesized originally as a solubilized Vitamin K substitute. It has been studied in squamous cell carcinoma as an inhibitor of mitosis (35).

The other compounds which were prepared have evidently not been examined

previously in biological work.

Methods.

I. Synthetic.

The radioactive phosphoryl chloride which was used as starting material in these preparations was obtained by a slight modification of the method of Axelrod (33). Specific activities were of the order of 6 mc/gm but greater specific activity can be obtained. Radioactive phosphoryl chloride has also been obtained by a number of other methods (4, 7, 12, 14, 17, 18, 38).

The radioactive organic compounds were made from the phosphoryl chloride by processes very similar to those described in the literature except as noted. Kosolapoff (31) gives a very thorough survey of preparative methods for organic phosphorus compounds of all types. Synthetic methods and references for the organic compounds used in the present work are listed as follows:

Triphenyl phosphine oxide, $(C_6H_5)_3PO$. This was obtained from phenylmagnesium bromide and phosphoryl chloride in ether solution (19).

Tributyl phosphine oxide, $(C_4H_9)_3PO$. (20).

Diphenyl ethyl phosphine oxide, $(C_6H_5)_2(C_2H_5)PO$. (21)

Diphenyl butyl phosphine oxide, $(C_6H_5)_2(C_4H_9)PO$. (21).

Diphenyl phosphinic acid, sodium salt, $(C_6H_5)_2POONa$. (22,23)

Cholesterol Phosphate, (24,25).

Phosphoric trianilide, $(C_6H_5NH)_3PO$. (26)

Dianilidophosphoric acid or phosphoric dianilide, sodium salt, $(C_6H_5NH)_2POONa$. (27,28).

Synkavit, Tetrasodium salt of 2-methyl-1-4-naphthohydroquinone diphosphate, $CH_3.C_{10}H_5(OPO(ONa)_2)_2$. (34).

Di(beta-aminoethyl)phosphoric acid monohydrochloride, $(NH_2.CH_2.CH_2.O)_2POOH,HCl$. (36).

Benzene phosphonic acid, sodium salt, $C_6H_5PO(ONa)_2$. (44).

Triphenyl phosphine oxide monosulfonic acid, sodium salt of probable formula $(C_6H_5)_2(C_6H_4.SO_2.ONa)PO$. This was obtained from strongly radioactive triphenyl phosphine oxide. 0.5 gms of the oxide was dissolved in 25 ml. of chlorosulfonic acid at 10°C and this solution heated to 100°C on a water bath for 10 minutes. It was then cooled in an ice bath and poured dropwise onto a large amount of crushed ice. The precipitated sulfonyl chloride was filtered off and boiled with water until complete solution occurred. This solution, after charcoal decolorization and filtration, was neutralized with sodium carbonate and evaporated to dryness on the steam bath. The white solid obtained was dried overnight in a vacuum desiccator and dissolved in water for use.

For characterization, the sulfonamide was prepared from a sample of non-radioactive oxide. A portion of the sulfonyl chloride (about 0.5 gms.) was digested with 50 ml. of concentrated ammonium hydroxide at 60°C for 1/2 hour. The solution was filtered and acidified with cold hydrochloric acid giving a somewhat gummy precipitate which was purified by reprecipitating the ammoniacal solution with acid several times and finally crystallizing from ether-petroleum ether. The product was washed with petroelum ether and air dried. It melted 105-115°C with some gas evolution and evident decomposition. Attempts to get a sharply melting product by further recrystallization failed.

Anal:	C	H	N
Calcd.	60.50	4.48	3.92
Found	59.88	4.85	3.61

for $C_{18}H_{16}NSPO_3$ or $(C_6H_5)2(C_6H_4.SO_2.NH_2)PO$.

By analogy with other orientations observed in substituted aromatic

phosphine oxides, it was thought that these sulfonated derivatives were meta compounds (31).

Mercapto diphenyl phosphinic acid, sodium salt of probable formula $(C_6H_5)(C_6H_4 \cdot SH)POONa$. 0.5 gms. of strongly radioactive diphenyl phosphinic acid was reacted with chlorosulfonic acid in a manner similar to that described for the oxide, with the difference that the solution was kept at 100°C for 3 hours before cooling and pouring onto crushed ice. The precipitated sulfonyl chloride (probably of the composition $(C_6H_5)C_6H_4 \cdot SO_2 \cdot Cl)POCl$) was dissolved in excess glacial acetic acid and boiled with 30-mesh zinc with intermittent addition of concentrated hydrochloric acid. After four hours refluxing, the solution was poured into ice and dilute hydrochloric acid mixture. The precipitated acid was purified by several depositions from its hot carbonate solution by adding hydrochloric acid and cooling. The least soluble material was rejected. It was then recrystallized from ether, m. p. 154.5-158°C. An analytical sample was recrystallized from water several times, rejecting the least soluble resinous material (which has a higher sulfur content). After air drying the acid had m. p. 162-163°C.

