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HYPOPHOSPHITES #114

MONOGRAPH

ON THE

HYPOPHOSPHITES

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HYPOPHOSPHITES

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HYPOPHOSPHITES

Summary

Popoviciu and Dariu (39) reported that the hypophosphites were first used in medicine in 1858 by Francis Churchill for treating tuberculosis. These compounds were supposed to stimulate the appetite and improve the blood.

Polk (36) also reported Churchill's use of hypophosphite syrups in treating tuberculosis. The dose consisted of 3 teaspoons per day to young children, along with cod liver oil and an aromatic elixir of calisaya. The syrups contained various mixtures of 256 g of a hypophosphite salt (calcium, manganese, potassium, or sodium), sugar, iron oxide, water, and hypophosphorous acid. Hypophosphorous acid was said to be normally found in the brain and other nervous tissue combined with glycerin and fat. The hypophosphites were thought to cause improvement in patients with tuberculosis, by replacing depleted supplies of hypophosphorous acid.

Panzer (32) found hypophosphorous acid in the stomach and intestine 6 hours after the oral administration of calcium hypophosphite to one dog. Hypophosphorous acid was found in trace amounts in the blood and kidneys of a second dog after 3 hours. Panzer thought that calcium hypophosphite was absorbed completely and excreted rapidly and that more of the compound was present than his tests for organ content could detect at that time (1902).

Paquelin and Joly (33) studied the urinary excretion of sodium hypophosphite. Doses of 0.5 g were given orally, twice a day for 5 days to a woman patient. Hypophosphite was excreted almost unchanged in the urine. The main action of hypophosphite was diuretic - urine output, density, urea, and phosphoric acid levels increased.

Panzer (32) gave 1 g calcium hypophosphite orally to 2 dogs. In the first dog, hypophosphite was detected up to 22 hours in the urine. In the second dog, hypophosphite was first detected in the urine 15 minutes after the administration of the compound. Panzer took the same dose himself and first detected hypophosphite in the urine 30 minutes later. The hypophosphites were no longer present after 2 days.

Strada (52) found that 81.29 to 83.85% of the 500 to 1000 mg/kg sodiumhypophosphite administered i.v. or s.c. to 2 rabbits was excreted within 3 days.

Another urinary excretion study was performed using one pregnant and one non-pregnant cow by Vasenius and Kallela (58). They injected 0.3 moles i.v. (half calcium hypophosphite and half sodium hypophosphite) into the cows and repeated the study 3 and 4 times respectively. The hypophosphites increased urine output and the excretion of reducing

substances. Two-thirds of the injected dose was excreted, half in 2 hours. Hypophosphite was not an adequate source of phosphorous for cows.

Engel (11) found that mice were sensitive to hypophosphite. The injection of 2 ml of a 1:10 solution produced death, after the animals experienced breathing difficulty and paralysis.

Hypophosphites in large doses were not fatal to guinea pigs, which may be resistant to the compound, according to Engel (11). It was also found that hypophosphites are less toxic than phosphites and other phosphorous containing solutions.

Nofre, Dufour, and Cier (30) reported that the acute toxicity of sodium hypophosphite in male, Swiss, albino mice, expressed as the LD_{50}/30 (the LD_{50} on the 30th day), was 1584 mg/kg (in 0.4 ml) given i.p. in aqueous solution.

Takahashi (53) gave male white rats, weighing 60 g, phosphoric acid-free basal diets containing 0.055% sodium hypophosphite for 4 months. After the first 10 days, 1% free hypophosphorous acid was also added to the basal feed. The rats showed almost no growth; 0.5% hypophosphite still did not produce weight gain. A level of 1.5 to 2% caused the death of the animals in a few days.

Meyer and Greenberg (27) fed young Sprague-Dawley rats a low-calcium diet for 14 days and then calcium hypophosphite supplements for 11 days. Control rats, fed the low-calcium diet for 25 days, all developed baldness. None of the rats given calcium hypophosphite became bald. The authors suggested that calcium hypophosphite may be a suitable supplement for calcium in the human diet, since it has an acceptable taste and does not affect the acid-base balance.

Popoviciu and Dariu (39) used hypophosphite syrup in treating 6 children with rickets and one with pleuro-peritonitis. The syrup was composed of potassium, sodium, calcium, iron salts (pyrophosphates and citrates), quinine, and phosphorous acid. The oral administration of an unspecified amount of the syrup for 4 to 6 1/2 weeks resulted in an improvement in the children's condition.

Sterner and Medes (51) found that 0.64 M sodium hypophosphite did not inactivate prothrombin when tested in vitro on rabbit plasma. The clotting times of control and hypophosphite-treated plasma were both one minute.

CALCIUM HYPOPHOSPHITE

Chemical Information

I. Nomenclature

A. Common Name

Calcium Hypophosphite

B. Chemical Name

Calcium Hypophosphite

C. Trade Name

None

D. Chemical Abstracts Registry No.

007-789-799

II. Empirical Formula

$Ca(H_2PO_2)_2$

Ca 23.57%; H 2.37%; O 37.63%; P 36.43%

III. Structural Formula

$Ca(H_2PO_2)_2$

IV. Molecular Weight

170.07

V. Specifications

None Available

VI. Description

A. General Characteristics

Calcium hypophosphite occurs as monoclinic, prismatic crystals or granular powder.

B. Physical Properties

Calcium hypophosphite is soluble in water, slightly soluble in glycerol, practically insoluble in alcohol. The aqueous solution is slightly acid. At temperatures above 300 degrees C, calcium hypophosphite evolves spontaneously-flammable phosphine. There is no melting point or boiling point, since it decomposes.

C. Stability

Keep in tightly closed containers.

VII. Analytical Methods

Two official analytical methods for hypophosphites in syrups, in the absence of phosphates, are available. In the first method, a sample is diluted with water, boiled twice with HNO_3, cooled, precipitated with NH_4OH in excess, and mixed with a few drops of HNO_3. Molybdate solution is then added to the treated sample until precipitation is complete. The solution is then filtered and washed with NH_4NO_3. The precipitate on the filter is dissolved in NH_4OH and hot water, neutralized with HCl, and cooled. A magnesia mixture is slowly added and then NH_4OH is added and allowed to stand overnight. The solution is again filtered and the precipitate washed with NH_4OH to remove Cl, then dried and combusted in an electric furnace. The cooled material is weighed as $Mg_2P_2O_7$ ($Mg_2P_2O_7$ X 0.6377 = P_2O_5). (A.O.A.C.)

The second method applies only when other reducing agents or phenolic compounds are not present. The sample to be tested is diluted with water and mixed with a KBr - $KBrO_3$ solution and H_2SO_4. After standing for 2 hours, KI is added, shaken, and the liberated iodine is titrated with 0.1N $Na_2S_2O_3$ to straw color. Then starch solution is added until the solution is colorless (1 ml 0.1 N $Na_2S_2O_3$ = 0.00165 g H_3PO_2; 1 ml 0.1N $Na_2S_2O_3$ = 0.00208 g $NH_4H_2PO_2$). (A.O.A.C.).

VIII. Occurrence

None Available

MAGNESIUM HYPOPHOSPHITE

Chemical Information

I. Nomenclature

A. Common Name

Magnesium Hypophosphite

B. Chemical Name

Magnesium Hypophosphite

C. Trade Names

None

D. Chemical Abstracts Registry No.

II. Empirical Formula

$Mg(H_2PO_2)_2 \cdot 6H_2O$

III. Structural Formula

$Mg(CH_2PO_2)_2 \cdot 6H_2O$

IV. Molecular Weight

262.41

V. Specifications

None Available

VI. Description

A. General Characteristics

Magnesium hypophosphite occurs as white, tetragonal, efflorescent crystals.

B. Physical Properties

Its solubility at 25 degrees C in cold water is 20 parts per 100 ml;

95% ethyl alcohol - slightly soluble. Magnesium hypophosphite decomposes at 100 degrees C and has a specific gravity of 1.59 at 13 degrees C.

C. Stability

Keep in tightly closed containers.

VII. Analytical Methods

See calcium hypophosphite

VIII. Occurrence

None Available

MANGANESE HYPOPHOSPHITE

Chemical Information

I. Nomenclature

A. Common Name

Manganese Hypophosphite

B. Chemical Name

Manganese Hypophosphite

C. Trade Names

None

D. Chemical Abstracts Registry No.

010-043-842

II. Empirical Formula

$Mn(H_2PO_2)\cdot 2H_2O$

91.12% anhydrous salt; 8.88% H20; 27.07% Mn; 2.98% H; 39.42% O; 30.53% P

III. Structural Formula

$Mn(H_2PO_2)\cdot 2H_2O$

IV. Molecular Weight

202.94; anhydrous weight 184.91

V. Specifications

Food Chemicals Codex

Assay	Not less than 97.0% of $Mn(PH_2O_2)_2$ after drying
Loss on drying	Not more than 9%
Limits of impurities	
Arsenic (as As)	Not more than 3 ppm
Heavy metals (as Pb)	Not more than 40 ppm
Lead	Not more than 10 ppm

VI. Description

A. General Characteristics

Manganese hypophosphite occurs as pink, odorless, almost tasteless crystals or powder, which is stable in air.

B. Physical Properties

Its solubilities are as follows:

water	1 g dissolves in 6.5 ml at 25 degress C
boiling water	1 g dissolves in 6 ml at 25 degrees C
alcohol	insoluble

When heated, manganese hypophosphite evolves flammable phosphine. The melting point of the anhydrous form is 150 degrees C.

C. Stability

Keep in well-closed containers. It may explode when triturated or heated when mixed with nitrates, chlorates, or other oxidizing agents.

VII. Analytical Methods

The sample to be tested is dried, diluted with water, and mixed with bromine, dilute sulfuric acid, and a saturated potassium iodide solution. The liberated iodine is titrated with 0.1 N sodium thiosulfate, using starch as the indicator. Each ml of 0.1 N bromine = 2.311 mg of $Mn(H_2PO_2)_2$ (Food Chemicals Codex).

VIII. Occurrence

None Available

POTASSIUM HYPOPHOSPHITE

Chemical Information

I. Nomenclature

A. Common Name

Potassium Hypophosphite

B. Chemical Name

Potassium Hypophosphite

C. Trade Names

None

D. Chemical Abstracts Registry Number

007-782-878

II. Empirical Formula

KH_2PO_2
37.56% K; 1.94% H; 30.74% O; 29.79% P; 63.41% H_3PO_2

III. Structural Formula

KH_2PO_2

IV. Molecular Weight

104.09

V. Specifications

None Available

VI. Description

A. General Characteristics

Potassium hypophosphite exists as white hexagonal crystals or as granular, deliquescent powder. It is odorless, pungent, and has a salty taste.

B. Physical Properties

Its solubilities are as follows:

water	1 g dissolves in 0.6 ml
alcohol	1 g dissolves in 9 ml
boiling alcohol	1 g dissolves in 5 ml

Its aqueous solution is neutral or slightly alkaline and it ignites evolving phosphine when heated in air.

C. Stability

Keep well closed. Potassium hypophosphite explodes when triturated with chlorates or other oxidizing agents.

VII. Analytical Methods

See Calcium Hypophosphite

VIII. Occurrence

None Available

SODIUM HYPOPHOSPHITE

Chemical Information

I. Nomenclature

A. Common Name

Sodium Hypophosphite

B. Chemical Name

Sodium Hypophosphite

C. Trade Names

None

D. Chemical Abstracts Registry Number

007-681-530

II. Empirical Formula

$NaH_2PO_2 \cdot H_2O$
Anhydrous salt 83.00%; H_2O 16.99%; H_3PO_2 62.26%; P 29.22%:
Na 21.69%; H 3.80%; O 45.28%

III. Structural Formula

$NaH_2PO_2 \cdot H_2O$

IV. Molecular Weight

106.01

V. Specifications

Not Available

VI. Description

A. General Characteristics

Sodium hypophosphite exists as white, deliquescent granules, which are odorless and have a salty taste.

B. Physical Properties

The solubilities of sodium hypophosphite at 25 degrees C are as follows:

cold water	100 parts/100 ml water
hot water	830 parts/100 ml water
glycerol	freely soluble
boiling alcohol	freely soluble
cold alcohol	freely soluble
absolute alcohol	slightly soluble
propylene glycol	9.7 g/100 g
NH_3	slightly soluble
ether	insoluble

When triturated with chlorates or other oxidizing agents sodium hypophosphite explodes. The aqueous solution is neutral. The melting point of sodium hypophosphite minus water is 200 degrees C.

C. Stability

Keep well closed.

VII. Analytical Methods

See Calcium Hypophosphite

VIII. Occurrence

None Available

Biological Data

I. Acute Toxicity

Substance	Animal	Sex & No.	Route	Dosage (mg/kg) Body Weight	Measurement	Ref.
sodium hypophosphite	Mouse	Male	i.p.	1584	*$LD_{50}/30$	30

* the LD_{50} on the 30th day, determined by a graphic statistical method.

Mice

Engel found that mice seem to be sensitive to hypophosphite, since small doses of a 1:10 solution (about 0.2 g or 2 ml, injection route not specified) were lethal. Intoxication symptoms included breathing difficulties, crippling, and death within an hour (11).

The acute toxicity of sodium hypophosphite in male, Swiss, albino mice were reported by Nofre, Dufour, and Cier. The toxicity was determined by the graphic statistical method and expressed as the $LD_{50/30}$ (the LD_{50} on the 30th day). The $LD_{50}/30$ of sodium hypophosphite (NaH_2PO_2) was 1 584.00 mg/kg i.p. The sodium hypophosphite was given in aqueous solution and the injection volume was 0.4 ml (30).

Guinea Pigs

Engel found that hypophosphite is usually less toxic than phosphite and other P solutions. The guinea pig was found to be very resistant to large doses (11).

II. Short-Term Studies

Rats

Tahahashi conducted a 4 month study in which male white rats, 50 g, were fed a basal diet containing 17% horse meat protein, 60% starch, 20% butter, 3% phosphoric acid-free inorganic salts, trace amounts of oryzanin, and 0.055% of the sodium salt of hypophosphorous acid. After 10 days 1% of free hypophosphorous acid was added to the basal feed. Both the control group fed the basal diet alone and the groups fed the basal diet plus 0.055% sodium hypophosphite exhibited almost no growth. When the hypophosphite level was increased to 0.5%, no growth occurred. A level of 1.5-2% sodium hypophosphite caused the rats to die in a few days (53).

Meyer and Greenberg fed 7 groups of 7 or 8 young Sprague-Dawley rats (64-101 g) dietary supplements of calcium hypophosphite for 11 days after being on a low-calcium diet for 14 days. Control groups were kept on the basic low-calcium diet for 25 days. The basic diet consisted of 70 parts yellow corn meal, 16 parts wheat gluten, 10 parts brewer's yeast, and 1 part NaCl with cod-liver oil as a vitamin D source (0.1 unit/g diet). Control rats, which had no calcium supplements, developed baldness. Calcium hypophosphite was found to be a suitable supplement for calcium in the diet, since none of the rats became bald when fed this compound. The authors suggested that calcium hypophosphite might be used in man for calcium therapy, since it has an acceptable taste and does not change the acid-base balance to alkaline as do carbonates. The exact amounts of calcium hypophosphite were not specified - only the amounts of calcium or phosphorus (27).

III. Long-Term Studies

No Information Available

IV. Special Studies

Therapy

Polk reported that Churchill used hypophosphites in treating phthisis (tuberculosis) in the form of the calcium, manganese, potassium, and sodium salts (256 g) and given as a syrup. The syrups contained other compounds such as iron oxide, sugar, water, and hypophosphorous acid. The dose given to children of 2 or 3 years of age was usually 3 teaspoons daily combined with an aromatic elixir of calisaya and cod liver oil. The reason for the success in treating tuberculosis with hypophosphites was explained by the fact that they aid in the replacement of hypophosphorous acid, which exists normally in the brain and other nervous tissues combined with glycerin and fat (36).

Popoviciu and Dariu discussed the use of hypophosphite syrup in the treatment of rickets in 6 children and pleuro-peritonitis in another child. The syrup is composed of potassium, sodium, calcium, iron salts (pyrophosphates and citrates), quinine, and phosphorous acid. The administration of an unspecified amount of the hypophosphite syrup (oral) for 4 to 6 1/2 weeks resulted in an improvement in the condition of the children. The number of white and red blood cells and the hemoglobin level increased. The calcium level and the phosphorus level both increased significantly in one child. The phosphorus level decreased in 4 of the 7 cases. It was not possible to draw definite conclusions about the effect of hypophosphites on calcium and phosphorus levels from the results (39).

