A STUDY OF THE ORGANIC PHOSPHATE FRACTION OF HUMAN URINE

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HISTORICAL SURVEY

As early as 1846 Ronalds found a portion of the phosphate of the urine present in organic combination. Sonitchewsky (1880) believed glycerophosphoric acid to be a common constituent of human urine, while Zeulzer (1881), Lepine and Eymonnet (1882) did further work on the organic phosphate excretion in normal and pathological cases. An excess was found in apoplexy, epilepsy and delirium tremens, and also in typhoid fever and acute pneumonia, while a decrease was noted in meningitis.

According to Bülow (1894) the oral or subcutaneous injection of glycerophosphates in dogs did not result in any increase in the organic phosphate excretion in the urine. Ceconi (1896) stated that 11 to 28 mg. of organic phosphate were excreted per day, and values above 20 mg. were indicative of pathological conditions. His contention further was that variation in the food intake did not affect the organic phosphate of the urine. Other workers supporting this view were Mandel and Oertel (1902)

and Symmers (1904). Keller (1900) found that fasting resulted in an increase in the organic phosphate excretion of the urine. In fatty degeneration of the liver, Lepine (1901) observed an abnormally large glycerophosphoric acid excretion, while Symmers (1904) found an excess in lymphatic leukemia and nervous diseases of the degenerative type. Plimmer, Dick and Lieb (1909-10), Mathison (1910), and Kondo (1910) found the excretion of organic phosphate to be very irregular and not dependent on the diet.

Among the compounds of phosphorous suggested as present in the organic phosphate portion are the following: α - and β -glycerophosphate, glycerophosphoric acid, hexose di-phosphate, creatine phosphate, adenosine phosphate, phospho-di-hydroxy acetone, the choline ester of sphingosine phosphoric acid, flavine-phosphoric acid, adenosinetriphosphoric acid, phosphoserine, and uridinphosphoric acid. There has been little done, however, in the way of isolation or separation of the organic phosphate portion. The present work was undertaken in an attempt to enlarge the knowledge of the organic phosphate fraction of the urine.

The earliest method used in determining phosphate was that of Pincus (1854) using uranyl acetate. The total phosphorous after fusion was determined by titration with

uranyl acetate and the inorganic phosphate by titration without fusion. Organic phosphate was calculated by difference. Symmers (1904) used uranyl nitrate as the precipitating reagent.

Youngburg and Pucher (1924) developed the magnesia mixture method for the determination of inorganic phosphate. The remaining organic phosphate portion was believed to exist as glycerophosphate. Using Briggs' method, Brain, Kay and Marshall (1928) found the amount of organic phosphate in urines of 27 normal persons varying from 0 to 0.28 mg. per cc.

Uhrbach (1931) compared his method with that of Bell and Doisy, Fiske and Subbarrow, Folin and Embden, and stated that his method for the determination of total and inorganic phosphate in urine using the step-photometer was superior. Walker (1931-32) found values of 0.02 to 2.10 mg. per cc. (mean 0.603) for inorganic phosphate, and 0 to 0.195 mg. per cc. (mean 0.018) for organic phosphate.

Youngburg (1932-33) determined inorganic phosphate by the molybdate sulfuric acid mixture with stannous chloride and a standard phosphate. The total phosphate was determined the same way, after oxidation with sulfuric acid and hydrogen peroxide. An improved

direct method for the quantitative determination of organic phosphate in the urine was evolved by Walker and Walker (1932-33). The normal output for 24 hours was determined as being 6 to 13 mg. with a mean value of 9 mg.

Rae (1936) introduced a modified and more rapid method of estimation of the organic phosphate portion of the urine using the magnesia mixture procedure of Youngburg and Pucher (1924). In the following work, Rae's (1937) method was used for the organic phosphate analysis, while King's (1932) method was used in determining the inorganic phosphate in the urine.

EXPERIMENTAL WORK

I. METHODS OF ANALYSIS

The solutions used in the analysis for phosphate were 60% perchloric acid, 8.33% ammonium molybdate, and 0.33% 1-amino-2-naphthol-4-sulfonic acid reagent. In preparing the amino-naphthol-sulfonic acid the following materials were used: 0.84 grams of the 1:2:4 acid, 50 grams of sodium bisulphite and 10 grams of sodium sulphite, made up to 500 cc. with distilled water.

The standard phosphate solution used for comparison was made by dissolving 2.1935 grams of pure potassium dihydrogen phosphate in 500 cc. of distilled water. This solution contains 1.0 mg. of phosphorous per cc. A weaker

standard solution was made by diluting 5 cc. of this stock solution up to 500 cc. with distilled water. 10 cc. of this solution contain 0.1 mg. of phosphorous. 1 cc. of chloroform was added to prevent bacterial contamination.

(a) Inorganic phosphate was estimated in the following way. An amount of the solution to be tested was measured into a 25 cc. volumetric flask and distilled water added up to 10 cc. 2 cc. of 60% perchloric acid, 1 cc. of ammonium molybdate and 1 cc. of the sulphonic acid reagent were added and distilled water up to 25 cc. A standard containing 0.1 mg. of phosphate was prepared the same way at the same time. The flask was shaken and the colors read after five minutes using a Duboscq colorimeter.