Anal:	C	H	S
Calc.	57.60	4.40	12.80
Found	57.08	4.15	13.15

for $C_{12}H_{11}PSO_2$.

It was assumed that this substance is also a meta-substituted derivative (31). For use, the acid was neutralized with sodium carbonate solution.

II. Biological.

After preparation, varying amounts of the above materials were given to albino rats of both sexes by intravenous administration, and in a few cases by intramuscular injection. Three or four animals per study were employed,

ranging in weight from 200 to 250 gms. The animals were sacrificed at one and four day periods afterwards, except as noted in Table (II). Specific activity of the injected solutions ranged from 1.5 to 17 uc/ml. Total volume of the injected solution per animal varied between 0.1 and 1 ml. The P^{32} solution used for comparison with the organic compounds contained added carrier phosphate. Those substances which were water-insoluble or sparingly soluble were administered in cottonseed oil. The acidic compounds were administered as saline or water solutions of their sodium salts at pH 7-8.5. The cholesterol phosphate was very insoluble and intractable in the dried form and was used therefore as a colloidal suspension in water which was never allowed to dry out during preparation. Vehicles used and the mg/kg injected are given in Table I.

After the animals were sacrificed, samples of tissues were prepared for radioactive assay. This was done by drying the weighed tissues and the remainder of the animal, as well as the urine and feces, at 100°C for two days and then ashing at 550°C for one day. Known weights of ash were taken for assay so that self absorption corrections could be made. The data obtained are summarized in Table II. The results are expressed as per cent of the dose administered per gram of organ or tissue wet weight and per cent of dose excreted in urine and feces. Corrections were made for recovery which varied from 85% to 111% of the dose administered. A Table (III) is also included of average wet weights of the organs and tissues and the ranges in these weights.

The fate of the compounds was studied in liver, spleen, gastro-intestinal tract, muscle, skin, and skeleton and in some cases in animals which were bearing lymphosarcoma, neurofibroma or fibrosarcoma tumor implants. Urine and feces were collected and counted also. Various other organs and remains are not included in the Table. A Geiger-Muller counter with thin window tube

was used for counting the samples. No attempt was made to reisolate any of the injected material from the tissues of the animals. Some of the compounds could have been metabolized or altered therefore in the animal body.

Results.

Numerical values are given in Table II. Each substance is described individually and relations or similarities indicated. In most cases distributions were different from that of simple inorganic phosphate. This is believed to mean biological stability of the compounds with respect to breakdown to phosphate, in these cases. The pattern of P^{32} distribution (as phosphate) characterized by considerable muscle, skeleton and liver uptake and moderate excretion rate is not duplicated by the organic compounds with the exception of one of the esters and to a certain extent by benzene phosphonate and di(beta-aminoethyl)phosphate. With the exception of cholesterol phosphate and Synkavit, all of the organic derivatives show an excretion rate much more rapid than inorganic phosphate up through the four day period studied.

Triphenyl phosphine oxide: This compound is rapidly excreted and is not retained to any extent in the rat tissues or in tumor. By the end of the fourth day 98-99% of the injected dose can be accounted for in the urine and feces. This high rate of excretion was observed also with some of the other compounds. 0.03% of the injected dose of the oxide was found in tumor (per gm. tissue) after one day.

Triphenyl phosphine oxide monosulfonic acid, sodium salt: This was prepared primarily to obtain a water soluble form of the parent oxide. The main difference in distribution between this substance and the unsulfonated oxide was greater urine/feces values for the water soluble sulfonate after one day periods (though this trend is reversed in the four day series). Very little

TABLE I

Compound	Vehicles	mg/kg
P^{32} & Carrier phosphate Na_2HPO_4	Saline solution	5.0
Cholesterol phosphate	Saline suspension	21
Synkavit	Saline solution	4.9
Benzene phosphonic acid Na salt	Saline solution	10
Mercapto diphenyl phosphinic acid, Sodium salt	Water solution	11.4
Dianilido phosphoric acid Na salt	Water solution	510
Diphenyl phosphinic acid Na salt	Saline solution	8.9
Diphenyl butyl phosphine oxide	Cottonseed oil	16
Triphenyl phosphine oxide	Cottonseed oil	10
Di(aminoethyl)phosphoric acid	Saline solution	64
Phosphoric trianilide	Cottonseed oil	123
Diphenyl ethyl phosphine oxide	Cottonseed oil	21
Tributyl phosphine oxide	Saline solution	8.7
Triphenyl phosphine oxide Monosulfonic acid Na salt	Water solution	319