Biochemical Aspects

I. Breakdown

No Information Available

II. Absorption - Distribution

Panzer studied the distribution of hypophosphite in 2 dogs after the oral administration of calcium hypophosphite. In one dog, the calcium hypophosphite was given 3 times, several days apart; the dog was sacrificed 6 hours after the last dose. The blood, GI tract, liver, kidneys, and brain were assayed for hypophosphorous acid. It was found in only the stomach and the intestine. The second dog was sacrificed 3 hours after the dose. No definite indication of hypophosphorous acid was found in the organs listed above, although a slight trace was found in the blood and kidneys. Panzer concluded that calcium hypophosphite was absorbed completely and excreted rapidly and that the tests for organ content that were used may not have been sensitive enough to detect the presence of hypophosphorous acid (32).

III. Metabolism and Excretion

Paquelin and Joly monitored the diet of a female subject for 15 days. Five grams of sodium hypophosphite were administered to her by mouth in divided doses of 0.5 g hypophosphite with each of 2 daily meals over 5 days (day 6 to 10). Urine output in 24 hours was increased from 1.135 to 1.205 (liters ?) and the density increased from 1.024 to 1.029. Urea excretion increased by 598 mg and phosphoric acid increased by 335 mg. The investigators reported finding hypophosphites to be excreted almost unchanged. The main action of the hypophosphites was diuretic (33).

Dog

Panzer studied the urinary excretion of calcium hypophosphite in two male dogs, weighing 10.1 and 7.4 kg. One gram of the hypophosphite was given orally and the presence of the substance in the urine was tested up to 22 hours in the first dog and for 3 hours in the second. In the first dog, no more hypophosphites were excreted after 22 hours. The study was repeated twice more in this animal, during which it was found that hypophosphite appeared in the urine 15 minutes after its administration. The appearance of hypophosphite after 15 minutes was also observed in the second dog (32).

Human

Panzer himself ingested one gram of calcium hypophosphite and found that the compound did not appear in the urine until 30 minutes later. The hypophosphites were no longer present in the urine after two days (32).

Strada administered sodium hypophosphite to 2 rabbits and measured the amount excreted in the urine. The following doses and routes were administered, after which urine was collected for 4 days:

Rabbit #		
I	1.	500 mg/kg i.v. in 10 ml of 10% sol. into a 2.0 kg rabbit
	2.	1000 mg/kg i.v. in 20 ml of 10% sol. into a 2.0 kg rabbit
II	3.	500 mg/kg s.c. in 75 ml of 10% sol. into a 1.5 kg rabbit
	4.	1000 mg/kg s.c. in 16 ml of 10% sol. into a 1.6 kg rabbit

Within 24 hours, 78.50 to 81.43% of the original sodium hypophosphite dose was excreted. Within 3 days, 81.29 to 83.85% had been eliminated in the urine. The difference in the route of administration of the sodium hypophosphite made no significant difference in its excretion (52).

Vasenius and Kallela injected 0.3 moles hypophosphite i.v. into each of 2 healthy cows - one pregnant (400 kg), the other non-pregnant (320 kg) - and used iodometric titration to determine the amount excreted in the urine. The study was repeated 3 times for the pregnant cow and 4 times for the non-pregnant one and the results pooled. Half of the hypophosphite dose was given as the sodium salt and the other half as the calcium salt. Half of the hypophosphite excreted was released in 1 1/2 to 2 hours. The total quantity excreted was 2/3 the injected dosage. The injection of hypophosphite increased urine output and the excretion of reducing substances. Hypophosphite was not found to be an adequate source of phosphorus (58).

IV. Effects on Enzymes and Other Biochemical Parameters

Clotting Time

Sodium hypophosphite was tested in vitro on rabbit plasma, in a concentration of 0.64 M, by Sterner and Medes. Both the control sample and the hypophosphite sample had a clotting time of 1 minute. It was concluded that sodium hypophosphite does not inactivate prothrombin (51).

V. Drug Interaction

No Information Available

VI. Consumer Exposure Information

Hypophosphites are used in foods as nutrients and/or dietary supplements. No information is available on the amount found in foods.

HYPOPHOSPHITES

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XXIV.

Aus dem Pharmakologischen Institut der Universität Greifswald.

Untersuchungen über den Wirkungsmechanismus des Phosphors.

Von

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(Mit 8 Kurven.)

(Eingegangen am 24. I. 1924.)

Aus zahlreichen Arbeiten ist das anatomische und pathologisch-physiologische Bild der Phosphorvergiftung aufs Beste bekannt[1]). Auch die Grundlagen seiner therapeutischen Anwendung sind durch das Experiment wenigstens zum Teil geschaffen. Nach wie vor aber ungeklärt bleibt die Frage wie eigentlich der Phosphor wirke. Bekanntlich sind hier drei Theorien aufgestellt. Nach der einen soll P als solcher wirken. Die Tatsache, daß in sehr akut verlaufenden Fällen schwerer P-Vergiftung, freier P sowohl im Blute wie auch in den Organen nachweisbar war, zusammen mit der angeblichen Ungiftigkeit der Oxydationsprodukte des P, hat zu dieser Anschauung geführt, die aber zweifellos insofern unbefriedigend ist, als man sich die Wirkung des elementaren P als solchen schwer vorstellen kann. Man kann allenfalls daran denken, daß die Sauerstoffavidität des in freier Form resorbierten P eine Schädigung überall da bedeuten müsse, wo er sich anhäuft, also insbesondere in den lipoidreichen Geweben und vor allem in der Leber. In der Tat hat man auch

1) Die ältere Literatur bei Munk und Leyden, Die akute P-Vergiftung, 1865, ferner bei Dybkowski, Beiträge zur Theorie der P-Vergiftung. Hoppe-Seylers med. chem. Unters. Berlin, 1866, S. 49—70. Die neuere Literatur, besonders auch über die Stoffwechselvorgänge bei P-Vergiftung, bei R. Tischner, Virchows Arch. 1904, Bd. 175, S. 90. Bei E. Frank und S. Isaak, Arch. f. exp. Path. u. Pharm. 1911, Bd. 64. Hirz, Zeitschr. f. Biolg. 1913, Bd. 60, S. 187. H. Rettig, Arch. f. exp. Path. u. Pharm. 1914, Bd. 76, S. 355.

gerade vom pathologisch-anatomischen, aber auch vom pathologisch-physiologischen Standpunkt aus, dieser Reduktionswirkung des P eine wesentliche Rolle zugeschrieben.

Die unleugbare Tatsache, daß P in wässeriger Lösung bei Gegenwart von O, also vor allem im Blute, aber auch in den Geweben, mit bestimmter Geschwindigkeit zu phosphoriger und unterphosphoriger Säure oxydiert[1]) wird, gab Anlaß zu der zweiten Hypothese, wonach der P nur insoweit wirke, als er in diese Oxydationsprodukte umgewandelt wird. Gegen diese Theorie wandten sich H. Schulz[2]) und Neumann[3]) auf Grund ihrer Befunde, nach denen sowohl unterphosphorige als phosphorige Säure ungiftig seien.

Diesem Befunde gegenüber konnte auch die Tatsache, daß phosphorige Säure nach P-Vergiftung im Blute und in den Geweben festgestellt wurde[4]), nicht beweisend sein. Schließlich sei noch die dritte Hypothese erwähnt, nach welcher der P im Organismus zu dem überaus giftigen PH_3 reduziert und dadurch erst wirksam werde. Mit Santesson und Malmgren sind wir der Meinung, daß eine solche Bedeutung des PH_3 nur in Ausnahmefällen gelten kann, in denen nach Aufnahme größter P-Mengen eine hyperakute Wirkung eintritt.

Betrachten wir die angeführten Hypothesen, so erscheint eine Reduktionswirkung des P durchaus wahrscheinlich, doch kann auch eine Beteiligung der Oxydationsprodukte nicht als ausgeschlossen gelten. Es ist zu bedenken, daß eine Bildung solcher Substanzen in den Geweben selbst eine durchaus andere Wirkung entfalten könnte als wenn man sie per os verabreicht, eine Anschauung, die schon H. Schulz[5]) vertreten hat. Wir hielten es daher für geraten, die Wirkungen der niederen Oxydationsprodukte des P auf überlebende Organe zu studieren. Auch schien es nötig, die Frage nach der toxischen Wirkung dieser Substanzen an ganzen Tieren erneut nachzuprüfen. Da die Phosphorsäuren nach Starkenstein[6]) als

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1) Vaclav Plavec, Pflügers Arch. 1904, Bd. 49, S. 1 und H. Vohl, Berl. Klin. Wochenschr. 1865, Nr. 32, S. 329; Nr. 33, S. 336.

2) H. Schulz, Arch. f. exp. Path. u. Pharm. Bd. 18, S. 174 und Bd. 23, S. 150.

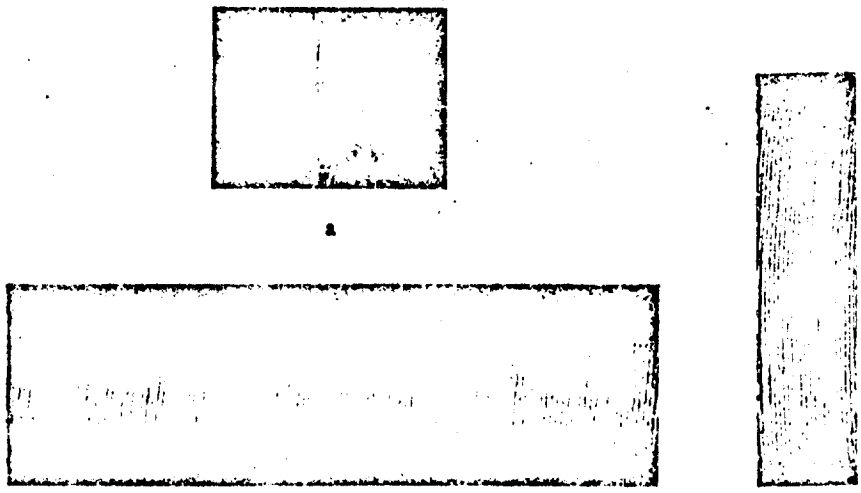
3) J. Neumann, Experimentelle Studien zur P-Vergiftung. Diss., Rostock 1886.

4) Selmi, Arch. d. Pharm. 1880, III., Bd. 17, S. 253; 1887, Bd. 19, S. 276. Hager, Handb. d. pharm. Prax. 1887, Bd. 2, S. 672. Hollefreund, zitiert bei A. Fischer, Pflügers Arch. 1903, Bd. 97, S. 578; diese fanden niedere Oxydationsstufen des P im Harn und den Organen.

5) H. Schulz, Arch. f. exp. Path. u. Parm. Bd. 23, S. 151.

6) Starkenstein, Ebenda 1914, Bd. 77, S. 45.

Ca-fällende Substanzen giftige Wirkungen ausüben und, in allerdings recht hohen Konzentrationen auch an überlebenden Organen wirksam sind; da ferner Staub[1]) vor kurzem die günstige Wirkung der Phosphate auf das überlebende Froschherz demonstriert hat, so könnte man bei jeder Untersuchung der Wirkung der niederen Oxydationsprodukte des P den Einwand erheben, daß das eigentlich wirksame dabei die durch Oxydation gebildete Phosphorsäure sei. Diese Anschauung haben schon Munk und Leyden[2]) auf Grund allerdings ganz ungeeigneter Experimente zu stützen gesucht. Wir werden jedoch zeigen, daß die Wirkungen insbesondere des Posphits sich zum Teil in qualitativer Hinsicht zum Teil hinsichtlich der wirksamen Dosis von denen des Phosphats wesentlich unterscheiden. Überdies ist zu bemerken, daß die Oxydation der phosphorigen Säure durch Gewebsbrei bei Anwesenheit von Sauerstoff nach unseren Beobachtungen überaus langsam verläuft. Wir werden auf die Frage einer spezifischen Phosphitwirkung noch wiederholt zurückkommen.

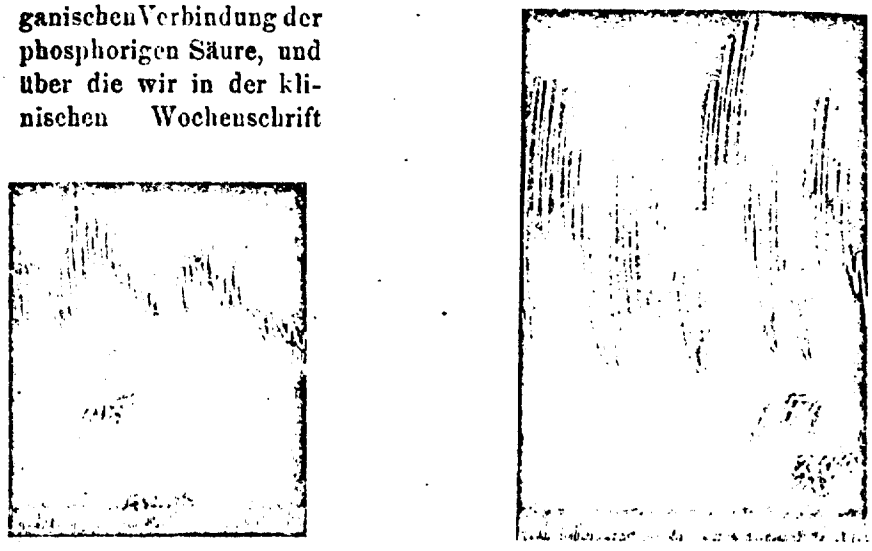


a b c

Kurve 1. Versuch 110a. 12. XI. 1923. Wirkung des Tonophosphans auf das Herz eines in Chloroformnarkose verendeten Kaninchens. Erst ½ Stunde nach Herausnahme und Durchströmung im Apparat nach Locke-Rosenheim begann das Herz vereinzelt sich zu kontrahieren, wie a zeigt. $5^h 02'$ und $5^h 06'$ oberhalb der Heizspirale je 2 ccm Tonophosphan 1:10000 injiziert, worauf die Herzaktion, wie b und c zeigen, innerhalb 10 Minuten ein bedeutendes Ausmaß erreicht.

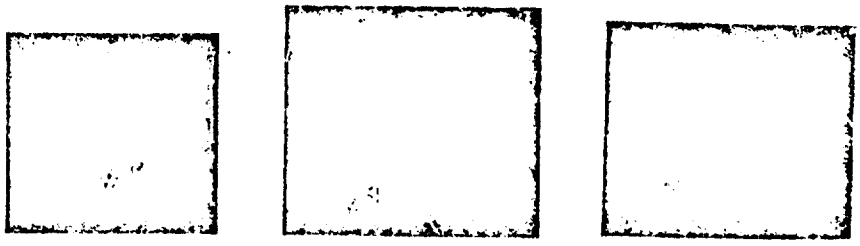
1) H. Staub, Phosphatwirkung am Herzen. Bioch. Zeitschr. Bd. 127, S. 255.
2) Munk und Leyden, a. a. O.

Unsere Untersuchungen wurden angeregt durch Befunde, die wir bei der Prüfung der pharmakologischen Wirkung des Tonophosphans erhielten, einer organischen Verbindung der phosphorigen Säure, und über die wir in der klinischen Wochenschrift



a b

Kurve 2. Versuch 12. 8. I. 1923. Tonophosphanwirkung am Katzendarm. a Darmbewegung ohne Tonophosphan. b 45 Minuten nach Zusatz von 0,5 ccm Tonophosphan 1% (↑).



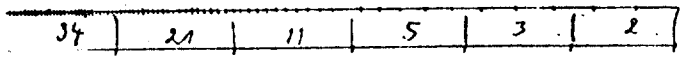
a b c

Kurve 3. Versuch 109. 27. IX. 1923. Tonophosphanwirkung am Meerschweinchenuterus. a 4^h 10′ Uterusbewegungen in reiner Lockelösung. b 4^h 30′ unter Tonophosphanwirkung (4^h 19′ und 4^h 23′ Tonophosphan 1:100 je 1 ccm auf 25 ccm Suspensionsflüssigkeit). c 5^h 50′ nach Ausspülung und Wechsel gegen reine Lockelösung: 5^h 32′.

kürzlich berichtet haben[1]). Es stellte sich in Versuchen an überlebenden Organen eine beträchtliche funktionssteigernde Wirkung heraus bei

1) Klin. Wochenschr. 2. Jahrg., Nr. 19, S. 872.

sonst fehlender Giftigkeit. Außerordentlich geringe Konzentrationen erwiesen sich als wirksam (1 : 10—1 : 100 Millionen). Diese Wirkung war besonders an geschädigten Organen erkennbar und war unter anderem dadurch gekennzeichnet, daß sie stets erst mit einer Latenzzeit von einigen Minuten zur Geltung kam. Beispiele der Wirkung des Tonophosphans zeigen die Kurven 1—3. Seither machten wir noch die durch eine Reihe gleich verlaufener Versuche gesicherte Beobachtung, daß Tonophosphan am Durchströmungsapparat nach Läwen-Trendelenburg eine reversible, erhebliche Gefäßverengerung schon in Konzentrationen 1 : 1 Millionen hervorruft (Kurve 4).