(b) Total phosphate was estimated as follows. The sample was measured into a digestion tube, 2 cc. of 60% perchloric acid were added and the contents of the tube digested on an electric heater. The contents of the tube turned brown and then colorless with the evolution of dense fumes. A few drops of nitric acid or 30% hydrogen peroxide hastened complete oxidation of organic material. Excess nitric acid or peroxide was driven off by further heating. The contents of the tube were then cooled and washed into 25 cc. volumetric flasks. 0.2 cc. of

perchloric acid was added to make up for the loss which occurred during heating and the analysis from this stage was the same as in (a).

(c) The difference between total and inorganic phosphate gives the organic phosphate. A more direct way is to use Rae's method. To 10 cc. of the urine magnesia mixture (without ammonia) was added, with 0.16 cc. of magnesia mixture for each mg. of inorganic phosphate present. To this mixture was added a volume of 1 N ammonium hydroxide equal to the magnesia mixture added. The liquid was well shaken and allowed to stand for ten minutes (pH 8.8-9.0). It was then filtered and a 3 cc. aliquot of the filtrate used for the organic phosphate determination as in (b). A test was made on 3 cc. of the remaining filtrate for completeness of precipitation.

II. METHODS OF PRECIPITATING ORGANIC PHOSPHATE

Since no definite methods of precipitating the organic phosphate portion of urine are given in the literature, various reagents were tried upon concentrated urines containing an appreciable amount of organically bound phosphate. Only three of the procedures from the many tried are described below.

A. Barium Hydroxide Method.

1. About one and a half litres of fresh urine were taken in the first trial and analysed for inorganic and organic phosphate. The values found were: inorganic phosphate, 0.65 mg. per cc.; organic phosphate, .006 mg. per cc. To a one litre portion of this urine 6.006 grams of magnesium chloride and 7.64 grams of ammonium chloride were added to remove the inorganic phosphate and the mixture stirred until the solid dissolved completely. Concentrated ammonia (13.52 cc.) was then added and the mixture well stirred. The resulting precipitate of magnesium ammonium phosphate was permitted to settle before filtering.

The filtrate (volume 1030 cc.) was acidified with sulfuric acid to a pH of 4.0 and evaporated to 130 cc. on a hot plate, using an electric fan to hasten the process. The dark brown precipitate which formed on standing was filtered off and aliquots of this liquid analysed for phosphate with the following results: inorganic phosphate, negative; organic phosphate, 0.038 mg./cc. or a total of 4.94 mg. of organic phosphate. Since the original urine contained a total of 6.0 mg. of organic phosphate there has occurred a loss of 18%.

Hot saturated oxalic acid was added in excess to the remaining filtrate to precipitate the urea in the

form of urea oxalate. After standing overnight the precipitate was filtered off, washed with a small amount of water and the washings added to the filtrate. This filtrate plus washings was analysed for organic phosphate. The amount found was 0.33 mg. per cc. or a total of 4.29 mg. The loss by occlusion in the urea oxalate was about 13%. The filtrate was divided into aliquots of 25 cc. and an attempt was made to discover whether soluble or insoluble barium salts of the organic phosphate are formed when a saturated baryta solution is added.

A typical result is given when a 25 cc. portion containing 0.82 mg. of organic phosphate was treated with an excess of cold saturated baryta (pH 11). A heavy brown precipitate of barium salts was formed and filtered off. The barium present in the filtrate was removed with dilute sulfuric acid and the liquid analysed for phosphate. The organic phosphate was 0.002 mg. per cc. or a total of 0.20 mg.

It appears that 0.62 mg. of the organic phosphate has been precipitated as the insoluble barium salt, or else has been adsorbed on the precipitate of barium salts. Approximately 24% of the organic phosphate remains in solution, while 76% is precipitated with the barium salts.

An attempt was made to recover the organic phosphate portion from the precipitate of barium salts. This was done by suspending the precipitate in a small volume of water and adding dilute sulfuric acid to remove the barium as barium sulfate. Consistent appearance of a white colloidal precipitate in the test samples and the small amount of organic phosphate present made it impossible to analyse the solution colorimetrically.

2. In the second trial urines were collected over a period of several weeks and analysed for inorganic and organic phosphate. The required amount of magnesia mixture and concentrated ammonia was added in each case to remove the inorganic phosphate present. The results of the analysis are given in Table I.