TABLE II

Fate of Organic Phosphorus Compounds in the Rat After Parenteral Administration. Data are Expressed as % of Administered Dose per gram of Wet Weight Tissues and % of Dose Excreted in Urine and Feces. One Day Series.[a]

	Liver	Spleen	Muscle	Skel.	Skin	GI Tract & Contents	Feces	Urine
P^{32} & Carrier Phosphate (2 day) Na_2HPO_4	0.55	0.43	0.21	0.97	0.14	0.40	5.1	27.7
Cholesterol Phosphate	2.9	2.0	-	0.33	0.08	0.42	0.72	5.24
Synkavit								
Benzene phosphonic acid Na Salt	0.38	0.36	0.12	0.54	0.08	0.30	2.5	60.7
Mercapto diphenyl phosphinic Acid. Sodium salt	0.10	0.03	0.23	0.05	0.03	1.16	9.52	69.1
Dianilido phosphoric acid Na salt								
Diphenyl phosphinic acid Na salt	0.03	0.01	0.01	0.09	0.04	0.09	24.7	63.8
Diphenyl butyl phosphine oxide	0.05	0.01	0.005	0.07	0.02	0.84	21.2	64.2
Triphenyl phosphine oxide	0.06	0.08	0.02	0.07	0.02	1.10	40.0	32.0
Di(aminoethyl)phosphoric acid	0.26	0.25	0.06	0.58	0.06	0.17	5.3	71.0
Phosphoric Trianilide	0.06	0.01	0.008	0.03	0.01	0.39	22.1	66.5
Diphenyl ethyl phosphine oxide	0.05	0.01	0.006	0.02	0.01	1.14	13.8	63.3
Tributyl phosphine oxide	0.007	0.01	0.002	0.01	0.002	0.018	9.96	89.2
Triphenyl phosphine oxide (IM)[b] Monosulfonic acid Na salt	0.009	0.007	0.001	0.0007	0.002	0.03	46.6	51.0

a. Data are corrected to 100% recovery
b. Intramuscularly

TABLE II

Fate of Organic Phosphorus Compounds in the Rat After Parenteral Administration. Data are Expressed as % of Administered Dose per gram of Wet Weight Tissue and % Excreted in Urine and Feces. Four Day Series.[a]

	Liver	Spleen	Muscle	Skel.	Skin	GI Tract & Contents	Feces	Urine
P^{32} and Carrier Phosphate (IM)[b] Na_2HPO_4	0.48	0.48	0.22	1.23	0.14	0.24	11.8	21.3
Cholesterol Phosphate (5 day)	1.23	1.16	-	0.3	0.05	0.10	6.4	8.8
Synkavit (3 day)	0.45	0.66	0.15	0.42	0.09	0.35	5.32	28.9
Benzene Phosphonic acid Na salt	0.28	0.29	0.13	0.75	0.06	0.15	7.99	55.2
Mercapto diphenyl phosphinic Acid Sodium salt								
Dianilido phosphoric acid Na salt	0.09	0.10	0.05	0.15	0.02	0.04	21.3	68.1
Diphenyl phosphinic acid Na salt	0.006	0.01	0.006	0.04	0.008	0.009	39.1	57.0
Diphenyl butyl phosphine oxide								
Triphenyl phosphine oxide	0.01	0.01	0.004	0.02	0.01	0,04	39.0	60.0
Di(aminoethyl)phosphoric acid (7 days)	0.12	0.14	0.04	0.33	0.02	0.04	12.8	72.9
Phosphoric trianilide (5 day)	0.03	0.01	0.003	0.02	0.005	0.03	34.2	63.1
Diphenyl ethyl phosphine oxide								
Tributyl phosphine oxide	0.004	0.01	0.002	0.008	0.002	0.019	15.6	83.5
Triphenyl phosphine oxide(IM)[b] Monosulfonic acid Na salt	0.001	0.007	0.001	0.001	0.0005	0.002	49.1	50.8

a. Data are corrected to 100% recovery.
b. Intramuscularly

TABLE III

Tissue and Organ Wet Weights (Gms.)