Kurve 4. Versuch 79. I. VI. 1923. Gefäßwirkung des Tonophosphans am Froschpräparat nach Trendelenburg. Auf 2 ccm Tonophosphan 1 : 1 Million sinkt die Tropfenzahl von 34 auf 2 in der Minute herab.

Da wir annahmen, daß es sich bei der Wirkung des Tonophosphans im wesentlichen um eine Phosphitwirkung handeln müsse, so führten wir dieselben Versuche auch mit Natriumphosphit durch. Hierbei zeigte sich in der Tat eine vollkommene Analogie der Wirkung mit der des Tonophosphans mit dem praktisch-therapeutisch allerdings sehr bedeutsamen Unterschiede, daß das Natriumphosphit im Gegensatz zu dem Tonophosphan nicht ungiftig ist.

I. Versuche mit Natriumphosphit.

1. Am suspendierten Froschherzen nach Straub. Kanülenfüllung 1 ccm.

Von unseren sehr zahlreichen Versuchen sei nur einer vom 30. VIII. 1923 in Form eines abgekürzten Protokolls wiedergegeben (Kurve 5).

Versuch 93.

Eskulentenherz, $2^3/_4$ Stunden nach Versuchsbeginn Nachlassen der Herztätigkeit. Reine Ringerlösung bringt keine Besserung. Natriumphosphit-Ringer 1 : 100 Millionen hebt die Herztätigkeit wesentlich für längere Zeit. 1 : 50 Millionen wirkt schon schädlich, 1 : 1000 führt zu sofortigem Herzstillstand. Nach Wechsel gegen reine Ringerlösung vollständige Erholung.

Dieser Versuch zugleich mit einer großen Zahl (18) gleich verlaufener zeigt, daß ein geschädigtes Froschherz durch geringe Phosphitdosen in seiner Tätigkeit erheblich gefördert, durch höhere Dosen

dagegen geschädigt wird. 1 : 1 Million ist in der Mehrzahl der Fälle schon schädlich gewesen.

a

b

Kurve 5. 30. . . 1923. Natriumphosphitwirkung am Froschherzen. a Günstige Wi' ung durch Na_2HPO_3 1 : 100 Millionen (↑). b Vollkommenes Versagen nach Na_2HPO_3 1 : 1000 (↑).

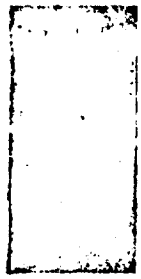
2. Am isolierten Säugetierherzen. Apparatur nach Locke-Rosenheim. Durchströmungsflüssigkeit nach Locke mit 1% Glukose. Temperatur 38—39°.

Abgekürztes Protokoll vom 5. IX. 1923. Kaninchenherz (Kurve 6).

Versuch 91.

Nachdem das Herz 1 Stunde gleichmäßig im Apparat gearbeitet hat, führen 2 ccm einer Phosphitlösung 1 : 1 Million, in den Schlauch oberhalb der Heizspirale injiziert, zu einer erheblichen Steigerung der Herzkontraktionen, die fast 2 Stunden anhält. 2 ccm Natriumphosphit 1 : 100000, an gleicher Stelle injiziert, schädigen das Herz. Ersatz der Durchströmungsflüssigkeit durch neue, phosphitfreie, führt zunächst fast zum Herzstillstand, der durch erneute Injektion von je 1 ccm Natriumphosphit 1 : 10 und 1 : 1 Million vorübergehend behoben werden kann.

a



b

Kurve 6. 5. IX. 1923. Natriumphosphitwirkung am Kaninchenherz. a $11^h 43'$ ohne Na_2HPO_3, 1 Stunde nach Beginn der Durchströmung. b $12^h 40'$ nach Injektion von 1 ccm Na_2HPO_3 1 : 1 Million in zwei Gaben: um $12^h 00'$ und $12^h 16'$.

Es beweisen diese Versuche, von denen wir insgesamt über 15 verfügen, daß auch am Säugetierherzen das Phosphit in geringen Dosen eine funktionssteigernde Wirkung entfaltet. Die schädliche Dosis liegt bei 1 : 100000. Dabei ist aber daran zu erinnern, daß durch die Zumischung der Injektionslösung zu der die Heizspirale durchströmenden Flüssigkeit eine weitere erhebliche und nicht abzuschätzende Verdünnung eintritt.

3. Versuche am überlebenden Katzendarm. Suspension in 25 ccm Tyrodelösung.

Abgekürztes Protokoll des Versuchs vom 10. IX. 1923.

Versuch 98.

Nachdem die Bewegungen des Darmes etwas nachgelassen haben, werden sie durch Zusatz von nur 2 ccm Natriumphosphit in Tyrode erheblich verstärkt bei gleichzeitiger mäßiger Steigerung des Tonus. Die Besserung hält 1 Stunde lang an. Wechsel gegen reine Tyrodelösung bringt starkes Sinken des Tonus und Nachlassen der Bewegungen. 1 Stunde später wird der nur wenig erholte Darm durch erneuten Zusatz von 2 ccm Natriumphosphit 1 : 100 Millionen wiederum erregt unter gleichzeitigem Tonusanstieg. Nachträgliche Steigerung der Dosis auf 1 : 100000 läßt keine Wirkung mehr erkennen.

Die Zahl unserer Darmversuche beträgt zwölf. Sie gaben bis auf wenige Ausnahmen alle das gleiche Resultat.

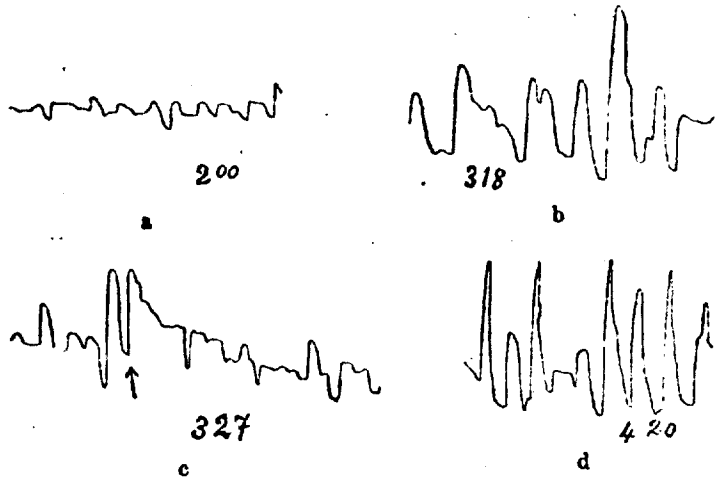
4. Versuche an der überlebenden Blase. Suspension in 25 ccm Lockelösung. Sauerstoffzufuhr. Temperatur 38—39°.

Abgekürztes Versuchsprotokoll vom 17. IX. 1923. Meerschweinchenblase.

Versuch 104.

Nachdem die zunächst guten Bewegungen der Blase sich etwas abgeschwächt haben, werden sie durch Zusatz von insgesamt 3 ccm Natriumphosphit 1 : 10 Millionen erheblich verbessert. Wechsel gegen reine Lockelösung wirkt verschlechternd auf die Aktion, die durch Zusatz von 2 ccm Natriumphosphit 1 : 100 Millionen wieder sehr deutlich gesteigert wird. Der Tonus ändert sich nicht. Nachdem die verbesserte Aktion längere Zeit angehalten hat, werden 2 ccm Natriumphosphit 1 : 1 Million zugesetzt mit dem Erfolg einer Beschleunigung der Bewegungen. Weitere Zusätze von 2 ccm der gleichen Lösung wirkten indessen schon leicht schädlich und die Erhöhung der Dosis durch Zufügen von Natriumphosphit 1 : 100000 und 1 : 10000 führten zu weiterer Verschlechterung der Aktion und Tonusnachlaß (Kurve 7).

Gesamtzahl der Versuche an der Blase acht. Ergebnisse gleich, wenn auch nicht immer gleich ausgeprägt.



Kurve 7. 17. IX. 1923. Phosphitwirkung auf Meerschweinchenblase. a 2^h 00′ in reiner Tyrodelösung. b 3^h 18′ nach Zusatz von 2 ccm Na_2HPO_3 1 : 10 Millionen um 2^h 52′. c 3^h 27′ nach Wechsel gegen reine Lockelösung (↑). d 4^h 20′ Zunahme nach Zusatz von 2 ccm Na_2HPO_3 1 : 10 Millionen um 3^h 50′.

5. Versuche am überlebenden Uterus. Suspension in 25 ccm **Lockelösung. Sauerstoffdurchleitung.** Temperatur 38—39°.

Gekürztes Protokoll vom 5. IX. 1923. Kaninchenuterus.

Versuch 95.

1 : 1 Millionen Natriumphosphit wirkt verstärkend auf die Bewegungen. 1 : 100000 ist deutlich schädlich.

Die Wirkung von Na_2HPO_3 am Uterus war ebenso deutlich wie beim Tonophosphan, wo wir sehr erhebliche Erregung feststellen konnten.

Die Gesamtheit der Versuche an überlebenden Organen zeigt also, daß Phosphit in sehr geringen Konzentrationen erregend, in höheren schädigend auf die Funktion einwirkt.

II. Versuche mit Hypophosphit.

Da neben phosphoriger Säure stets auch unterphosphorige Säure entsteht, wenn P bei Gegenwart von Sauerstoff mit Wasser in Berührung kommt, so haben wir auch dessen Wirkung auf überlebende Organe untersucht. Bemerkenswerterweise ist Hypophosphit fast

gänzlich wirkungslos. Am isolierten Froschherzen macht es erst in einer Konzentration von 1 : 100 eine Schädigung. Das Herz wird derart durchlässig, daß innerhalb 1 Minute bei fortwährendem Nachfüllen der Kanüle 2—4 ccm Flüssigkeit hindurchlaufen, während zugleich die Aktion aufhört. Unmittelbar nach Wechseln der Flüssigkeit gegen reine Ringerlösung wird die Durchlässigkeit wieder normal und das Herz beginnt wieder zu schlagen, ohne daß von einer dauernden Schädigung etwas zu merken wäre.

III. Lösungen von Phosphor.

Wenn eine alkoholische Lösung von Phosphor 1 : 1000 mit Ringer- oder Lockelösung verdünnt wird, so erhält man eine klare Lösung, die im wesentlichen phosphorige- und unterphosphorige Säure enthält und etwa mit dem Blut nach Resorption von P vergleichbar sein mag. Da wir mit diesen Lösungen nur in höchsten Verdünnungen arbeiteten, kommt der saure Charakter der Substanzen praktisch gar nicht in Betracht. Diese Lösungen erwiesen sich als besonders wirksam.

1. Versuche am Froschherzen.

Gekürztes Protokoll vom 11. IV. 1923. Eskulent.

Versuch 36.

Eine Lösung von 1 : 1 Million bewirkt in kurzer Zeit starke Schädigung des Herzens: Nachlassen der Kontraktionen, dann Arythmie, schließlich systolischen Stillstand. Wechsel gegen reine Ringerlösung bringt das Herz wieder zum Schlagen, beseitigt aber die Arythmie nicht völlig. Einführen einer P-Lösung in Ringer 1 : 400 Millionen beseitigt die Arythmie und fördert die Aktion, Wechsel gegen reine Ringerlösung setzt sie wieder stark herab und erneute Zufuhr von P 1 : 100 Millionen hebt sie wieder so, daß sie der Anfangsaktion gleich wird.

Sämtliche Versuche, insgesamt zehn, hatten das prinzipiell gleiche Ergebnis: Förderung in kleinsten, Hemmung in größeren Dosen.

2. Versuche am überlebenden Säugetierherzen. Anordnung wie oben, in Lockelösung.

Abgekürztes Protokoll vom 16. IV. 1923. Kaninchenherz.

Versuch 40.

Injektionen von zuerst 2 ccm P-Lösung 1 : 10 Millionen, dann 2 ccm 1 : 5 Millionen verbessert die von Anfang an mäßige Herzaktion erheblich. Injektionen von 1 : 1000 bringt das Herz sofort zum Stillstand. Mit nach-

fließender P-freier Lösung kommt es wieder in Tätigkeit. Die später nachlassende Tätigkeit konnte durch Injektion von 2 ccm der Lösung 1 : 10 Millionen nochmals wesentlich gebessert werden.

Gesamtzahl der Versuche neun, Ergebnisse identisch.

3. Versuche am überlebenden Darm.

Abgekürztes Protokoll vom 25. IV. 1923. Kaninchendarm in Tyrodelösung.

Versuch 52.

Der von Anfang an nicht gut arbeitende Darm erlangt unter P 1 : 100 Millionen optimale Tätigkeit. Lösungen von 1 : 1 Million und besonders von 1 : 100000 wirken erheblich schädigend. Bei Wechsel gegen reine Tyrodelösung hört die Tätigkeit des Darmes fast völlig auf, worauf Zusatz von 0,2 ccm P 1 : 100000 zur Suspensionsflüssigkeit sie erneut stark anregte.

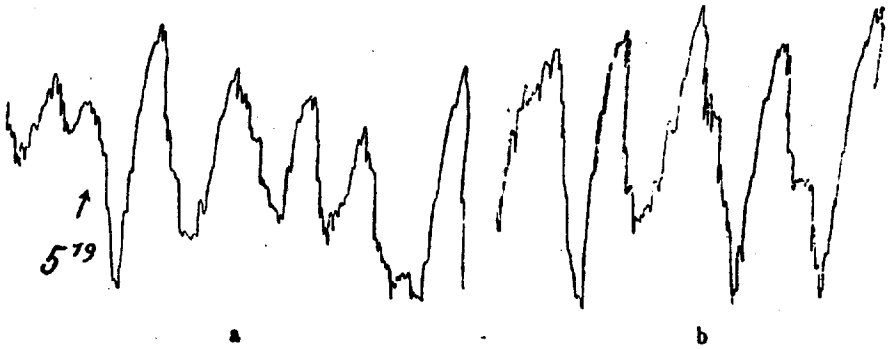
Gesamtzahl der Versuche zehn.

4. Versuche am überlebenden Uterus.

Abgekürztes Protokoll vom 3. V. 1923. Kaninchenuterus (Kurve 8).

Versuch 65.

Nach längerer gleichmäßiger Tätigkeit werden die Bewegungen durch Zusatz von 1 ccm P-Lösung 1 : 40 Millionen erheblich verstärkt. Der Tonus steigt langsam. Nach Wechsel gegen reine Lockelösung sinkt der Tonus zunächst stark ab. Nachdem er konstant geworden ist, machen viermal 1 ccm der Lösung 1 : 40 Millionen Verstärkung der Bewegungen, aber keine Tonussteigerung mehr.



Kurve 8. 3. V. 1923. Wirkung von P-Lösung auf Uterus von Kaninchen. a Bei (↑) Zusatz von 1 ccm P-Lösung 1 : 40 Millionen. b 25 Minuten später.

Auch aus den Versuchen mit P-Lösungen ergibt sich die funktionssteigernde Wirkung kleinster Dosen und die schädigende Wirkung der größeren. Da in den Lösungen lediglich die phosphorige Säure wirksam sein kann, so bestätigen sie die mit Natriumphosphit angestellten Versuche.

Vergleichen wir die mit phosphoriger Säure erzielten Resultate mit denen, die Staub mit Phosphat erhielt, so fällt vor allem der Unterschied in der wirksamen Dosis auf. Staub arbeitete mit N/100 Phosphatgemisch. N/100 primäres Natriumphosphat entspricht einer Konzentration von 1 : 46000, N/100 sekundäres Phosphat einer Konzentration von etwa 1 : 1000. Mit beiden Phosphaten hat Staub am Froschherzen sehr günstige Beeinflussung der Funktion erreicht. Ich selbst habe im Hinblick hierauf auch einige Versuche mit Phosphat an Säugetierorganen gemacht, die aber negativ verliefen. Die Zahl der Versuche ist indessen eine zu geringe, als daß sie Schlüsse gestattete. Es muß aber besonders darauf hingewiesen werden, daß unsere Darmversuche sämtlich in Tyrodelösung vorgenommen wurden, die schon von vornherein einen Phosphatgehalt von 0,05 g auf 1 l, d. h. also von 1 : 20000 hatte und daß hierin Phosphit in einer Konzentration von 1 : 10 Millionen sich als wirksam erwies. Es kann somit nicht an eine sekundäre Wirkung von aus Phosphit entstehendem Phosphat gedacht werden.

IV. Über die Toxizität der O-Verbindungen des P.

Nachdem wir festgestellt hatten, daß dem Phosphit an überlebenden Organen eine erhebliche Wirkung zukommt, schien uns eine erneute Prüfung seiner Wirkung am ganzen Tiere notwendig im Vergleich zur Wirkung des Hypophosphits sowie des P selber. Hierbei zeigte sich, daß entgegen den Ergebnissen von H. Schulz und von Neumann das Phosphit bei subkutaner Zufuhr zweifellos giftig ist. Das Präparat war, wie eine genaue Untersuchung mittels des Verfahrens von Marsh zeigte, völlig frei von As.