TABLE I

Volume of Urine cc.	Specific Gravity	Phosphate mg/cc Inorganic	Organic
2000	0.1025	0.70	0.008
2000	0.1029	0.83	0.006
3650	0.1029	1.13	0.007
4000	0.1027	0.94	0.008
4000	0.1020	0.67	0.006
4000	0.1023	0.70	0.006
4000	0.1030	1.10	0.009
4000	0.1028	1.12	0.009
4000	0.1030	.98	0.008
4000	0.1027	.97	0.006

The urine, freed from inorganic phosphate, was acidified to a pH of 4 with 6 N hydrochloric acid and evaporated to a syrup in a large dish on an electric heater using a strong air current from a fan to speed up evaporation. The preliminary procedure adopted as an economy measure was to concentrate the urine by the partial freezing of two-litre portions in a metal cone, discarding the frozen part which consisted mainly of water, and collecting the unfrozen organic phosphate containing syrup. The volume of concentrated urine was

1100 cc. containing 0.20 mg. organic phosphate per cc. or a total of 220 mg. The urea was removed as urea oxalate by adding hot saturated oxalic acid in calculated excess. The volume of the filtrate was 1200 cc. containing 0.17 mg. of organic phosphate per cc. or a total of 204 mg.

The formation of barium salts with baryta was used on a 50 cc. sample which contained 0.17 mg. of organic phosphate per cc. or a total of 8.5 mg. An excess of hot baryta was added to the concentrate to a pH of 11. The precipitate of barium salts was filtered and dried. The barium present in the filtrate was removed by precipitation with dilute sulfuric acid. Analysis for organic phosphate in the filtrate was negative. The powdered barium salts were then treated with a little dilute hydrochloric acid with evolution of carbon dioxide due to the presence of carbonates and filtered. The barium present was then removed as barium sulfate with a small quantity of dilute sulfuric acid. The object of this procedure was to secure an acid soluble fraction from the barium precipitate. The volume of solution was 300 cc. containing no inorganic phosphate and 0.013 mg. organic phosphate per cc., or a

total of 3.9 mg. About 46% of the organic phosphate was recovered as the acid soluble fraction.

The same procedure was repeated on 830 cc. of the concentrated urine containing 0.17 mg. organic phosphate per cc. or a total of 141.1 mg. The weight of barium salts recovered was 130.5 grams. The total organic phosphate in the filtrate after the removal of the insoluble barium precipitate was 23.3 mg., leaving 117.8 mg. of organic phosphate brought down in the barium precipitate. The acid soluble fraction of organic phosphate recovered from the barium salts contained a total of 82.5 mg. of organic phosphate. Baryta precipitated 84% of the organic phosphate present in the urine concentrate, of which 70% was recovered as the acid soluble fraction of the barium precipitate.

B. Basic Lead Acetate Method

1. A 100 cc. urine concentrate sample with an organic phosphate content of 0.045 mg. per cc., or a total of 4.5 mg. (inorganic phosphate free) was used as a starting material. Hot saturated basic lead acetate solution was added at neutrality. A heavy precipitate of lead salts formed, which was filtered, suspended in a small volume of water and decomposed with hydrogen sulfide. Analysis showed that 2.2 mg. of organic phosphate remained in the

filtrate, while 2.0 mg. were precipitated with the lead salts. Basic lead acetate at neutrality brings down about 44% of the organic phosphate.

2. In a second attempt a 50 cc. sample of concentrated urine containing a total of 3.5 mg. of organic phosphate was used. The procedure was the same as in 1 only that more care was taken to have an excess of the basic lead acetate and greater caution exercised in washing the precipitates and their decomposition. The filtrate from the lead salts contained 1.12 mg. of organic phosphate while the organic phosphate recovered from the lead salts was 2.42 mg. With greater care about 70% of the organic phosphate was precipitated with basic lead acetate at neutrality and recovered in the lead-free form.

C. Cadmium Chloride Method

100 cc. of urine concentrate containing 0.06 mg. of organic phosphate per cc., or a total of 6.0 mg., were used in this trial. 200 cc. of 95% ethyl alcohol-cadmium chloride suspension were added. The mixture was let stand overnight with the formation of a heavy yellow precipitate (weight 20 grams). The precipitate was suspended in a small volume of water and the cadmium chloride removed with hydrogen sulfide. The cadmium sulfide was filtered off and the filtrate analysed, giving no organic

phosphate. The filtrate from the alcoholic cadmium chloride precipitation was freed from cadmium with hydrogen sulfide and analysis gave 2.7 mg. of organic phosphate. About 45% of the organic phosphate can be accounted for in the filtrate remaining after the cadmium chloride precipitation. The method gave poor results in its present form.

III. COMPARISON OF THE METHODS OF PRECIPITATION OF THE ORGANIC PHOSPHATE PORTION.

In the baryta method the suspension of the insoluble barium salts secured by the action of barium hydroxide on concentrated urine in water and decomposition with dilute sulfuric acid to remove the barium brought about the formation of a highly colored solution. The color formed interfered with the blue color of the perchloric acid method of analysis. However, between 46% and 70% of the organic phosphate was recovered as the acid soluble fraction of the barium precipitate.

Using the basic lead acetate as the precipitant, between 44% and 70% of the organic phosphate was precipitated either in the form of the insoluble lead salt or due to adsorption of the lead precipitate. Factors which favor more complete precipitation in this method were (1) use of highly concentrated urines, (2) working

in slightly alkaline solution. The basic lead acetate method seemed preferable because it did not give as much interfering color in the final solution and because it generally resulted in a greater percentage of the organic phosphate being precipitated.