Organ	Average Wts.	Range of Wts.
Liver	10.1	7.3 - 12.5
Spleen	0.9	0.5 - 1.9
Muscle	10.9	7.3 - 13.6
Skeleton	125	91 - 181
Skin	29.8	23 - 48
GI Tract & Contents	18.5	13.5 - 24.9

organ retention occurred in those organs measured and the compound passed rapidly from the body.

Tributyl phosphine oxide: This soluble product was rapidly excreted showing practically no retention in any organ measured. The oxide gave the highest urinary excretion values of any compound tested. The ratio of urine to feces concentrations averaged about 8/1.

Diphenyl ethyl and diphenyl butyl phosphine oxides: The distribution of these two analogous oxides was similar. In both cases, the amounts of activity found in the gastro-intestinal tract were considerably higher than those observed with the other compounds. Other than in the tract, little retention occurred. It is noteworthy that the urinary excretion values were higher in these two cases than in the case of the triphenyl derivative. This may be related to the somewhat greater water solubility of these two oxides.

Diphenyl phosphinic acid, sodium salt: The values obtained with this compound resembled those found with the oxides, especially the mixed diphenyl alkyl phosphine oxides. As with these compounds, the acid is comparatively rapidly removed from the system. The minor amounts of activity in skeleton, skin and muscle are not believed to be significant. Tumor activity was also low, about 0.01% of the dose.

Mercapto diphenyl phosphinic acid, sodium salt: This substance was prepared in an attempt to modify the distribution of diphenyl phosphinic acid by including an SH group in the molecule. In this way it was hoped to effect protein binding of the compound through its sulfhydryl group. Whether or not this was successful, the results were somewhat different from those obtained with the unsubstituted acid. Higher activity values were found for the GI tract and skeleton and lower values for feces. The uptake by tumors of this

mercapto acid (0.08 to 0.11% per gm.) was much greater than that of the parent unsubstituted acid (0.01% per gm.) but all of these tumor values were comparatively small. Comparing the two types of tumors tested with the mercapto acid, higher activities were obtained for neurofibroma as against fibrosarcoma.

Benzene phosphonic acid, sodium salt: The distribution of this substance in the animal body resembled that of phosphate more closely than that of the other compounds containing a C-P link. It was excreted at a greater rate than phosphate however and organ values were lower. Whether or not clevage occurred at the C-P bond was not determined but the great stability of this compound and analogous substances (31) may argue against this. A comparison of benzene phosphonate with diphenyl phosphinate shows little similarity as the latter passed through without evident retention by any organ.

Cholesterol phosphate: This compound was excreted considerably more slowly than any other phosphorus derivative including phosphate itself. High liver and fairly high tumor values were observed. The tumor used was lymphosarcoma and it took up 0.95% of the administered dose per gm. tissue. The tabulated data indicates that not much of the ester was hydrolyzed to cholesterol and phosphate.

Phosphoric trianilide: This anilide appeared to be excreted largely without breakdown to phosphate and showed distribution results similar to those of the oxides and diphenyl phosphinate. The resistance of this compound to enzymatic cleavage would be in line with its general chemical inertness (29).

Dianilido phosphoric acid, sodium salt (Phosphoric acid dianilide, sodium salt): Judging from the distribution results, this compound was excreted mostly without hydrolysis to phosphate. This was somewhat surprising in this case, considering the instability of the anilide with respect to breakup by chemical

agents, especially acids. Higher muscle, skeleton and liver values found with this dianilide as compared to the trianilide may indicate some hydrolysis.

Di(beta-aminoethyl) phosphoric acid monohydrochloride: Results obtained with this secondary ester indicate a small amount of hydrolysis to phosphate but most is apparently excreted without cleavage. The ester is excreted at a much faster rate than phosphate.

Synkavit, Tetrasodium salt of 2-methyl-1-4-naphthohydroquinone diphosphate: This was the only compound tested which showed clearly a large amount of hydrolysis or degradation to inorganic phosphate. Here virtually all of both phosphate groups were split off and the resulting activity distributed as inorganic phosphorus. Tumor animals employed showed tumor localization of the activity but only to an extent similar to that obtained with inorganic phosphate.

Discussion.

It was assumed by analogy with the corresponding arsenic compounds, that the toxicity of pentavalent (tetracovalent) phosphorus types would be less than that of the trivalent compounds, so only the former were examined in this work. No naturally occurring compound having a phosphorus-carbon linkage is known and those tested here seem to be largely resistant to cleavage at this point.