Abgekürzte Protokolle.

Zwei Temporarien, A männlich, 23,2 g Gewicht; B weiblich, 19,95 g Gewicht.

Beide erhalten am 7. III. 1923 um 11^h 00′ 0,5 ccm Natriumphosphat 1 : 100 in den Brustlymphsack, um 3^h 45′ 1 ccm, um 7^h 10′ nochmals 1 ccm, ohne wesentliche Wirkung. Am 4. VII. werden um 10^h 45′ 1 ccm, um 11^h 30′ 0,15 ccm injiziert. Um 12^h 00′ zeigt A fibrilläre Zuckungen an Bauch und Beinen, bei B treten krampfartige Kontrakturen

auf. Beide Tiere bewegen sich mühsam. A bekommt nochmals 1 ccm. Um 1^h 15′ zeigen beide Tiere fibrilläre Muskelzuckungen und Streckkrämpfe bei jedem Bewegungsversuch. Am 5. VII. um 4^h 35′ haben die Zuckungen bei beiden Tieren aufgehört. Bewegungen kriechend. Um 8^h 45′ dulden die Tiere Rückenlage. Am 6. VII. ist A tot, B hat sich völlig erholt. A hatte im ganzen 5 ccm, B 4 ccm der Lösung 1 : 100, also 5 bzw. 4 cg erhalten. Die Sektion ergibt bei A ein in Diastole stillstehendes Herz von mittlerer Füllung. Die Leber von derber Konsistenz, grauer Farbe und ziemlich blutarm. — Niere sehr blaß. Im Abdomen eine serös blutige Flüssigkeit. In anderen Fällen wurde das Herz in Systole stillstehend gefunden und Leber und Niere waren bei akut verlaufenden Vergiftungen eher hyperämisch.

Im allgemeinen genügten 0,1 g Phosphit um Frösche innerhalb weniger Stunden zu töten. Wintertiere sind etwas widerstandsfähiger, gehen aber auch nach 1—2 Tagen zugrunde. Mäuse kommen nach 0,15—0,2 g subkutan innerhalb weniger Stunden zum exitus. Bei Meerschweinchen genügten 0,2—0,5 g. Große Tiere zeigen sich aber oft recht widerstandsfähig. Man muß dann mehrere Tage 0,5 g geben um tödlichen Ausgang zu erhalten. In einem Falle gab ich 4 Wochen hintereinander einem kräftigen Meerschweinchen jeden 2.—3. Tag 2 ccm Natriumphosphit 1 : 10, also jedesmal 0,2 g ohne Änderungen seines Zustandes feststellen zu können. Erst in der 5. Woche starb das Tier nach einer Einzeldosis von 0,2 g. Das Ergebnis der histologischen Untersuchungen siehe weiter unten.

In einem anderen Versuche erhielt ein Meerschweinchen in den Tagen vom 17.—19. X. 1923 insgesamt 0,8 g Phosphit subkutan. Am 20. X. wurden ihm 0,2 ccm auf einmal injiziert, kurz danach erkrankt es schwer. Es wird paretisch, zeigt Tränenfluß, Diarrhoe und Polyurie, zittert und schreit bei jeder Berührung. Am nächsten Tage ist es schwer krank und geht mittags ein.

Die histologische Untersuchung der Organe, die wir dem hiesigen pathologischen Institut verdanken, ergab weder bei den schnell noch bei den allmählich innerhalb einiger Tage eingegangenen Fröschen etwas Besonderes. Vor allem fehlten Verfettungserscheinungen in Leber und Niere. Auch bei dem im zuletzt geschilderten Versuche innerhalb von 4 Tagen eingehenden Meerschweinchen zeigte sich nichts von Bedeutung. Dagegen ergab die histologische Untersuchung des oben erwähnten 4 Wochen lang mit Phosphit behandelten Tieres folgenden Befund:

Lungen: Vereinzelte Blutungen in den Alveolen. **Niere:** Starke Blutfüllung der Glomeruli, vereinzelte Blutaustritte in der Nierenrinde. **Leber:** Mäßige aber unverkennbare Verfettung der Leberzellen und erhebliche Hyperämie.

Genau wie die Wirkung des Phosphits, nur intensiver und schneller im Verlauf, erweist sich die Wirkung von wässerigen P-Lösungen. Frösche gehen nach Injektion von 1 mg P in alkoholischer Lösung innerhalb weniger Stunden ein. Bei Anwendung einer Lösung von 1 : 10000, mit Wasser oder Ringerlösung aus der alkoholischen Stammlösung 1 : 1000 hergestellt, ergibt sich das gleiche Bild. Die Erscheinungen sind Lähmungen, die mit ataktischen Bewegungen beginnen und mit völliger Bewegungslosigkeit enden. Fibrilläre Zuckungen sind selten, dagegen wurden mehrfach Zuckungen ganzer Muskelbündel beobachtet. Die zur Kontrolle mit den entsprechenden Mengen Alkohols gespritzten Tiere erholten sich stets vollständig.

Abgekürztes Protokoll eines Versuches an der weißen Maus.

28. VI. 1923. Injektion von zweimal 0,5 ccm einer P-Lösung 1 : 10000. Zunächst ohne deutliche Wirkung. Am 29. VI. morgens erhält das Tier 0,25 ccm einer alkoholischen P-Lösung 1 : 1000. Innerhalb von 45 Minuten schwere Vergiftungserscheinungen: Lähmung, Atemnot, schließlich Atemstillstand und Tod innerhalb 1 Stunde nach der Injektion. Die Sektion ergab auch hier histologisch keinerlei Veränderungen an den Organen.

Erheblich weniger giftig als Phosphit und P-Lösungen erweist sich das Hypophosphit. Frösche halten selbst wiederholte Injektionen von Lösungen 1 : 10 während mehrerer Tage aus. Selten kann man mit Lösungen 1 : 5 Exitus herbeiführen. Meerschweinchen erwiesen sich als anscheinend völlig resistent, selbst gegenüber sehr großen Dosen. Lediglich Mäuse sind, aus unbekannten Gründen, ziemlich empfindlich. Schon nach 2 ccm einer Hypophosphitlösung 1 : 10 gingen sie wiederholt mit ganz ähnlichen Erscheinungen ein wie nach Phosphitvergiftung.

Vergleichsweise haben wir auch eine Anzahl von Fröschen mit Natriumphosphat vergiftet. Das Bild ist hier besonders durch die langanhaltenden fibrillären Zuckungen in allen Muskeln gekennzeichnet. Von Bedeutung für die wiederholt erörterte Frage, ob etwa das Phosphit lediglich nach Oxydation zu Phosphat wirke, ist die durch Starkenstein gefundene Tatsache, daß man die Phosphatvergiftung durch $CaCl_2$ aufheben bzw. verhüten kann. Dagegen konnten wir die tödliche Wirkung des Phosphits durch $CaCl_2$ nicht beeinflussen. Es verschwinden lediglich die beim Phosphit weit spärlicheren und vielleicht auch auf Ca-Entzug beruhenden fibrillären Zuckungen, der Tod trat aber trotzdem ein. Es muß demnach auch

aus diesem Grunde dem Phosphit eine besondere, von der des Phosphats verschiedene Wirkung zugeschrieben werden. Die Einwirkungen, die Starkenstein mit Phosphat an isolierten Kalt- und Warmblüterherzen, sowie am Kaninchendünndarm fand, ließen sich ebenfalls durch $CaCl_2$ aufheben. Überdies verwandte er N/5-Lösungen von Phosphat, entsprechend einer Konzentration von etwa 1 : 50, welche Dosis mit den von uns als wirksam befundenen geringen Phosphitmengen gar nicht in Vergleich gesetzt werden kann.

V. Zur Theorie der Phosphorwirkung.

Wir haben gezeigt, daß das Phosphit eine stark wirksame Substanz ist. Es erhebt sich die Frage, inwieweit dieser Befund geeignet ist zur Erklärung der Phosphorwirkungen beizutragen. In dieser H[illegible]t ist das Ergebnis der histologischen Untersuchungen von Belang. Wie wir zeigen konnten, bekommt man auch bei protrahiertem Verlauf der Phosphitvergiftung keine jener auffälligen degenerativen Organveränderungen, wie sie für die akute, vor allem aber die chronische P-Vergiftung charakteristisch sind. Andererseits ist es bekannt, daß bei Vergiftungen mit sehr hohen P-Dosen und sehr akutem Verlauf ebenfalls keine Organveränderungen festzustellen sind. Wir halten es daher für wahrscheinlich, daß in diesen Fällen die Überschwemmung mit Phosphit die Todesursache ist, zumal wir feststellten, daß hohe Phosphitdosen schwer schädigend insbesondere auf das Herz einwirken, dessen Lähmung auch bei jenen hyperakuten Fällen von P-Vergiftung als Todesursache betrachtet wird. So hat H. Meyer[1]) experimentell Herzlähmung durch P erzielen können zu einer Zeit, da noch keinerlei degenerative Organveränderungen eingetreten waren.

Anders bei der chronischen Vergiftung. Hier, wie bei den nicht allzu akuten Fällen, ist es ohne Zweifel die reduzierende Wirkung des P an den Stellen, wo er gespeichert wird, welche die Hauptrolle spielt. Durch die langanhaltende Entziehung von Sauerstoff, die bei der stetigen und langsam fortschreitenden Oxydation des P an jenen Stellen eintreten muß, werden die charakteristischen degenerativen Erscheinungen insbesondere die fettige Degeneration, verursacht, die ja allgemein als eine Folge der Sauerstoffverarmung betrachtet werden.

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1) H. Meyer, Arch. f. exp. Path. u. Pharm. Bd. 14, S. 313, zitiert auch bei v. Maschka, Wien. Med. Wochenschr. 1884, Nr. 21, S. 648, der ebenfalls Herzlähmung bei sehr schnell und ohne nachweisbare anatomische Veränderungen verlaufenden P-Vergiftungen annimmt.

Was aber schließlich die therapeutische Wirkung kleinster Phosphordosen betrifft, so glauben wir, daß hierbei der Phosphitwirkung eine wesentliche Rolle zukommt. Die funktionsanregenden Wirkungen minimaler Phosphitdosen, wie wir sie in unseren Versuchen nachwiesen, entsprechen der therapeutischen Indikation der P-Medikation, und es erscheint in dieser Hinsicht besonders wichtig, daß Wegner auch mit Phosphit wie mit Phosphor, Begünstigung des Knochenwachstums erzielte.

Solche Betrachtungsweise muß notwendig zu dem Schluß führen, daß bei der therapeutischen Anwendung des P lediglich kleinste Dosen anzuwenden sind und daß sie zur Erreichung der erwünschten Wirkung auch ausreichen. Die mit Recht wegen ihrer Gefahren gefürchtete Anwendung größerer Dosen muß nicht nur, sondern sie kann auch vermieden werden, ohne eine Beeinträchtigung der therapeutischen Wirkung.

Zusammenfassung.

1. Es werden kurz die über den Wirkungsmechanismus des P im tierischen Organismus bestehenden Theorien besprochen.

2. Kurze Wiedergabe der Versuchsergebnisse mit Tonophosphan an überlebenden Frosch- und Säugetierorganen. Tonophosphan regt die Thätigkeit mangelhaft schlagender Frosch- und Säugetierherzen an. Ebenso findet eine Steigerung der Arbeitsleistung bei isolierten, überlebenden Därmen, Blasen und Uteri von Säugetieren statt.

3. Die Annahme, daß es sich beim Tonophosphan um eine Phosphitwirkung handle, wird durch Wiederholen derselben Versuchsreihen mit Natriumphosphit bestätigt. Auch Natriumphosphit vermag in starken Verdünnungen in gleichem Sinne wie Tonophosphan zu wirken, doch besteht ein Unterschied darin, daß Tonophosphan selbst in einer Konzentration von 1 : 100 nicht schädigend wirkt, Phosphit dagegen in einer Konzentration 1 : 1000 die Bewegungen überlebender Organe zum Stillstand bringt.

4. Wässerige Lösungen von P hergestellt aus einer alkoholischen Stammlösung 1 : 1000) wirken wie Phosphit, nur müssen noch stärkere Verdünnungen angewandt werden, da Konzentrationen von 1 : 1 Million zum mindesten aber 1 : 100000 bereits sehr starke Schädigungen der Organe verursachen.

5. Mit Hypophosphit konnte an keinem Organe ein günstiger Effekt einwandfrei beobachtet werden. Auch Schädigungen waren am Froschherzen nur bei Benutzung einer Konzentration von 1 : 100 zu erzielen.

6. Es wird gezeigt, daß Phosphit- und Phosphatwirkung nicht identisch sind.

7. Eine Nachprüfung der Toxizität von Phosphit an Fröschen, weißen Mäusen und Meerschweinchen zeigt, daß bei subkutaner Applikation von 1—3 ccm einer Lösung 1 : 10 bei Fröschen und Mäusen, 1—5 ccm bei Meerschweinchen, die Tiere innerhalb weniger Stunden eingehen. Mit Hypophosphit konnte tödliche Vergiftung nur bei Fröschen mit größten Dosen und bei Mäusen beobachtet werden.

8. Auf Grund der gefundenen Tatsachen wird der Versuch einer Erklärung der P-Wirkung im Organismus gemacht.

Arch. exptl. Path. Pharm. 102, 289-304 (1924)

RESEARCH ON THE MECHANISM OF ACTION OF PHOSPHORUS

From the Pharmacological Institute of the University of Greifswald

by

Dr. Kurt Engel
Institute Assistant

(with 8 graphs)

(Submitted on January 24, 1924)

Numerous works have taught us about the anatomical and pathological picture of phosphorus intoxication[1]. Also, the fundamentals of its therapeutic use have been laid at least in part by various experiments. However, now as previously, the question as to the actually manner in which phosphorus acts remains unclarified. Three theories, as is known, have been proposed. According to one, P is supposed to act only as phosphorus. The fact that, in very acute cases of P-intoxication, free P was detectable in both the blood and in the organs, together with the ostensible innocuousness of P's oxidation products, led to this view, which is, however, unsatisfactory insofar as it is difficult to imagine the action of elementary P as such. In any case, we can assume that the oxygen avidity of P absorbed in free form must indicate harm everywhere where it accumulates, thus especially in the lipoid-rich tissues and above all in the liver. Indeed, from the pathological-anatomical, and also the pathological-physiological point of view, a significant role has been ascribed to this reduction effect of P.

The undeniable fact that P in an aqueous solution in the presence of O, thus in the blood especially, but also in the tissues, is oxidized with a certain speed into phosphoric and subphosphoric acid[2], led to the second hypothesis, according to which P acts only insofar as it is converted into these oxidation products. H. Schulz[3] and Neumann[4] contested this theory on the basis of their findings, according to which both subphosphoric and phosphoric acid are non-toxic.

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1 Older literature: Munk and Leyden, Die akute P*Vergiftung, 1865; also Dybkowski, Beiträge zur Theorie der P-Vergiftung. Hoppe-Seylers med. chem. Unters. Berlin, 1866, p. 49-70. New literautre, especially concerning metabolic processes in the case of P-intoxication: R. Tiscenner, Virchows Arch. 1904, Bd. 175, S. 90. Bei E. Frank und S. Isaak, Arch. f. exp. Path. u. Pharm. 1911, Bd. 64. Hirz, Zeitschr. f. Biolg. 1913, Bd. 60, S. 187. H. Rettig, Arch. f. exp. Path. u. Pharm. 1914, Bd. 76, S. 365.

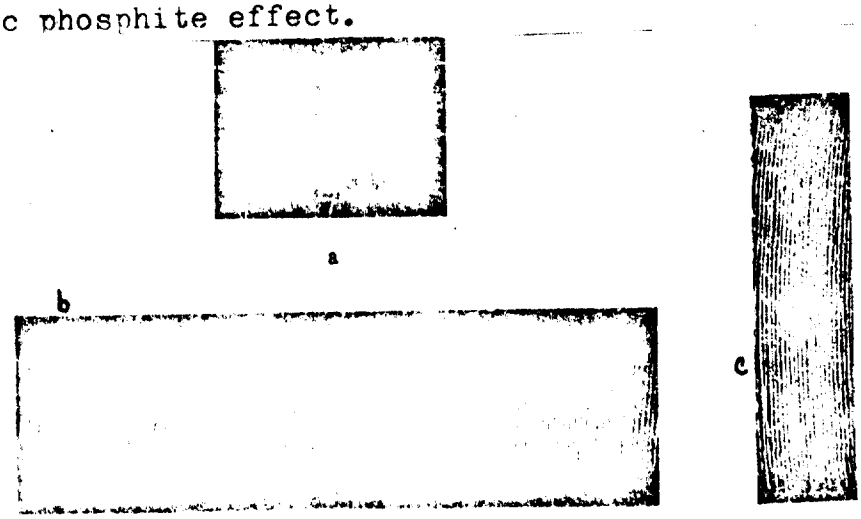
2 Vaclav Plavec, Pflügers Arch. 1904, Bd. 49, S. 1 und H. Vohl, Berl. Klin. Wochenschr. 1865, Nr. 32, S. 329; Nr. 33, S. 336.