IV. USE OF DECOLORISING AGENTS

The perchloric acid method of phosphate analysis was satisfactory for clear urines, but in the case of concentrated urines much difficulty was encountered in reading the colorimeter due to interfering colored substances present. In an attempt to remove the colored material from concentrated urine a number of decolorising agents was investigated.

Ordinary animal charcoal contains inorganic phosphates which render it unfit for this purpose. However, these were removed by boiling 10 grams of charcoal with about 15 cc. of 10% hydrochloric acid for ten minutes, filtering, and washing repeatedly with hot distilled water till the filtrate was clear from chlorides. When repeated tests on the filtrate showed no organic and inorganic phosphate present the charcoal was dried in an oven at 100° C. and was ready for use. About 0.4 gram of this acid-washed charcoal was added to 5 cc. of the concentrated urine. It is preferable to work at neutrality when using the charcoal.

Lloyd's reagent, while effective when used with dilute urines, was a poor decoloriser when used with concentrated urines. It further had a tendency to lose some of its silicate to the urine upon standing, with a resulting interference in the color test. Lampblack and wood charcoal were found to be poor decolorisers.

V. ACTION OF SOLVENTS ON THE ORGANIC PHOSPHATE PORTION

Ether. 5 cc. of concentrated urine containing 0.3 mg. of organic phosphate (inorganic phosphate free) were extracted with 2 cc. of ether. A jelly-like mass was formed at the junction of the two layers, which was quickly pipetted off. The organic phosphate present in the ether extract was found to be 0.06 mg. giving 19.5% of the organic phosphate extractable with ether. The gel forming at the junction of the ether-urine mixture disappears on standing, and with rapid removal of the layer after shaking as much as 30% of the organic phosphate may be recovered.

Benzene. Extraction with benzene also results in the formation of a gel. Only 10% of the organic phosphate may be removed in this solvent.

VI. ENZYME ACTION ON THE ORGANIC PHOSPHATE PORTION

A. Preparation of Kidney Phosphatase

150 grams of fresh pork kidney were chopped up and

ground thoroughly with quartz. About 100 cc. of distilled water were added and the mixture left overnight in a cool place. 200 cc. more water were added, the mixture filtered through cotton wool, and the filtrate set aside with a few cubic centimeters of chloroform.

B. Action on Sodium α-Glycerophosphate

The enzyme was first used on a sample of Boots' calcium α-glycerophosphate after converting it into the sodium salt by adding an equivalent amount of sodium oxalate. For the control experiment, two 1 cc. portions of the sodium α-glycerophosphate solution containing an approximate amount of the α-glycerophosphate at a pH of 8.3 were taken, water added up to 10 cc., 5 cc. of 20% trichloracetic acid and 1 cc. of the enzyme preparation were added to each. The trichloracetic acid precipitated the enzyme from the solutions with no conversion of organic phosphate taking place. The precipitates were filtered off, the total volumes being 16 cc. each. Total and inorganic phosphate content were determined on 3 cc. portions of the final volumes. The analysis gave the amount of organic phosphate present with no enzyme action, as well as any inorganic phosphate which may have been present.

In the enzyme experiment, two 1 cc. portions of

the sodium α-glycerophosphate were adjusted to a pH of 8.3 and water was added up to 10 cc. 1 cc. of the enzyme preparation was added to each and the test tubes placed in a water bath at 37° C. for thirty minutes. At the end of this time the enzyme had exerted its maximum effect and 5 cc. portions of 20% trichloracetic acid were added to each to precipitate the enzyme from the solution. After filtering the precipitate off, analysis on 3 cc. portions for the total and inorganic phosphate showed that complete conversion of organic phosphate into inorganic phosphate had occurred.

C. Action on Organic Phosphate in the Urine

50 cc. of fresh urine were analysed for inorganic phosphate which was then removed with magnesia mixture and ammonia. Two 10 cc. portions were brought to a pH of 8.3 and the controls performed using 1 cc. of kidney phosphatase. The enzyme action on two 10 cc. portions was noted, following the procedure described above for sodium α-glycerophosphate. It was found that 58% of the organic phosphate in fresh urine was hydrolysed by kidney phosphatase. Rae (1937) found that about 50% of the organic phosphate was hydrolysed. With concentrated urines only 6% was found to be hydrolysed.

VII. URINARY PHOSPHATASE

Kutscher and Wohlbergs (1936) have shown that there is an enzyme in urine capable of splitting the organically-bound phosphorous. Men excrete 3-5 times as much of this enzyme as women because of an admixture with the prostatic secretion, which also contains an active phosphatase. It is active in acid but not in alkaline medium and is somewhat specific in its action. Heating at 60° C. for 5 minutes completely destroys it. At a pH of 8.5 the enzyme is activated by magnesium ions.