All five phosphine oxides studied were rapidly removed from the body, the more water soluble types showing greater urinary excretion. This behavior and the lack of organ retention indicates the biological stability of these substances, which is also in agreement with their chemical inertness. Triphenyl phosphine oxide can be distilled unchanged at atmospheric pressure at a temperature of over 400°C indicating the degree of chemical stability possessed by some members of this class.

The two phosphinic acids, with similar rapid excretion rates probably also are not being broken down to phosphate to appreciable extents. This applies also to phosphoric trianilide and probably to the dianilide.

The ratios of skeleton values to excretion values which can be calculated for the oxides, phosphinic acids and anilides, are much smaller than the corresponding values for inorganic phosphate. This is due to lower bone deposition and to greater excretion rates.

The case of benzene phosphonic acid is somewhat different from the others as the organ uptake shows some resemblance to phosphate, though the urine excretion is considerably greater. Considering the stability of the compound and general chemical evidence (31, pg 143), it would not be expected that much breakdown of this molecule would occur in the body. If this supposition is valid the distribution results remain to be explained, however.

Substitution of the benzene phosphonic acid molecule by introducing various groups on the aromatic ring may alter the distributions obtained. Modification of phosphoric acid in a similar way is not possible. In this connection, some testing of the para-amino derivative(phosphanilic acid) has been done by Pendse and Bhide (9), who found high urine and feces values and low blood concentrations with orally administered non-radioactive material in mice. Their distribution results would tend to support the supposition that the aryl phosphonic acid type of molecule is not degraded to a large extent in the animal body.

Primary phosphoric acid esters and amides are hydrolyzed in most cases into their constituents by phosphatases or phosphoamidases. Among the many examples known may be cited glycerophosphates, phosphorylcholine and the known reversible synthesis of such metabolic products as hexose phosphates and

phosphocreatine. On the other hand, a number of secondary and most of the tertiary esters seem to be resistant to hydrolysis by enzymes. Jackson (36) has shown that the sulfanilyl derivative of di(aminoethyl) phosphoric acid (a secondary ester) is resistant to hydrolysis by two types of phosphatases both of which were active against beta-glycerophosphate. The stability of di-isopropyl phosphate (2) and of tricresyl phosphate (12) may also be mentioned. In the present work, di(aminoethyl) phosphoric acid appears to be fairly stable. This effect is evidently due to the property of phosphatases of preferentially hydrolyzing mono esters.

The data obtained for Synkavit, which contains two primary phosphate ester groups, leads to the conclusion that both of these groups are nearly entirely cleaved from the molecule.

The cholesterol ester is apparently stable to hydrolysis and is probably being handled largely as a colloid by the reticulo-endothelial system. The low solubility and colloidal properties probably influence its stability to phosphatases.

The problem of the hydrolysis of substituted amides of phosphoric acid in vivo is apparently similar to the case of the esters. The naturally occurring phosphagens, which are monoamides are easily broken down and resynthesized. The dianilide, used in this work, is more resistant to hydrolysis and the trianilide is stable.

A phosphoamidase has been demonstrated to exist in the body by Gomori and others (30, earlier articles are referred to here). Its concentration was highest in CNS gray matter and malignant tumors as demonstrated by histological staining techniques. However this enzyme was active against an amide containing the $-N-P(O)-NH_2$ grouping where the NH_2 is labile, whereas those tested here

contain aromatic rings bound to each nitrogen atom. Gomori's substrate was p-Cl.C_6H_4.NH.PO(NH_2)(OH)(para-chlorophenyl diamido phosphoric acid). Rorig (41) has recently shown that the preparative method used gives this substance and not p-Cl.C_6H_4.NH.PO$(OH)_2$ as was formerly believed. The latter compound (para-chlorophenyl amido phosphoric acid) has lately been prepared (42) and has been shown to be different from the compound used by Gomori. The only known naturally occurring compounds containing the N-P link are the phospho derivatives of creatine, creatinine and arginine and an unknown substance from certain annelids (47).

Summary.

Representative members of six different classes of organic phosphorus compounds were synthesized containing radioactive phosphorus as a tracer. These compounds, in suitable form for biological use, were injected by parenteral routes into albino rats for the purpose of determining their fate in the animal body. After one and four day periods, the animals were sacrificed and the distribution of the tagged substances determined by counting the radioactivity of the dried and ashed tissues and organs. Conclusions concerning the biological stability of the injected materials are given where supporting experimental evidence is obtained.

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