3 H. Schulz, Arch. f. exp. Path. u. Pharm. Bd. 18, S. 174 und Bd. 23, S. 150.

4 J. Neumann, Experimentelle Studien zur P-Vergiftung. Diss., Rostock 1886.

This finding could not even be proved by the fact that phosphoric acids were determined in the blood and the tissues after P-intoxication.[1] Finally, the third hypothesis should be mentioned. According to this theory, the phosphorus in the organism is reduced to the thoroughly toxic PH_3, and only then becomes active. With Santesson and Malmgren, we believe that such a significance can be ascribed to PH_3 only in exceptional cases, in which a hyper-acute effect appears after the intake of large amounts of P.

If we consider these three proposed hypotheses, the reduction effect of P seems to be thoroughly probable, but we cannot completely exclude the possibility of a participation of the oxidation products, either. It is possible that a formation of such substances in the tissues could itself unfold a completely different action from that seen when it is administered orally. This view was represented by H. Schulz[2]. We therefore considered it wise to study the effects of the lower oxidation products of P on surviving organs. It also seemed necessary to examine once again the question of the toxic action of these substances on whole animals. Since according to Starkenstein[3] the phosphoric acids exercise toxic effects as Ca precipitating substances and, in high concentrations also act on surviving organs, and since Staub[4] recently demonstrated the favorable effect of phosphates on the surviving frog heart, the objection could be raised, for every examination of the action of the lower oxidation products of P, that the actually active substance in the case is the phosphoric acid formed by oxidation. Munk and Leyden[5] tried to support this view with experiments that were, however, not quite appropriate. Nonetheless, we will show that the effects of phosphite especially differ significantly from those of phosphate, partly in a qualitative respect, and partly with respect to the effective dose. Here it should be noted that the oxidation of the phosphoric acid by tissue sludge in the presence of oxygen takes place very slowly, according to our observations. We will return to the question of a specific phosphite effect.



Graph 1. Experiment 110a. 11/12/1923. Effect of tonophosphane on the heart of a rabbit killed in chloroform narcosis. The heart began to contract ½ hour after removal and subjection to flow in a Locke-Rosenheim apparatus, as seen in a. $5^h02'$ and $5^h06'$ above the heating coil 2 ccm tonophosphane 1:10,000 injected, whereupon the heart action, as seen in b and c, reaches a significant degree.

Footnotes to page 2.

1 Selmi, Arch. d. Pharm. 1880, III., Bd. 17, S. 253; 1887, Bd. 19, S. 276. Hager, Handb. d. pharm. Prax. 1887, Bd. 2, S. 672. Hollefreund, zitiert bei A. Fischer, Pflügers Arch. 1903, Bd. 97, S. 578; diese fanden niedere Oxydationsstufen des P im Harn und den Organen.*

2 H. Schulz, Arch. f. exp. Path. u. Parm. Bd. 23, S. 151.

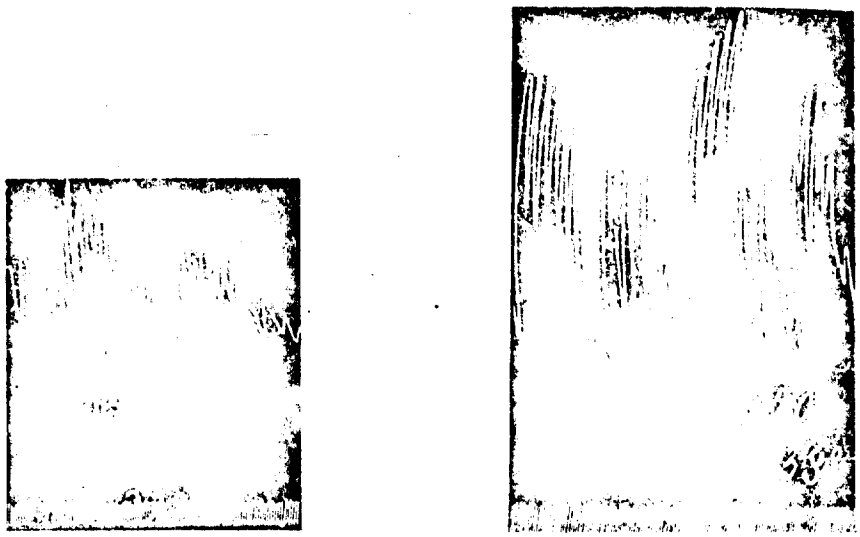
3 Starkenstein, Ebenda 1914, Bd. 77, S. 45.

4 H. Staub, Phosphatwirkung am Herzen. Bioch. Zeitschr. Bd. 127, S. 255.

5 Munk und Leyden, a. a. O.

* These authors found lower oxidation stages of P in the urine and in the organs

Our research was stimulated by findings which we made in testing the pharmacological effect of tonophosphane, an organic compound of phosphoric acid, and about which we have reported briefly in the Klinische Wochenschrift.[1] In experiments on surviving organs, a considerable function-increasing effect appeared, with otherwise absent toxicity. Extraordinarily small



a b

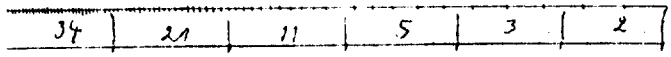
Graph 2. Experiment 12. 1/8/1923. Tonophosphane effect on the cat intestine. a: intestinal movement without tonophosphane. b: 45 minutes after administration of 0.5 ccm tonophosphane 1% ( ).

a b c

Graph 3. Experiment 109. 9/27/1923. Tonophosphane effect on the guinea pig uterus. a: $4^h10'$ uterus movements in pure rolling solution. b: $4^h30'$ under tonophosphane effect ($4^h19'$ and $4^h23'$ tonophosphane 1:100 every 1 ccm to 25 ccm suspension liquid). c: $5^h50'$ after washing and change to pure rolling solution: $5^h32'$.

concentrations were shown to be effective (1:10 -1:100 million). This effect was especially recognizable in harmed organs and was characterized among other things, by the fact that it always did not appear until after a latency time of several minutes. Graphs 1-3 represent examples of the effect of tonophosphane. Since then, we have made the observation, confirmed by a series of simultaneous experiments, that tonophosphane in the flow apparatus of Löwen-Trendelenburg produces a reversible, appreciable vessel narrowing in concentrations as small as 1:1 million (graph 4).

1) Klin. Wochenschr. 2. Jahrg., Nr. 19, S. 872.



Graph 4. Experiment 79. 6/1/1923. Vessel action of tonophosphane on the frog preparation of Trendelenburg. Upon 2 ccm tonophosphane 1:1 million, the drop count falls from 34 to 2 in one minute.

Since we assumed, that in the case of tonophosphane effect, we must be dealing with essentially a phosphite effect, we performed the same experiments with sodium phosphite. Here we did in fact see a clear analogy between the effect of sdium phosphite and that of tonophosphane, with the difference, however, vey significant in a practical therapeutic sense, that the sodium phosphite, in contrast to tonophosphane, is not non-toxic.

I. Experiments with Sodium Phosphite

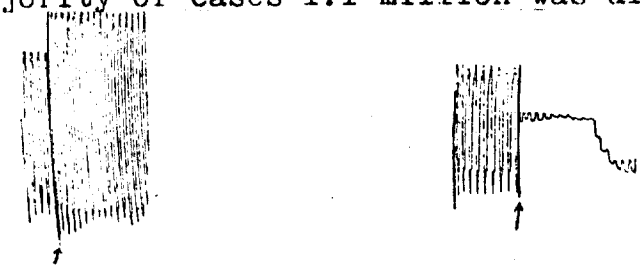
1. On the suspended frog heart of Straub. Tubule filling, 1 ccm.

Of our numerous experiments, we repeat here only the one of August 30, 1923, in the form of a shortened report. (Graph 5)

Experiment 93

Esculant heart, 2 3/4 hours after beginning of experiment, abatement of heart activity. Pure Ringer solution produces no improvement. Ringer sodium phosphite 1:100 million increases the heart activity significantly for some time. 1:50 million is harmful, 1:1000 leads to immediate cessation of heartbeat. After change to pure Ringer solution, complete recovery.

This experiment, together with a number of simultaneous ones (18) shows that a damaged frog heart is assisted considerably in its activity by small phosphite doses, but is damaged by larger doses. In the majority of cases 1:1 million was already harmful.



a b

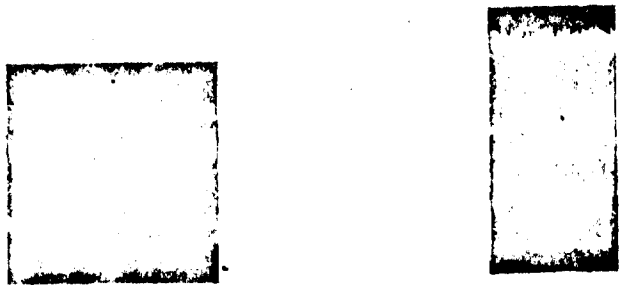
Graph 5. August 30, 1923. Sodium phosphite effect on the frog heart. a: favorable effect due to Na_2HPO_3 1:100 million (↑). b : complete stop after Na_2HPO_3 1:1000 (↑).

2. On an isolated rabbit heart. Locke-Rosenheim Apparatus. Flow liquid of Locke with 1% glucose. Temperature 38 -39°.

Shortened report of September 5, 1923. Rabbit heart (graph 6).

Experiment 94

After the heart had worked regularly in the apparatus for 1 hour, 2 ccm of a 1:1 million phosphite solution, injected into the tube above the heat coil, led to a considerable increase in heart contractions, which continued for nearly 2 hours. 2 ccm 1:100,000 million sodium phosphite, injected at the same spot, damage the heart. When the flow fluid is replaced by a new, phosphite-free one, this leads at first to a nearly complete stoppage of the heart, which can then be avoided by another injection of 1 ccm 1:10 million and 1 ccm 1:1 million sodium phosphite.



a b

Graph 6. 9/5/1923. Sodium phosphite effect on the rabbit heart.
a: $11^h43'$ without Na_2HPO_3, 1 hour after beginning of flowthrough.
b: $12^h40'$ after injection of 4 ccm Na_2HPO_3 1:1 million in two doses, at $12^h00'$ and $12^h16'$.

These experiments, 15 of which we report here, prove that phosphite in small doses exercises a function-increasing effect on mammal hearts as well. The harmful dose is 1:100,000. Here it should be remembered that by mixing the injection solution with the fluid flowing through the heat coil, another considerable and significant dilution is effected.

3. Experiments with the surviving cat intestine. Suspension in 25 ccm tyrode solution.

Shortened report of the Experiment of September 10. 1923.

Experiment 98

After the movements of the intestine have abated somewhat, they are strengthened considerably by the addition of only 2 ccm sodium phosphite in tyrode; at the same time, the tonus is also increased considerably. This improvement continues for 1 hour. Change to pure tyrode solution leads to marked sinking of the tonus and abatement of the movements. 1 hour later, the intestine, which has recovered only slightly, is again stimulated with simultaneous tonus increase by the addition of 2 ccm sodium phosphite 1:100 million. Subsequent increase of the dose to 1:100,000 produces no new effect.

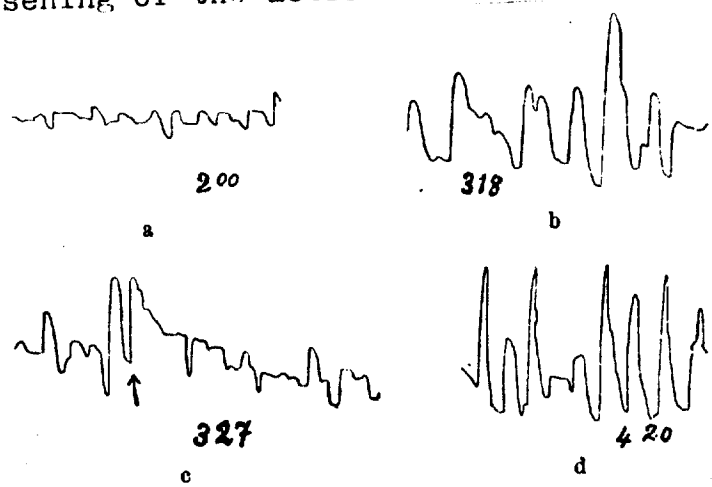
We performed twelve intestinal experiments. With only a few exceptions, they all yielded the same result.

4. Experiments on the surviving bladder. Suspension in 25 ccm Locke solution. Oxygen administration. Temperature 38 - 39°.

Shortened experiment report of September 17, 1923. Guinea pig bladder.

Experiment 104

After the movements of the bladder, which were good at first, weakened somewhat, they are improved considerably by the addition of a total of 3 ccm sodium phosphite 1:10 million. Change to pure Locke solution has a worsening effect on the action that is increased very clearly by the addition of 2 ccm sodium phosphite 1:100 million. The tonus does not change. After the improved action has continued for some time, 2 ccm sodium phosphite 1:1 million are added, leading to anacceleration of the movements. Further additions of 2 ccm of the same solution, however, begin to act harmfully, and increasing the dose by adding 1:100,000 and 1:10,000 sodium phosphite leads to further worsening of the action and abatement of tonus (graph 7).



Graph 7. 9/17/1923. Phosphite effect on guinea pig bladder. a: $2^h00'$ in pure tyrode solution. b: $3^h18'$ after addition of 2 ccm Na_2HPO_3 1:10 million at $2^h52'$. c: $3^h27'$ after change to pure Locke solution. (↑). d: $4^h20'$ increase after addition of 2 ccm Na_2HPO_3 1:10 million at $3^h50'$.

The total number of experiments on the bladder was eight. Results identical, though not always equally marked.

5. Experiments on the surviving uterus. Suspension in 25 ccm Locke solution. Introduction of oxygen. Temperature 38 -39°.

Shortened report of September 5, 1923. Rabbit uterus.

Experiment 95

1:1 million sodium phosphite acts strengtheningly on the movements. 1:100,000 is clearly harmful.

The effect of Na_2HPO_3 on the uterus was as clear as was the case with tonophosphane, where we were able to determine very marked stimulation.

All the experiments on surviving organs thus show that phosphite in very small concentration acts in a stimulating manner; in higher concentrations, it damages function.

II. Experiments with Hypophosphite

Since, besides phosphoric acid, subphosphoric acid also forms when P comes into contact with water in the presence of oxygen, we examined the effect of this latter on surviving organs. Notably enough, hypophosphite is nearly completely without effect. It causes damage to a frog heart only in a concentration of 1:100. The heart slows down so much that within 1 minute, upon continuous filling of the tubules, 2-4 ccm liquid pass through it, while action ceases at the same time. Immediately after a change of the liquid to pure Ringer solution, the permeability becomes normal again, and the heart begins to beat again, and there is no notice of any lasting damage.

III. Phosphorus Solutions

When an alcohol solution of phosphorus, 1:1000 is diluted with Ringer or Locke solution, we obtain a clear solution that contains phosphoric and subphosphoric acid, and can be compared somewhat with the blood after absorption of P. Since we worked with these solutions only in their greatest dilutions, the acid character of the substances does not really enter the picture here. These solutions were found to be especially effective.

1. Experiments on the frog heart

Shortened report of April 11, 1923. Esculant.

Experiment 36

A solution of 1:1 million causes marked damage to the heart in a short time: abatement of contractions, then arhythmia, finally systolic arrest. Change to pure Ringer solution starts the heart beating again, but does not completely remove the arhythmia. Introduction of a P solution in Ringer 1:400 million erases the arhythmia and promotes action; change to pure Ringer solution decreases it markedly again, and another addition of P 1:100 million raises it again, so that it is identical to the initial action.

All the experiments, ten in total, had the same principal result: promotion in small, inhibition in large doses.

2. Experiments on surviving mammal hearts. Set-up as above, in Locke solution.

Shortened report of April 16, 1923. Rabbit heart

Experiment 40

Injections first of 2 ccm P-solution 1:10 million, then of 2 ccm 1:5 million improve the moderate heart action considerably. Injections

of 1:1000 bring the heart to a standstill immediately. It resumes activity when solution without P is poured through. The later abating activity could again be improved significantly by the injection of 2 ccm of the 1:10 solution.

Total number of experiments, nine; results identical.

3. Experiments on the surviving intestine

Shortened report of April, 25, 1923. Rabbit intestine in tyrode solution.

Experiment 52.

The intestine, which was working well from the beginning, reached optimal activity under the effect of 1:100 million P. Solutions of 1:1 million and especially of 1:100,000 act in a damaging manner. Upon change to pure tyrode solution, the activity of the intestine cease nearly entirely, whereupon addition of 0.2 ccm P 1:100,000 to the suspension liquid stimulates them markedly again.