To note the effect of long standing on fresh urine at room temperature (25° C.), three 100 cc. portions of fresh urine were freed from the inorganic phosphate with magnesia mixture and ammonia, and put away in corked Erlenmeyer flasks at room temperature. The initial organic phosphate content was 0.73 mg. while after 8 days the inorganic phosphate content was 0.55 mg. It was impossible to secure an organic phosphate analysis due to the insignificant amount present. About 75% of the organic phosphate present in the urine was hydrolysed on standing for 8 days at room temperature in contact with magnesium ions and possible urinary phosphatase.

VIII. THE RELATION BETWEEN ORGANIC PHOSPHATE EXCRETION AND DIET

Previous workers have maintained that a diet rich

in organic phosphate exerted no effect on the organic phosphate excretion in the urine. The results of the following diet experiment do not support this contention. The diet was for a period of one week,with a male subject twenty-four years of age as the test object. The food intake consisted of the regular three meals a day, and in addition eight eggs, one pint of cream and generous quantities of ice cream per day.

TABLE II

Urinary Phosphate Excretion.

	Day	Mg. per 100 cc.	Mg. per 24 hours	% increase in 24 hours over normal
Normal		0.70	7.45	
	1	1.33	14.83	99.06
	2	1.29	13.28	78.25
High Organic Phosphate Diet	3	1.50	15.16	103.49
	4	1.32	13.99	87.78
	5	1.61	14.40	93.28
	6	1.53	15.54	108.58
	7	1.38	11.5	54.36

The mean increase in 24 hours over normal was 89% for a person on a diet rich in organic phosphate. It would be desirable to repeat this experiment with a large group.

SUMMARY AND CONCLUSIONS

1. Two methods of precipitation of the organically bound phosphate portion in normal human urine are given. Barium hydroxide precipitated 76% and 84% of the organic phosphate, of which 70% in the latter case was recovered in the free form. Using basic lead acetate, 44% and 70% of the organic phosphate were recovered. The lead salt method was preferable, giving less color interference during colorimetric analysis.

2. Purified animal charcoal, phosphate-free and in neutral solution, may be used to decolorise the urine.

3. Kidney phosphatase hydrolysed about 58% of the organic phosphate present in fresh urine in 30 minutes at 37° C.

4. From 15% to 20% of the organic phosphate present in fresh urine can be extracted with ether in the form of a jelly-like mass.

5. The organic phosphate excretion in the urine increased from 54% to over twice the normal value following the ingestion of a diet rich in organically bound phosphorous.

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Abstract of Thesis for MA

THE URINARY PHOSPHATE IN THE PSYCHOSES

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THE URINARY PHOSPHATE IN THE PSYCHOSES

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I N T R O D U C T I O N

The high content of phosphorus containing compounds in brain and nervous tissue and its universal distribution through all living cells has led to much research dealing with the role of phosphorus in animal metabolism. Embden, Myerhof and others have shown that a definite correlation exists between the conversion of carbohydrates into lactic acid and the presence of phosphates in the contraction of muscle. Phosphorus compounds also play an important part in protein metabolism. Further it has been shown by Robison that the presence of certain organic phosphates will improve the ossification of bone in vitro. Phosphatides, which are phosphorus containing fats, have been found to play an important role in blood clotting, in the metabolism of fat and in the permeability of cell membranes.

There are many sources of phosphorus available to the animal body. Among these are the inorganic phosphates, found in fruit or milk, which exist as phosphates, phosphites,

and pyrophosphates; the phosphatides, which are found in both animal and plant tissues, for example, lecithin, found in egg yolk and nerve tissue, and kephalin, found in the brain; the nucleoproteins, nucleic acid and the nucleotides, occurring in proteins. In addition there are the phosphoproteins contained in the caseinogen of milk and the vitellin of egg yolk; and the simple esters of phosphoric acid, such as the hexose phosphates, the glycero-phosphates, and the phosphoglycerates. Since phosphorus is found abundantly in nerve tissue throughout the body it is probable that in mental disease a relationship might exist between the psychotic state of an individual and his phosphorus metabolism. An indication of any disturbance in the latter would be noted in the urinary phosphate excretion.

HISTORICAL REVIEW

Mairet (1884) in studying the phosphorus metabolism of manic-depressives found an increased total urinary phosphate excretion over normal in both the agitated and depressed states. Lailler (1884) found that in acute delirium or mania there was a marked excess of phosphorus in the urine, in hypomania a slight excess only, while in mild depression the urine was normal.

Modica and Audenino (1901) found a reduction in the inorganic phosphate of the urine in ten cases of mental disease. Removal of the frontal lobes of two healthy guinea pigs and two healthy dogs caused the alkaline earth phosphates of the urine to decrease and finally disappear, while there was also a reduction in the total phosphorus. Folin and Shaffer (1902) made a study of the metabolic changes accompanying the cycle of a manic-depressive patient. They found an increased amount of phosphorus and in explanation suggested that during the agitated state the system is unable to utilize a part of the phosphate absorbed from the digestive tract resulting in an increased urinary excretion. In the depressed state, however, the system repairs the loss sustained on the preceding excited days and there is a corresponding diminution in the phosphate excretion. The nervous disturbance was believed to be the result of this abnormal periodicity in the assimilation of phosphates. After making a careful study of the urines of insane patients and numerous controls, Folin, Shaffer, and Hill (1904) concluded that there was insufficient experimental data recorded in literature to warrant any connection between the urinary phosphate excretion and mental disorder.