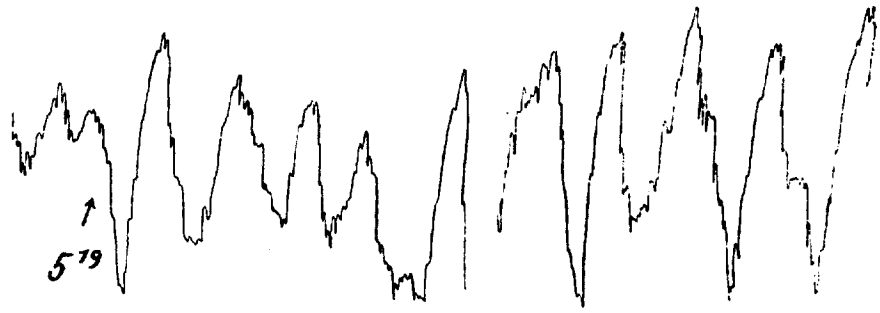
Total number of experiments, ten.

4. Experiments on the surviving uterus

Shortened report of May 3, 1923. Rabbit uterus (Graph 8).

Experiment 65

After long-lasting regular activity, the movements are strengthened considerably by the addition of 1 ccm P solution 1:40 million. The tonus rises slowsly. After change to pure Locke solution, the tonus sinks markedly at first. Afterwards, it becomes constant; then 4 administrations of the 1:40 million solution cause a strengthening of the movements, but no furtherincrease in tonus.



Graph 8. May 3, 1923. Effect of P-solution on the uterus of a rabbit. a: upon (↑) addition of 1 ccm P-solution 1:40. b: 25 minutes later.

The experiments with P-solutions also reveal that small doses have a function-promoting effect, while the larger doses have a damaging effect. Since in the solutions only the phosphoric acid can be active, these experiments confirm the experiments performed with sodium phosphite.

If we compare the results obtained with phosphoric acid with those that Staub obtained with phosphate, we notice above all the difference in the effective dose. Staub worked with N/100 phosphate mixture. N/100 primary sodium phosphate corresponds to a concentration of 1:46,000, N/100 secondary phosphate to a concentration of about 1:1000. Staub obtained very favorable effects on the function of the frog heart with both phosphates. I myself have also performed a fewexperiments with phosphate on mammal organs, which turned out negative. The number of experiments was, however, too small to allow any conclusions to be drawn. However, here it must especially be pointed out that our intestine experiments were all performed in tyrode solution, which already has a phosphate content of 0.05 g to 1 liter, thus 1:20,000, and that herein phosphite was found to be active in a concentration of 1;10 million. Thus, we cannot assume any secondary action of phosphate forming from phosphite.

IV. On the Toxicity of the O-compounds of P.

After we had determined that phosphite has a considerable effect on surviving organs, it seemed necessary to us to perform another test on its effect on the whole animal, in comparison to the effect of hypophosphite or P itself. Here it was shown that, in contrast to the results of H. Schulz and Neumann, phosphite administered subcutaneously is undoubtedly toxic. A precise examination according to the method of Marsh showed that the preparation was completely free of As.

Shortened report.

Two temporaries, A male, 23.2 g weight; B female, 19.95 g weight.

Both received 0.5 ccm sodium phosphate 1:100 in the breast lymph sack on March 7, 1923, at 11:00, 1 ccm at 3:45, another 1 ccm at 7:10, no significant effect. On July 4, 1 ccm was injected at 10:45, 0.15 ccm at 11:30. At 12:00, A manifests fibrillary convulsions in the stomach and legs; B manifests cramp-like contractions. Both animals move with difficulty. A receives another 1 ccm. At 1:15, both animals manifest fibrillary muscle twitches and extended cramps with each attempt at movement. On July 5, at 4:35, the spasms have stopped for both animals. Movements creeping. At 8:45, the animals are lying on their backs. On July 6 A dies, B has recovered completely. A received a total of 5 ccm, B 4 ccm of the 1:100 solution; thus they received 5 or 4 cg. Autopsy reveals a half-filled heart stopped in diastole for A. The liver is of rough consistency, gray in color, and is poor in blood. Kidneys very pale. A serous bloody liquid in the abdomen. In other cases, the heart was found stopped in systole, and the liver and kidneys were rather more hyperemic in the face of acute intoxication.

In general, 0.1 g phosphite were enough to kill frogs within a few hours. Winter animals are somewhat more resistant, but also perish after 1-2 days. Mice perish within a few hours after 0.15 - 0.2 g injected subcutaneously. 0.2-0.5 g were enough in the case of

guinea pigs. However, large animals are often quite resistant. 0.5 g must be administered for several days to cause death. In one case, I gave a strong guinea ;ig 2 ccm sodium phosphite 1:10 every 2-3 days for 4 successive weeks, thus 0.2 g each time, without being able to determine any change in its condition. The animal did not die until the fifth week, after a single dose of 0.2 g. The result of the histological examination are given below.

In another experiment, a guinea pig received a total of 0.8 g phosphite subcutaneously between the 17th and 19th of October, 1923. On the 20th of October, 0.2 ccm were injected once; shortely after this, the animal became seriously ill. It became paretic, showed tears, diarrhea and polyuria, shivered and cried when touched. On the next day it was very ill and perished toward noon.

The histological examination of the organs, for which we thank the Institute, revealed nothing unusual in the case of either the frog that died rapidly or the one that perished gradually over a few days. Above all, there were no phenomena of fat accumulation in the liver and kidneys. Likewise, in the case of the guinea pig that died within 4 days as described in the above experiment, therewas nothing significiant. On the other hand, the histological examination of the above-mentioned animal treated for 4 weeks with phosphite revealed the following finding:

Lungs: single bleedings in the alveoli. Kidneys: intense blood filling of the glomeruli, single blood phenomena in the kidney cortex. Liver: moderate but undeniable fat accumulation in the liver cells and considerable hyperemia.

The effect of aqueous P-solutions is found to be exactly like the effect of phosphite, only more intense and more rapid. Frogs perish within a few hours after injection of 1 mg P in an alcohol solution. When a solution of 1:10000, produced with water or Ringer solution from the alcohol original 1:1000 solution, is used, the same picture is found. The phenomena are ~~crippling~~ paralysis that begin with atactic movements and end with complete immobility. Fibrillary spasms are rare, but on the other hand, spasms of entire muscle masses were observed several times. The animals injected with the corresponding amounts of alcohol for control purposes always recovered fully.

Shortened report of an experiment on a white mouse.

6/28/1923. Injection of 0.5 ccm P-solution 1:10,000 twice. At first no clear effect. On June 29, in the morning, the animal receives 0.25 ccm of an alcohol P-solution, 1:1000. Within 45 minutes, heavy phenomena of intoxication: ~~crippling~~ paralysis, difficulty in breathing, then cessation of breathing and death within 1 hour after injection. Autopsy revealed no histological changes in the organs.

Hypophosphite is found to be considerably less toxic than phosphite and P-solutions. Frogs can tolerate even repeated injections of 1:10 solutions, for several days. Death can rarely be cause with 1:5 solution

Guinea pigs seemed to be fully resistant, even to large doses. For unknown reasons, only mice are rather sensitive. After only 2 ccm of a hypophosphite solution 1:10, they always perished, with phenomena entirely similar to those manifested after phosphite intoxication.

For purposes of comparison, we also poisoned a number of frogs with sodium phosphate. The picture is characterized above all by the long-lasting fibrillary spasms in all muscles. Significant for the repeatedly raised question as to whether phosphite acts only after oxidation to phosphate, is the fact found by Starkenstein, that phosphate intoxication can be annulled or prevented by $CaCl_2$. On the other hand, we were not able to affect the fatal effect of phosphite with $CaCl_2$. Only the fibrillary spasms, which in the case of phosphite are far more sparse and possibly also due to Ca removal, disappear; death occurs nevertheless. Thus, for this reason too, phosphite must be attributed with a special effect, different from that of phosphate. The effects that Starkenstein found with phosphate on isolated cold and warm-blooded hearts, as well as on the rabbit small intestine, could also be annulled with $CaCl_2$. Beyond this, he used N/5 solutions of phosphate, corresponding to a concentration of about 1:50, a dose that can hardly be compared with the small phosphite amounts that we found to be effective.

V. On the Theory of Phosphorus Action

We have shown that phosphite is a very active substance. The question is then raised, as to how suited this finding is to contributing to the clarification of phosphorus effects. In this respect, the results of the histological examinations are significant. As we were able to show, even in the case of protracted phosphorus intoxication, none of those striking degenerative organ alterations are found, that are so characteristic of acute, and above all chronic P-intoxication. On the other hand, it is known that in the case of intoxications with very high doses of P, and very acute poisoning, no organ alterations can be determined. We therefore consider it probable that in these cases, the overwhelming with phosphite is the cause of death, since we determined that high phosphite doses act in a heavily damaging manner on the heart especially; the damage to the heart is also observed as the cause of death in hyperacute cases of P-poisoning. Thus H. Meyer[1] was able to cause heart stoppage with P at a time when no degenerative organ alterations appeared.

The case is different for chronic intoxication. Here, as in the not very acute cases, the chief role is undoubtedly played by the reducing effect of P in those places whereit is stored. By means of a long-lasting removal of oxygen, which must occur during the constant and slow oxidation of P in those places, the characteristic degenerative phenomena, especially fatty degeneration, are caused; they are generaly considered to be a consequence of oxygen loss.

1) H. Meyer, Arch. f. exp. Path. u. Pharm. Bd. 14, S. 313, zitiert auch bei v. Maschka, Wien. Med. Wochenschr. 1884, Nr. 21, S. 648, der ebenfalls Herzlähmung bei sehr schnell und ohne nachweisbare anatomische Veränderungen verlaufenden P-Vergiftungen annimmt.

As concerns, finally, the therapeutic effect of small doses of phosphorus, we believe that the phosphite effect plays a significant role. The function-stimulating effects of minimal phosphite doses, as we have determined them in our experiments, correspond to the therapeutic indication of P-medication, and in this respect it seems especially important that Wegner obtained favorable effects of bone growth with both phosphite and phosphorus.

These observations must necessarily lead to the conclusion that in the case of the therapeutic use of P, only smallest doses should be used, and that they are in fact sufficient to achieve the desired effect. The use of larger doses, which is justifiably feared because of its danger, not only must, but can be avoided, without affecting the therapeutic action.

Summary

1. The mechanism of action of P in the animal organism is discussed, as well as theories concerning this.
2. Short survey of the test results with tonophosphane on surviving frog and mammal organs. Tonophosphane stimulates the activity of poorly beating frog and mammal hearts. There is also an increase in operation in the case of isolated, surviving, intestines, bladders and uteruses of mammals.
3. The assumption that tonophosphane has a phosphite effect is confirmed by repeating the same series of experiments with sodium phosphite. Sodium phosphite in strong dilutions can act the same way as tonophosphane, but there is a difference, in that tonophosphane does no damage even in a concentration of 1:100, while phosphite in a concentration of 1:1000 stops the movements of surviving organs.
4. Aqueous solutions of P (produced from an alcohol original solution of 1:1000) act like phosphite, only even stronger dilutions must be used, as concentrations of 1:1 million at least, and even 1:100,000 cause heavy damages to the organs.
5. No favorable effect could be observed on any organ with hypophosphite. Damages to a frog heart could be produced only by using a concentration of 1:100.
6. It is shown that phosphite and phosphate effect are not identical.
7. A testing of the toxicity of phosphite on frogs, white mice and guinea pigs reveals that upon subcutaneous injection of 1-3 ccm of a 1:10 solution to frogs and mice, 1-5 ccm to guinea pigs, the animals die within a few hours. Fatal intoxication with hypophosphite could be observed only in frogs after largest doses, and in mice.
8. On the basis of the facts found, the experiment is considered an explanation of the action of P in the organism.

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17072. Value of Calcium Hypophosphite and Other Calcium Compounds as Calcium Supplements in Calcium-Low Diets.

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The value of hypophosphites as therapeutic agents has never been objectively demonstrated and Marriott's criticism of their use[1] has largely been accepted. However, calcium hypophosphite is well worth while being considered as a means of convenient calcium administration, where such therapy is desired, because it is high in this element (23%), does not have the objectionable taste of most calcium salts and, in contrast to gluconate, is adequately soluble. The hypophosphite ion, as a stable acid, balances the alkalinity of the calcium, which effect persists even if there should occur a transformation into phosphoric acid. Therefore, the salt does not shift the acid-base equilibrium to the alkaline side as is the case with salts of carbonate producing acids. The original purpose of the present investigation was a comparison of calcium hypophosphite with dicalcium phosphate and calcium gluconate as a supplement in a cal-

[1] Marriot, McKim, *J. Am. Med. Assn.*, 1916, 76, 486.

cium-poor diet. According to Marriot's report the hypophosphite anion is largely excreted unchanged; for that reason, the hypophosphite P was regarded as non-available in our first tests. When the early observations suggested that this might not be the case, a study of the influence of readily available P and hypophosphite P at varying Ca/P ratios was included.

Experimental. Groups of 7 to 8 Sprague-Dawley rats were placed on a diet prepared according to Sobel, Rockenmacher and Kramer,[2] which consists of 70 parts yellow corn meal, 16 wheat gluten, 10 brewer's yeast, and 1 NaCl with the addition of cod liver oil to supply 0.1 unit vitamin D per gram. Analysis showed a content of 0.03% Ca and 0.33% P, which is in accord with the figures given by these authors. All animals were kept for 14 days on this diet as a depletion period, and the control group for an additional 25 days. Additions of calcium hypophosphite, gluconate and secondary phosphate were used in the first comparative tests (A to E in table). Nucleic acid was used in some groups to equalize the content in available phosphorus without adding a phosphate. The additions in groups F to O served to vary the Ca/P ratios as widely as the P content in the basic diet would permit, with proper distinction of P, known to be utilized, and hypophosphite P.

The rats at the beginning of the depletion period were of an age specified for vitamin D assay in the U.S.P. It turned out that the range of permissible variations in weight and age, as set for that test, was too wide than was best suited for these experiments. The rats of the last group were apparently a few days older (Nos. A_3 to O), and consequently had larger calcium reserves. For that reason, each group can be compared only with its own control group. The animals were weighed at the beginning and end of the 25 days' test period, then killed and the femora dissected. The bones, dried at 80°, were freed of all adhering tissue, weighed, incinerated and the ash determined.

Results. It is evident that the assimilation of calcium is about the same whether supplied as hypophosphite, gluconate, phosphate or carbonate. While 130 mg Ca per 100 g diet is inadequate for maximal calcification of the bones, 530 mg gave results equal to 730 and 1030 mg as shown in Groups I to O. The final body weight at the end of the test period was within the same range in all groups disregarding the calcium intake, with exception of group A_1. While it is possible that the smaller animals were more sensitive to Ca deficiency, it must be pointed out that group B, with equally low Ca feeding, showed only a slight depression in weight. The weight and ash content of the femora reflect more definitely any calcium deficiency than the body weight.

The experiments on Ca/P ratio were limited by the 330 mg P content in the basic diet, which was found to be high enough to prevent any acute P deficiency. As a consequence the results appear to be exclusively a function of the Ca supply. A ratio of Ca/P of 1:2.54 (Group F) was positively not detrimental to bone formation, and neither was one of 1:4.9, (Group H) assuming that hypophosphite P is utilized. This, however, we were unable to prove because of the impossibility to produce a P deficiency with this diet and of the latitude in tolerated Ca/P ratios.

Shohl[3] has claimed that a low Ca high P ratio lowers the ash content in the bones of the rats. He used, indeed, a ratio of 1:16, but only by sacrificing the absolute Ca content in the diet. When 0.12% Ca was given with 2% P the ash was 36%, but with 0.12% Ca and 0.5% P the ash was the same. This is in keeping with our conclusion that the quantity of Ca and not the Ca/P ratio is the limiting factor. Since, according to Shohl's statement, excessive quantities of phosphorus are toxic, it is difficult to prove a detrimental effect of a low Ca/P ratio at an adequate Ca intake.

Some of the ulnae and radii of the A_1 to E groups were examined by the Ag line test as used in vitamin D assay, but no differences were detected.

It seems that bone formation is not the most

[2] Sobel, A. E., Rockenmacher, M., and Kramer, B., *J. Biol. Chem.*, 1945, **158**, 475.

[3] Shohl, Alfred, *J. Nutr.*, 1936, **11**, 275.

TABLE I.

	Addition to basic diet	Mg Ca per 100 g	Mg P per 100 g	Ratio, Ca/P	Wt. at beginning of test period	Wt. at end of test period	Femora wt., mg	Ratio femora mg to wt. g	% ash in femora
A_1		30	330	1:11	68.9 ±7.6	9[illegible].9 ±10.8	301.5 ±22.5	3.13 ±0.51	31.24 ±1.71
B	Nucleic acid	30	330 77	1:13.6	72.5 ±10.1	110 ±14.[illegible]	320.5 ±16.4	2.91 ±0.30	31.45 ±0.68
C	$CaHPO_4$ $2H_2O$	30 100	330 77	1:3.13	60.7 ±2.2	122.8 ±8.8	352.7 ±14.6	2.87 ±0.13	41.73 ±1.74
D	$Ca(H_2PO_2)_2$ Nucleic acid	30 100	330 77 (155)	1:3.13 (1:4.3)	64.9 ±3.9	134.[illegible] ±[illegible].4	395.6 ±18.8	2.93 ±0.15	40.76 ±1.08
E	Ca gluconate Nucleic acid	30 100	330 77	1:3.13	61.1 ±3.8	115.9 ±9.0	378.7 ±23.0	3.28 ±0.18	41.86 ±1.53
A_2		30	330	1:11	83.9 ±7.8	131.7 ±7.9	268.4 ±16.5	2.04 ±0.08	30.70 ±0.40
F	$Ca(H_2PO_2)_2$	30 100	330 (155)	1:2.54 (1:3.73)	83.2 ±9.0	135.1 ±13.8	341.5 ±27.5	2.54 ±0.17	39.22 ±1.27
G	Ca gluconate	30 100	330	1:2.54	88.4 ±10.8	135.9 ±9.8	329.3 ±32.9	2.42 ±0.19	39.96 ±1.01
H	$Ca(H_2PO_2)_2$ $Na(H_2PO_2)$ H_2O	30 100	330 (155) (155)	1:2.54 (1:4.9)	83.5 ±3.1	131.4 ±9.2	324.6 ±17.2	2.48 ±0.12	40.49 ±1.29
A_3		30	330	1:11	95.2 ±7.7	129.3 ±10.7	231.2 ±39.7	2.56 ±0.23	36.9 ±1.2
I	$Ca(H_2PO_2)_2$	30 1000	330 (1550)	1:0.32 (1:1.8)	96.0 ±8.4	121.2 ±10.7	472.6 ±45.1	3.93 ±0.12	52.5 ±1.2
K	Ca gluconate	30 1000	330	1:0.32	97.2 ±5.0	128.8 ±19.1	466.9 ±59.4	3.65 ±0.29	51.6 ±2.0
L	Nucleic acid $Ca(H_2PO_2)_2$	30 1000	330 300 (1550)	1:0.61 (1:2.0)	96.7 ±9.4	125.2 ±9.3	476.6 ±24.6	3.82 ±0.24	53.0 ±0.8
M	Nucleic acid $Ca(H_2PO_2)_2$	30 700	330 300 (1085)	1:0.9 (1:2.4)	96.6 ±2.8	125.3 ±11.3	458.9 ±35.7	3.66 ±0.18	52.4 ±1.1
N	$Ca(H_2PO_2)_2$	30 500	330 (775)	1:0.62 (1:2.09)	101.4 ±7.6	140.3 ±13.6	504.2 ±44.9	3.60 ±0.24	53.2 ±1.6
O	$CaCO_3$	30 1000	330	1:0.32	90.8 ±6.4	132.8 ±8.2	462.1 ±61.2	3.48 ±0.10	53.4 ±2.2

In the columns of Ca and P values, the first figure represents the quantities contained in the basic diet, the following figures are added Ca and P. The parentheses signify hypophosphite P, or in the Ca/P ratio, the inclusion of hypophosphite P in the total P value. The second figures in the data of results are standard deviations $S = \pm \sqrt{\frac{\Sigma(d^2)}{n-1}}$.

sensitive indicator of a deficiency or unbalance of Ca and P. Most of the control rats developed a considerable degree of baldness. This was still more accentuated in Group B, which points to a further damage caused by the change in the Ca/P ratio from 1:11 to 1:13.6. It also appeared markedly in Group K, receiving calcium gluconate without additional P, but was absent in E, where the gluconate was balanced with P. None of the other groups showed loss of hair. By comparing the Ca/P ratio in Group K (1:0.32) with those of the other groups showing baldness (Ca/P, 1:11;1:13.6) it may be concluded that the disproportion in Ca/P ratio to either extreme has a similar effect. The non-appearance of baldness in Group I may point to some assimilation of hypophosphite P, since otherwise this group would have the same Ca/P ratio as K. More data will be required to make a positive statement on this point.

Summary. Calcium hypophosphite is well suited to serve as a supplement for dietary calcium. To what extent the hypophosphite phosphorus is utilized by the organism remains uncertain. The ratio Ca/P in the diet may be varied within a wide range without affecting the calcification of the bones, provided the absolute quantities of each constituent are adequate. Extreme disproportion of Ca and P in either direction seems to cause loss of hair, as was observed in rats receiving either very low or very high relative levels of calcium.

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TOXICOLOGY. COMPARATIVE GENERAL TOXICITY OF THE MINERAL ANIONS IN THE MOUSE.

by

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transmitted by

Henri Hermann

The acute toxicities of 32 mineral anions were determined, after IP injection in the mouse of the LD50 on the 30th day. There exists for the elements of groups 5b, 6b and 7b of the periodic classification a relation of the values of the toxicity depending upon the degree of observation.

It was recently shown (1), following a study on the acute general toxicity of 42 cations, in the mouse, that there exists depending on the atomic number a periodicity of the values of toxicity similar to that of the Mendeleeff classification. The toxicity of the anions has until now been the subject of only studies of limited scope, in general carried out on lower beings (2,3), without any well defined relation being established. This is what encouraged us to undertake this work with the goal of determining if, in the mouse, the toxicity of the anions could obey a relation comparable or not to that previously found with cations.

We found on the mouse (Swiss, albinos, male, from 20 to 25g), this study required the use of about 3,000 animals. The toxicity criterion chosen was the LD50 in the 30th day (LD50/30) established by the graphic statistical method (4) on logarithm-probit paper (5) expressed with the typical variations (6) in milliatoms-grams of element per body kilogram.

The salts used are all of commercial origin (pure reagents); it is a question of sodium salt whose aqueous solutions were prepared extemporaneously in concentrations calculated so as to inject IP in all cases an identical value (0.40 ml per mouse of 20 g).

Two particular cases must be mentioned: sodium selenide, a very autoxydizable body, was dissolved in distilled water previously deoxydized by vacuum and by a nitrogen steam, and the solutions were

preserved in sealed flasks under inert atmosphere (nitrogen); because of the weak solubility of sodium perborate, a double volume of solution, 0.80 ml per mouse of 20 g was injected.

The results obtained, ~~LD~~50/30 expressed in milligrams of salt per kilogram of mouse, are included in Table 1.

It nonetheless seemed to us interesting to evaluate the ~~LD~~50 in milliatoms-grams (mat-g) of the basic element taking into account its degree of oxidation and to establish the relative toxicities in relation to that of the chloride ion taken as a unit. These values are shown by following the order of the increasing toxicities in Table 2.

We were not able to find with the anions, as for mutations, a periodicity of the toxicity in relation to the atomic number. However, the graphic interpretation of the results (Figure 1 : semi-logarithmic scale)makes it possible to observe that there exists with the elements of groups 5b, 6b and 7b of the periodic classification a relation in the increase of intoxicity in relation to the degree of oxidation.

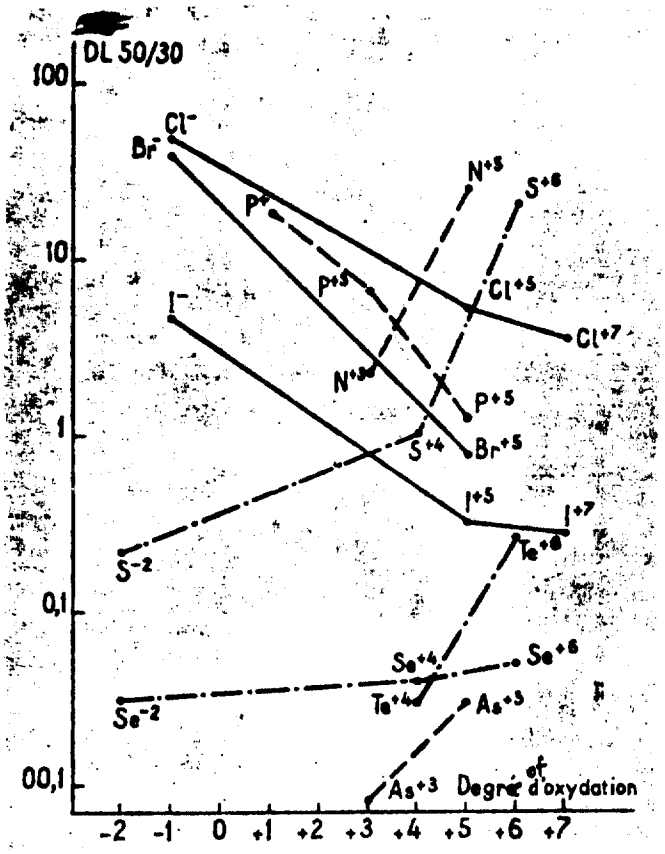
The toxicity of elements in group 7b, that is the halogenes, is an increasing function of the degree of observation; by contrast, the toxicity of the elements of the oxygen and nitrogen family is a decreasing function, with the exception of the phosphorous compounds whose toxic affect follows a evolution to that of the halogene derivatives.

a Sel utilisé.	b $DL_{50}/30$ (mg de sel/kg de souris).	Sel utilisé.	$DL_{50}/30$ (mg de sel/kg de souris).
NaF	49,00	Na_2SeO_4, $10H_2O$	18,45
$NaCl$	2603,25	Na_2TeO_3, $5H_2O$	7,48
$NaClO_2$	596,12	Na_2TeO_4, $2H_2O$	73,87
$NaClO_4$	550,80	$NaNO_2$	158,70
$NaBr$, $2H_2O$	5488,13	$NaNO_3$	2975,00
$NaBrO_3$	140,35	NaH_2PO_2	1584,00
NaI	869,42	Na_2HPO_3, $5H_2O$	2052,00
$NaIO_3$, $5H_2O$	86,37	Na_2HPO_4, $12H_2O$	429,80
$NaIO_4$	57,75	Na_2AsO_2	1,17
$NaOH$	40,00	Na_3AsO_4, $7H_2O$	9,33
Na_2S, $9H_2O$	52,84	$NaCN$	4,90
Na_2SO_3, $7H_2O$	277,20	Na_2CO_3	116,60
Na_2SO_4	[illegible]7,00	$Na_2B_4O_7$, $7H_2O$	450,08
$NaHSO_4$, H_2O	190,32	$NaBO_3$, $4H_2O$	538,37
$Na_2S_2O_3$	226,22	Na_2CrO_4	32,40
Na_2Se	3,99	Na_2MoO_4, $2H_2O$	133,07
Na_2SeO_3, $5H_2O$	9,21		

Table 1. Key: a) Salt used, b) ~~LD~~DL50/30 (mg of salt/kg of mouse).

a Élément de base.			
b Degré d'oxydation.	c Forme anionique.	d $DL_{50}/30$ (mat-g d'élément de base).	e Toxicité relative.
Cl^{-}	Cl^{-}	44,500 ± 0,72	1
Br^{-}	Br^{-}	39,500 ± 0,50	1,12
N^{+5}	NO_3^{-}	35,000 ± 0,80	1,27
S^{+6}	SO_4^{--}	23,500 ± 0,50	1,89
P^{+}	$H_2PO_2^{-}$	18,000 ± 0,80	2,47
P^{+3}	HPO_3^{--}	9,500 ± 1,28	4,68
I^{-}	I^{-}	5,800 ± 0,66	7,67
Cl^{+5}	ClO_3^{-}	5,600 ± 0,60	7,95
B^{+3}	$B_4O_7^{--}$	4,720 ± 0,26	9,43
Cl^{+7}	ClO_4^{-}	4,500 ± 0,48	9,89
B^{+3}	BO_3^{-}	3,500 ± 0,34	12,71
N^{+3}	NO_2^{-}	2,300 ± 0,18	19,36
S^{+7}	$S_2O_8^{--}$	1,900 ± 0,10	23,42
S^{+6}	HSO_4^{-}	1,400 ± 0,05	31,80
P^{+5}	HPO_4^{-}	1,200 ± 0,10	37,10
S^{+4}	SO_3^{--}	1,100 ± [illegible]	40,47
C^{+4}	CO_3^{--}	1,100 ± [illegible]	40,47
F^{-}	F^{-}	1,000 ± [illegible]	44,50
O^{-2}	OH^{-}	1,000 ± [illegible]	44,50
Br^{+5}	BrO_3^{-}	0,930 ± [illegible]	47,87
Mo^{+6}	MoO_4^{--}	0,550 ± [illegible]	80,91
I^{+5}	IO_3^{-}	0,300 ± 0,03	148,40
I^{+7}	IO_4^{-}	0,270 ± 0,02	164,88
Te^{+6}	TeO_4^{--}	0,270 ± 0,03	164,88
S^{-2}	S^{--}	0,220 ± 0,02	202,36
Cr^{+6}	CrO_4^{--}	0,200 ± 0,04	222,50
C^{-4}	CN^{-}	0,100 ± 0,02	445,20
Se^{+6}	SeO_4^{--}	0,050 ± 0,01	890,40
Se^{+4}	SeO_3^{--}	0,035 ± 0,01	1 272,00
Se^{-2}	Se^{--}	0,032 ± 0,005	1 391,25
As^{+5}	AsO_4^{---}	0,030 ± 0,005	1 484,00
Te^{+4}	TeO_3^{-}	0,024 ± 0,002	1 855,00
As^{+3}	AsO_2^{-}	0,009 ± 0,0002	4 946,66

Table 2. Key: a) basic element, b) degree of observation, c) anion form, d) LD50/30 (mat-g of basic element) and e) relative toxicity.


DL 50/30
100
10
1
0,1
00,1
Cl⁻
Br⁻
I⁻
P⁺
N⁺⁵
S⁺⁶
P⁺⁵
Cl⁺⁵
Cl⁺⁷
N⁺³
P⁺⁵
S⁺⁴
Br⁺⁵
I⁺⁵
I⁺⁷
Te⁺⁶
S⁻²
Se⁺⁴
Se⁺⁶
Te⁺⁴
As⁺⁵
Se⁻²
As⁺³
Degré d'oxydation
-2 -1 0 +1 +2 +3 +4 +5 +6 +7

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(*) Séance du 24 juin 1963.
(1) P. Bienvenu, C. Nofre et A. Cier, *Comptes rendus*, 256, 1963, p. 1043.
(2) A. P. Mathews, *Amer. J. Physiol.*, 10, 1903, p. 290.
(3) J. R. E. Jones, *J. Exp. Biol.*, 18, 1941, p. 170.
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(5) P. Bonet-Maury, *C. R. Soc. Biol.*, 137, 1943, p. 400.
(6) J. T. Lichtfield et J. W. Fertig, *Bull. John Hopkins Hosp.*, 68, 1941, p. 276.

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Nofre-'63
Bib No. 30

Compt. Rend. 257(3): 791-794. 1963.

TOXICOLOGIE. — *Toxicité générale comparée des anions minéraux chez la Souris.* ~~Note (*) de MM.~~ CLAUDE NOFRE, HENRI DUFOUR et ANDRÉ CIER, ~~transmise par M.~~ Henri Hermann.

Les toxicités aiguës de 32 anions minéraux ont été déterminées, après injection intrapéritonéale à la Souris, par la DL_{50} au 30e jour. Il existe pour les éléments des groupes V*b*, VI *b* et VII *b* de la classification périodique une correspondance des valeurs de la toxicité en fonction du degré d'oxydation.

Il a été montré récemment [1], à la suite d'une étude sur la toxicité générale aiguë de 42 cations, chez la Souris, qu'il existe en fonction du numéro atomique une périodicité des valeurs de toxicité analogue à celle de la classification de Mendéléev. La toxicité des anions n'a fait l'objet jusqu'ici que d'études de portée limitée, effectuées en général sur des êtres inférieurs [2], [3], sans qu'il ait pu être établi de relation bien définie. C'est ce qui nous a incités à entreprendre ce travail dans le but de rechercher si, chez la Souris, la toxicité des anions pouvait obéir à une relation comparable ou non à celle précédemment trouvée avec les cations.

Réalisée sur la Souris (Swiss, albinos, mâle, de 20 à 25 g), cette étude a nécessité l'emploi de 3 000 animaux environ. Le critère de toxicité choisi a été la DL_{50} au 30e jour ($DL_{50}/30$) établie par la méthode statistique graphique [4] sur papier logarithme-probit [5] exprimée avec les écarts types [6] en milliatomes-grammes d'élément par kilogramme corporel.

TABLEAU I.