In studying the excretion of phosphoric acid in urine in the psychoses, Tsuchiya (1924) found in

neurasthenics an average excretion 2.4 times the normal. In eighteen manic-depressive cases there was an average increase of twice the normal, both in excited and depressed states. The examination of five epileptic specimens before and shortly after an attack gave low values preceding and high values shortly after the fit, with an average about twice the normal. In dementia praecox the values were close to the normal, while in excited catatonics the average was about twice normal.

More recent work by Weil and Liebert (1937) on the phosphorus content of the blood serum during an epileptic seizure in man has shown that during the seizure the inorganic phosphate content of the serum was increased by 34%. Analyses of the blood serum were made during the aura preceding the convulsion, during the attack itself, and after the seizure. Experimental injection of rabbits with an emulsion of thujone produced convulsive seizures and an accompanying increase in the inorganic and organic phosphates of 98 and 37% respectively. Upon administering curare into the muscles, further injections of thujone did not affect the phosphate content. Their final conclusion was that in man the tonic-clonic contractions of the muscles during an epileptic fit are the source of the increased serum phosphate.

In view of the indefinite results reported by various workers in literature regarding the urinary excretion of phosphate in the psychoses it was thought advisable to investigate the matter more fully using improved methods of analysis and noting at the same time any abnormal physical or pathological condition of the patient in addition to his true mental type. It was further noticed that many of the previous workers were quite ambiguous in their use of nomenclature for the various mental types examined, and in view of recent advances in experimental psychiatry much more care has to be given to this phase of the problem.

With regard to the problem of arteriosclerosis, it has been observed that the level of the calcium content of the blood possibly is a determining factor in the amount of calcification that results from a given arterial damage. Etienne and Robert (1911) and Anderson (1925) contend that there is no evidence of any hypercalcaemia in old persons to account for the arterial calcification of senility. Labbé (1930), however, reports an increase in the blood calcium in some cases of arteriosclerosis. Bollag (1911) investigated seventeen cases of arteriosclerosis and found urinary calcium retention in two and marked loss in fourteen. He found that whereas normal excretion for adults varied from 0.15 to 0.5 gm. daily, in arteriosclerosis a greater variability was noticed, the values ranging from 0.0032 to 1.57 gm. daily

excretion. Page and Menschick (1932) state that the amount of phosphorus in the aorta increases parallel to the calcification (that is, phosphorus in organic combination), whereas other workers have reached the opposite conclusion. (Baldauf, 1906.) In view of these diverse findings, several of the urines from seniles (arteriosclerotics) were examined for calcium as well as phosphorus and results are given in the next section.

EXPERIMENTAL WORK

The inorganic phosphate present in the urine was estimated using King's (1932) method. Rae's (1936) modification of Youngburg and Pucher's (1924) method for the determination of organic phosphate was followed with satisfactory results.

*All samples of urine were collected in the morning between 5 and 7 A.M. and represent the night urine. 50 cc. aliquot portions of each were secured. The analyses were carried out the same day and were done in duplicate.

a. Inorganic phosphate.

To 0.2 cc. of the urine in a 25 cc. volumetric flask distilled water was added up to 10 cc.,

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*All patients were on the same average diet. Collection of urine samples in the early morning would tend to eliminate the factor of exercise.

2 cc. of 60% perchloric acid, 1 cc. of 8.33% ammonium molybdate and 1 cc. of 0.33% amino-naphthol-sulfonic reagent were added, and water up to 25 cc. A standard containing an appropriate amount of phosphate was prepared the same way at the same time. The contents of the flask were shaken, and after five minutes the colors were compared, using a Duboscq colorimeter.

b. Organic phosphate.

To 10 cc. of the urine was added a calculated volume of magnesia mixture without the ammonia (0.16 cc. magnesia mixture for each mg. of inorganic phosphate) and an equal volume of normal ammonium hydroxide. The mixture was well shaken and allowed to stand for ten minutes (pH 8.8-9.0). After filtering, a 3 cc. portion of the filtrate was used for the organic phosphate analysis and a test was made on 3 cc. of the remainder to note whether any inorganic phosphate had not been precipitated. 2 cc. of 60% perchloric acid were then added to the organic phosphate sample, and the mixture digested on an electric heater. A drop or two of nitric acid was added to hasten the oxidation of organic matter. The contents of

the tube were then cooled, and transferred by washing into a 25 cc. volumetric flask. 0.2 cc. of perchloric acid was added to make up for loss of acid by evaporation. The procedure from then on was the same as the inorganic phosphate analysis. The results obtained are given in Table I (page 9).

c. Urinary calcium.

A number of calcium determinations was made on the urines of arteriosclerotics to note whether any retention of calcium occurred. In view of the predominantly low phosphate excretion in the urines of seniles it seems probable that the calcium might be retained to enter into combination with the phosphate in forming calciforic deposits throughout the circulatory system as a result of a deranged metabolism.

TABLE I

The Inorganic and Organic Phosphate Content of the Urine in the Psychoses.

File #	Mental Classification Psychosis	Sub-type	Patient Age	Sex	Pathology, Physical Condition	Content, mg./100 cc. Inorganic	Organic
1	Schizophrenia,	paranoid	33	M	Good physical condition.	116.9	1.51
2	Schizophrenia,	paranoid	26	M	Healthy.	65.2	.86
3	Schizophrenia,	paranoid	59	M	Moderate sclerosis.	60.9	1.37
4	Schizophrenia,	paranoid	56	M	CaOx crystals in urine.	44.38	.89
5	Schizophrenia,	paranoid	59	M	Good physical condition.	57.33	.90
6	Schizophrenia,	catatonic	36	M	Enlarged septic tonsils.	104.7	2.23
7	Schizophrenia,	catatonic	21	M	No organic dysfunction; in state of acute excitement.	243.0	2.78
8	Schizophrenia,	paranoid	32	M	History of haematuria; albumin very heavy.	143.9	1.28
9	Schizophrenia,	paranoid	52	M	Well-built.	99.34	1.17
10	Schizophrenia,	catatonic	24	M	Intent physically.	61.62	.96
11	Schizophrenia,	simple	20	M	No defect.	76.05	.84
12	Schizophrenia,	paranoid	23	M	Good health.	26.37	1.22
13	Schizophrenia,	simple	32	M	No abnormality.	88.24	1.88
14	Schizophrenia,	simple	21	M	Attack of 'flu.	88.3	1.26
25	Schizophrenia,	paranoid	51	M	Good condition.	96.50	1.0
27	Schizophrenia,	paranoid	38	M	Some arteriosclerosis. Good health.	92.02	.95

TABLE I (Continued)

File #	Mental Classification Psychosis Sub-type	Patient Age	Sex	Pathology, Physical Condition	Content, mg./100 cc. Inorganic	Organic
29	Schizophrenia, catatonic	60	M		70.0	2.35
31	Schizophrenia, hebephrenic	36	M		115.4	1.44
15	Manic-depressive	35	M	Hernia.	65.37	.85
16	Manic-depressive	58	M	Moderate thickening of arteries. Atrophy of both testicles.	25.62	1.46
17	Manic-depressive	50	M	Asthenic type.	50.33	.61
18	Manic-depressive	58	M	No peripheral arteriosclerosis	(0 (20.83	0 1.12
19	Manic-depressive	57	M	Good physical condition.	110.5	.86
20	Manic-depressive	62	M	Cerebral and peripheral arteriosclerosis	20.12	.88
21	Manic-depressive	59	M	Marked sclerosis; hemiplegia; CaOx crystals in urine.	69.16	.88
22	Manic-depressive	65	M	Peripheral arteriosclerosis.	99.33	1.08
23	Manic-depressive	49	M	Malformation of chest.	53.05	1.70
24	Manic-depressive	45	M	Good condition.	115.0	.58
52	Manic-depressive	45	M	Varicosities of both legs; arteriosclerosis.	47.02	1.08
General Paralysis of the insane						
43	G. P. I.	28	M	No organic dysfunction	83.33	1.11
44	G. P. I.	51	M	No organic dysfunction.	76.75	.66
45	G. P. I.	49	M	Slight arteriosclerosis.	106.3	.78

Underlining ##15-24 and 52 indicates state of patient at time.

TABLE 1 (Continued)

File #	Mental Classification Psychosis Sub-type	Patient Age	Sex	Pathology, Physical Condition	Content, mg./100 cc. Inorganic	Organic
46	G. P. I.	25	M	Good physical condition.	46.88	.74
47	G. P. I.	43	M	Good physical condition.	98.05	1.60
Seniles						
26	Senile, paranoid	54	M	Atrophy of both legs; arteriosclerosis; CaOx crystals and pus cells in urine.	132.40	3.01
28	Senile, paranoid	60	M	Arteries not definitely sclerosed; good physical condition.	49.7	1.46
30	Senile	70	M		33.7	1.65
32	Organic brain disease	49	M	Moderate arteriosclerosis.	55.16	1.12
33	Psychosis with cerebral arteriosclerosis	80	M	Extreme restlessness.	91.46	.70
34	Psychosis with cerebral arteriosclerosis	68	M	Sclerosis of radials and brachials. Restlessness.	84.29	.64
35	Senile psychosis	75	M	Arteries tortuous.	42.86	.69
36	Psychosis with cerebral arteriosclerosis	76	M	Obese; definite peripheral arteriosclerosis; CaOx crystals in urine.	18.87	.78
37	Psychosis with cerebral arteriosclerosis	79	M	Arteriosclerosis.	48.33	.62
38	Senile, paranoid	80	M		35.0	1.25
39	Psychosis with cerebral arteriosclerosis	90	M	Enlarged heart; good health.	53.03	1.32
40	Psychosis with cerebral arteriosclerosis	62	M	Peripheral arteriosclerosis.	41.0	1.61

TABLE I (Continued)

File #	Mental Classification Psychosis Sub-type	Patient Age	Sex	Pathology, Physical Condition	Content, mg./100 cc. Inorganic	Organic
41	Psychosis with cerebral arteriosclerosis	70	M	Prostatic enlargement.	41.09	1.34
Other Psychoses						
42	No psychosis; morphine addict	39	M		134.2	1.03
48	Organic brain disease	56	M	Cerebral arteriosclerosis (tumour?)	76.33	1.08
49	Undiagnosed (organic brain lesion?)	38	M	Moderate thickening of arteries.	63.60	1.05
50	Mental deficiency without psychosis	15	M		89.28	1.14
51	Homicidal, suicidal, depressed	39	M		59.06	.85
53	Mental deficiency with psychosis	40	M	Peripheral arteriosclerosis.	149.5	.63
54	Psychoneurosis	28	M	Good health.	87.06	1.30
55	Psychopathic inferiority	35	M	Hyperactive; complete deafness.	85.71	1.18
56	Psychosis with brain disease	61	M		89.28	1.43
57	Psychosis with Huntington's chorea	44	M		78.53	2.12
58	Mental deficiency with psychosis	20	M		125.0	1.30

The method used in the determination of calcium was that of Schwartz (1936), which is a slight modification of the ordinary calcium oxalate precipitation procedure.

The urine was first diluted according to its calcium content, so that a 5 cc. aliquot of the resulting mixture contained calcium ion within the limits of the reagent for calcium. Thus in the case of a urine high in calcium, 1 volume of urine was added to 4 volumes of water. For a urine low in calcium, 1 volume of water was added to 4 volumes of urine.

Table II gives the results secured as well as the corresponding phosphate values.

TABLE II.

Patient file number	Calcium mg./100 cc.	Phosphorus, mg./100 cc. Inorganic	Organic
20	23.5	20.12	.88
18	21.2	20.83	1.12
35	.40	42.86	.69
36	.71	18.87	.78
41	.55	41.09	1.34
26	2.52	132.4	3.01
38	.85	35.0	1.25

COMMENTS AND CONCLUSIONS

1. The normal phosphate excretion as determined by Rae (1936) was inorganic phosphate 102.3 mg./100 cc. and organic phosphate 1.20 mg./100 cc. In the eighteen schizophrenics examined the average excretion was 91.84 mg./100 cc. inorganic phosphate, and 1.38 mg./100 cc. organic phosphate. In patient #7 in a state of acute catatonic excitement the value was more than twice the normal, whereas in schizophrenics of the paranoid type the values were below normal. It seems that excessive muscular activity favors an increased urinary phosphate excretion, whereas a lowered basal metabolic rate results in a decreased urinary phosphate excretion.

2. In the eleven manic-depressives the average excretion was 56.19 mg./100 cc. of inorganic phosphate and 0.92 mg./100 cc. of organic phosphate, an inorganic value about one-half of the normal. In this respect the results differ from those obtained by former workers who claim an increased urinary phosphate excretion in both the manic and depressed states of the psychosis. It was further noted that the manic or agitated state of this psychosis favors an increased metabolic activity and hence the phosphate excretion in these cases is normal or increased above normal, as noticed in patients ##19, 22 and 24. In the depressed phase the values are considerably below normal, in one case (#18) an analysis for phosphorus gave a negative result.

3. In five cases of general paralysis caused by syphilis the average was 82.26 mg./100 cc. of inorganic phosphate and 0.98 mg./100 cc. of organic phosphate. These values are slightly below the normal.

4. The greatest deviation from the normal was apparent in the senile group (thirteen). The average values obtained were 55.91 mg./100 cc. of inorganic phosphate and 1.24 mg./100 cc. of organic phosphate. Individual values vary from 18 to 50% below normal. Either there is a marked retention of phosphates held in combination with calcium in the walls of the arteries, or else a decreased food intake results in the decreased phosphate excretion.

5. In the seven seniles examined for calcium excretion in the urine there is retention in five of them, ##35, 36, 41, 26, 38; whereas in two of the cases the excretion is normal. There is a correspondingly low inorganic phosphate output in all cases except #26, an individual with a marked pathological disturbance.

6. The average for the remaining psychoses of the mild type (eleven) is 94.32 mg./100 cc. for inorganic phosphate and 1.20 mg./100 cc. of organic phosphate, which is within the normal limits.

7. From the data secured it appears that increased muscular activity in the manic, agitated or catatonic state of the

psychoses results in an increased urinary phosphate excretion. It would be interesting to investigate more fully the reverse of the condition, i.e. to note whether a disturbance in the phosphorus metabolism (produced by injection of phosphorus compounds into the muscle) would speed up or slow down muscular activity with accompanying mental symptoms.

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