Sel utilisé.	$DL_{50}/30$ (mg de sel/kg de souris).	Sel utilisé.	$DL_{50}/30$ (mg de sel/kg de souris).
NaF	49,00	Na_2SeO_4, $10H_2O$	18,45
NaCl	2603,25	Na_2TeO_3, $5H_2O$	7,48
$NaClO_2$	596,12	Na_2TeO_4, $2H_2O$	73,87
$NaClO_4$	550,80	$NaNO_2$	158,70
NaBr, $2H_2O$	5488,13	$NaNO_3$	2975,00
$NaBrO_3$	140,35	NaH_2PO_2	1584,00
NaI	869,42	Na_2HPO_3, $5H_2O$	2052,00
$NaIO_3$, $5H_2O$	86,37	Na_2HPO_4, $12H_2O$	499,80
NaIO	57,75	Na_2AsO_2	1,17
NaOH	40,00	Na_3AsO_4, $7H_2O$	9,33
Na_2S, $9H_2O$	52,84	NaCN	4,90
Na_2SO_3, $7H_2O$	277,20	Na_2CO_3	116,60
Na_2SO_4	3337,00	$Na_2B_4O_7$, $7H_2O$	450,08
$NaHSO_4$, H_2O	193,32	$NaBO_2$, $4H_2O$	538,37
$Na_2S_2O_8$	226,22	Na_2CrO_4	37,40
Na_2Se	3,99	Na_2MoO_4, $2H_2O$	133,07
Na_2SeO_3, $5H_2O$	9,21		

Les sels utilisés sont tous d'origine commerciale (réactifs purs); il s'agit de sels de sodium dont les solutions aqueuses ont été préparées extemporanément à des concentrations calculées de façon à injecter dans tous les cas, par voie intrapéritonéale, un volume identique (0,40 ml par souris de 20 g).

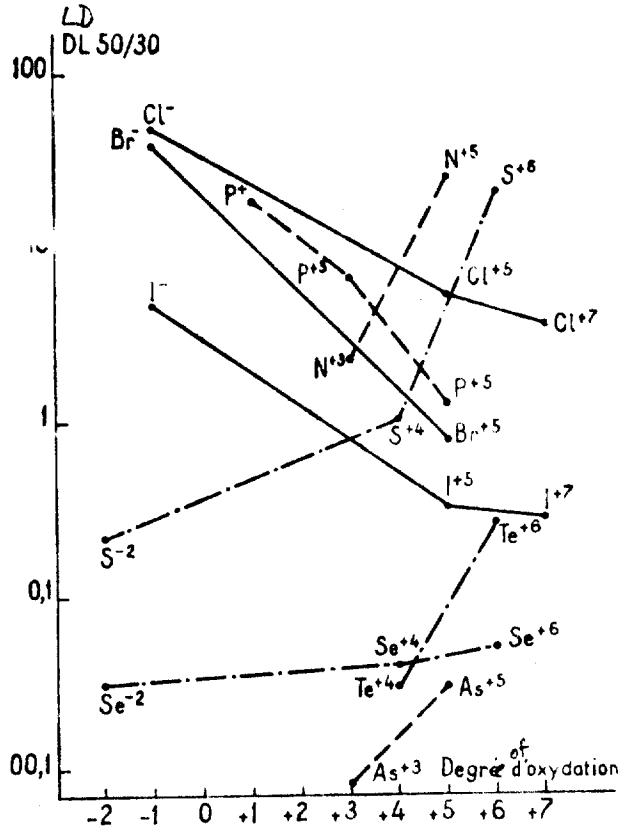
TABLEAU II.

a Élément de base.			
b Degré d'oxydation.	c Forme anionique.	d $DL_{50}/30$ (mat-g d'élément de base).	e Toxicité relative.
Cl^{-1}	Cl^-	44,500 ± 0,72	1
Br^{-1}	Br^-	39,500 ± 0,50	1,12
N^{+5}	NO_3^-	35,000 ± 0,80	1,27
S^{+6}	SO_4^{--}	23,500 ± 0,50	1,89
P^{+1}	$H_2PO_2^-$	18,000 ± 0,80	2,47
P^{+3}	HPO_3^{--}	9,500 ± 1,28	4,68
I^{-1}	I^-	5,800 ± 0,66	7,67
Cl^{+5}	ClO_3^-	5,600 ± 0,60	7,95
B^{+3}	$B_4O_7^{--}$	4,720 ± 0,26	9,43
Cl^{+7}	ClO_4^-	4,500 ± 0,48	9,89
B^{+3}	BO_2^-	3,500 ± 0,34	12,71
N^{+3}	NO_2^-	2,300 ± 0,18	19,36
S^{+7}	$S_2O_8^{--}$	1,900 ± 0,10	23,42
S^{+6}	HSO_4^-	1,400 ± 0,05	31,80
P^{+5}	HPO_4^{--}	1,200 ± 0,10	37,10
S^{+4}	SO_3^{--}	1,100 ± 0,11	40,47
C^{+4}	CO_3^{--}	1,100 ± 0,04	40,47
F^{-1}	F^-	1,000 ± 0,15	44,50
O^{-2}	OH^-	1,000 ± 0,05	44,50
Br^{+5}	BrO_3^-	0,930 ± 0,06	47,87
Mo^{+6}	MoO_4^{--}	0,550 ± 0,04	80,91
I^{+5}	IO^-	0,300 ± 0,03	148,40
I^{+7}	IO_4^-	0,270 ± 0,02	164,88
Te^{+6}	TeO_4^{--}	0,270 ± 0,03	164,88
S^{-2}	S^{--}	0,220 ± 0,05	202,36
Cr^{+6}	CrO_4^{--}	0,200 ± 0,04	222,50
C^{-4}	CN^-	0,100 ± 0,02	445,20
Se^{+7}	SeO_4^{--}	0,050 ± 0,01	890,40
Se^{+4}	SeO_3^{--}	0,035 ± 0,01	1 272,00
Se^{-2}	Se^{--}	0,032 ± 0,005	1 391,25
As^{+5}	AsO_4^{---}	0,030 ± 0,005	1 484,00
Te^{-2}	TeO_3^{--}	0,024 ± 0,002	1 855,00
As^{+3}	AsO_3^{---}	0,009 ± 0,0002	4 946,66

Deux cas particuliers doivent être signalés : le séléniure de sodium, corps très autoxydable, a été dissous dans de l'eau distillée préalablement désoxygénée par le vide et par un courant d'azote, et les solutions ont été conservées en ampoules scellées sous atmosphère inerte (azote); par suite de la faible solubilité du perborate de sodium, il a été injecté un volume double de solution, soit 0,80 ml par souris de 20 g.

Les résultats obtenus, $DL_{50}/30$ exprimés en milligrammes de sel par kilogramme de souris, sont réunis dans le tableau I.

Il nous a paru toutefois intéressant d'évaluer les DL_{50} en milliatomes-grammes (mat-g) de l'élément de base compte tenu de son degré d'oxydation et d'établir les toxicités relatives par rapport à celle de l'ion chlorure prise comme unité. Ces valeurs sont portées en suivant l'ordre des toxicités croissantes dans le tableau II.



Nous n'avons pas pu retrouver avec les anions, comme pour les cations, une périodicité de la toxicité en fonction du numéro atomique. Cependant, l'interprétation graphique des résultats (*fig.* 1 : échelle semi-logarithmique) permet d'observer qu'il existe avec les éléments des groupes V*b*, VI*b* et VII*b* de la classification périodique une correspondance dans l'accroissement de la toxicité en fonction du degré d'oxydation.

La toxicité des éléments du groupe VII*b*, c'est-à-dire des halogènes, est une fonction croissante du degré d'oxydation; par contre, la toxicité des éléments de la famille de l'oxygène et de l'azote est une fonction décroissante, exception faite des composés du phosphore dont l'effet toxique suit une évolution parallèle à celle des dérivés halogénés.

(*) Séance d[illegible] juin 1963.

(1) P. [illegible]ENVENU, G. NOFRE et A. CIER, *Comptes rendus*, 256, 1963, p. 1043.
(2) A. P. MATHEWS, *Amer. J. Physiol.*, 10, 1903, p. 290.
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(5) P. BONET-MAURY, *C. R. Soc. Biol.*, 137, 1943, p. 400.
(6) J. T. LICHTFIELD et J. W. FERTIG, *Bull. John Hopkins Hosp.*, 69, 1941, p. 276.

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Z. Untersuch. Nahr. U. Genussm. 5:11. 1902.

[illegible] of the Calcium Salt of Hypophosphorous Acid in the Animal Organism

by

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[illegible] from the university laboratory for medical chemistry in Vienna.

When we are faced with finding chemical evidence of a phosphorus poisoning and we have not been able to discover elemental phosphorous in our tests then, as we know, a search for phosphorous acid is indicated. For this purpose we have, as a rule, used the Dusart-Blondlot test[1]. Dusart had earlier demonstrated, however, that the application of this method revealed the same findings for the hypophosphorous acids as it did for the phosphorous acid. Since the salts of hypophosphorous acid are used in therapeutic practice we should not -- without further testing -- accept the conclusion that phosphorous acid is present when the Dusart-Blondlot method yields a positive finding in a given case suspected phosphorous poisoning. Rather, we must must always keep in mind the possibility that the afflicted individual had ingested a hypophosphite. It seemed worthwhile, therefore, to obtain some particulars about how the salts of hypophosphorous acid behave in the animal organism.

First Blondlot (2) and later Paquelin and Jolly (3) found that hypophosphite fed to an animal appears again intact in the urine. It occured to me to question particularly whether and for how long these salts remain in the animal's body after they are ingested.

[1] Op. cit. 43, 1126.
[2] Op. cit. 52, 1197.
[3] Op. cit. 36, 1505.

I especially wanted to learn whether the salts can be discovered in the urine of the animal. To this end I conducted several studies with dogs, in which the animals ingested the calcium salt of hypophosphorous acid.

A male dog weighing 10.1 kg received at 11 AM on 17 April one gram of calcium hypophosphite by mouth. The urine excreted in the course of the following 6 hours (about 100 ccm) was collected, placed in a hydrogen generating tank in which diluted sulfuric acid acted on zinc, and the escaping gas was passed through a solution of silver nitrate. The dark brown residue formed in the solution was washed by decanting with water and tested in a very small hydrogen generating tank which was so adapted that the escaping gas was passed rapidly over a piece of pumice stone impregnated with lye; the gas was then released by way of a porcelain test tube, where it was ignited. It burned with a bluish, green flame, indicating the presence of a hydrogen phosphorous compound, in this case hypophosphorous acid. All reagents used in the study were, of course, tested for their purity beforehand.

The urine (about 100 ccm) collected up to the next morning (9 AM, 18 April) was subjected to the same procedure. It also yielded a bluish, green-colored flame. Urine collected subsequent to this time, i.e. up to the morning of 19 April did not show a similar reaction. Nor did urine collected during the two days that followed.

Thus the excretion of hypophosphites in the urine was ended after 22 hours. In order to learn how long it took the salt to make

[illegible] in the urine, the same animal was again fed one gram of calcium hypophosphite and, in intervals of one-half hour duration after the ingestion, urine was collected by means of a catheter. After the first half hour, the urine already showed the distinctive reaction to the presence of hypophosphorous acid.

Several days later (on 25 April) the studies were repeated with the same animal. First, we tested in order to make sure that the urine was free from hypophosphite. Then the procedures were repeated, only this time catheterization samples were taken in one-quarter hour intervals following ingestion. Urine extracted after one-quarter hour also showed the distinctive green flame, though the flame was not as strong in color as in the preceding test. In this testing, no hypophosphorous acid could be demonstrated on the next day following the ingestion of the salt.

On 30 April, the dog again received one gram of calcium hypophosphite and was destroyed 6 hours later by bleeding from the carotid artery. The blood, along with the most important organs, namely the entire gastro-intestinal tract, the liver, both kidneys, and the brain were tested for hypophosphorous acid. To this end, the organs were shredded (in the case of the stomach and the intestine the contents were mechanically removed); they were then irrigated with water and allowed to stand overnight at room temperature. On the following day they were filtered and the filtrates were subjected to the same tests that we described above for the testing of the urine. Hypophosphorous acid could not be demonstrated in the blood or in any of the organs except the stomach and intestine. These produced the distinctive green-coloration of the hydrogen flame.

To check the validity of these findings, I repeated the tests with a second dog. To a male dog weighing 7.4 kg, I again administered one gram of calcium hypophosphite orally. As before, the presence of hypophosphorous acid could again be detected in the urine after one-quarter hour. This time, the dog was destroyed three hours after feeding by bleeding from the carotid. Conjointly, urine was collected from the bladder and tested for hypophosphorous acids; it produced a very sharp green-coloration in the hydrogen flame.

From the dead animal, blood, liver, both kidneys, brain, and gastrointestinal tract were removed and tested. As with the first animal, the contents of the gastrointestinal tract were completely removed by means of a strong water-irrigation. Further, the shredded organs were warmed with water in the water bath. This time, the tests yielded no decisive indication of the presence of hypophosphorous acid. When the kidneys and blood were tested, we observed a weak green-coloration of the hydrogen flame, but the evidence was not clear enough to allow for a certain conclusion.

In order to obtain some information about the excretion of this salt in man, I myself ingested one gram of calcium hypophosphite. By the way, I suffered no ill effects. After one quarter hour, no hypophosphorous acid was yet present in the urine, but it was distinctly present after one-half hour. Urine collected on the following day still contained hypophosphite; only on the second day following was the urine free of the salt. With man, therefore, the excretion

appears to last somewhat longer.

From the tests described we learn, therefore, that with dogs, [illegible] calcium hypophosphite is quickly and quite completely absorbed; it traverses the organism without meeting any appreciable restraint anywhere in the body and is very rapidly excreted. In any case, the excretion ought to be ended within 24 hours. The fact that no hypophosphite was found in the organs I would not interpret as absolute evidence that none of the salt had been there. Rather I would observe that the Musart-Blondlot test is not sensitive enough to demonstrate the small traces of hypoph[illegible]rous acid that might be contained in the organs. Since the hydrogen flame test is capable of revealing the smallest trace of hydrogen-phosphorous compounds we used it in our procedures by applying it to the silver-phosphorous residue. The fact that hypophosphorous acid was found in the first tests of the stomach and the intestine may be explained by the possibility that vestiges of the intestinal content were present at the testing.

From our studies, the following findings permit applications for forensic practice.

If phosphorous poisoning is suspected and it can be proved that the possibly poisoned person ingested no hypophosphites for several days before his death, then we do not need to take the presence of hypophosphorous acid into consideration.

However, if this fact cannot with certainty be established,

then the urine and the contents of the gastrointestinal tract should be tested for phosphorous acid. If a hypophosphite has been ingested, the findings of the test will not be impaired for the following reasons which appear conclusive. Hypophosphorous acid could not be demonstrated in the organs of animals as small as our two test dogs following the ingestion of such a comparatively large amount as one gram. There is therefore a much smaller possibility that it will be detectable in such a comparatively larger organism as man following the ingestion of medicinal doses of the salt which are much smaller that the amount ingested by the dogs.

Panzer-1902
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Z. Untersuch Nahr. U. Genussm. 5:11. 1902.

Ueber das Verhalten von unterphosphorigsaurem Calcium im thierischen Körper.

BUREAU OF CHEMISTRY U.S. DEPT OF AGRICULTURE

Von

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Wenn es sich um den chemischen Nachweis einer Phosphorvergiftung handelt, und elementarer Phosphor in den Untersuchungsgegenständen nicht aufgefunden wird, so pflegt man, wie bekannt, nach phosphoriger Säure zu suchen. Hierzu bedient man sich in der Regel des Dusart-Blondlot'schen Verfahrens. Nun hat Dusart[1]) schon nachgewiesen, dass die unterphosphorige Säure bei Anwendung seiner Methode dieselben Erscheinungen zeigt, wie die phosphorige Säure. Die unterphosphorigsauren Salze werden aber zu therapeutischen Zwecken verwendet und deshalb ist, wenn in einem gegebenen Falle die Dusart-Blondlot'sche Methode ein positives Ergebniss liefert, nicht ohne Weiteres der Rückschluss auf die Anwesenheit von phosphoriger Säure, bezw. auf eine Phosphorvergiftung gestattet, sondern es muss immer noch die Möglichkeit in Betracht gezogen werden, dass dem betreffenden Individuum Hypophosphite einverleibt wurden. Es schien daher wünschenswerth, einige Aufschlüsse darüber zu erlangen, wie sich unterphosphorigsaure Salze im thierischen Organismus verhalten.

Schon Blondlot[2]) und später Paquelin und Joly[3]) fanden, dass, wenn Thieren ein Hypophosphit einverleibt wird, dieses im Harne wieder erscheint. Mir kam es hauptsächlich darauf an, die Frage zu beantworten, ob und wie lange diese Salze, wenn sie dem Thierkörper einverleibt werden, darin verbleiben, namentlich aber, ob sie dann in den Organen nachzuweisen sind. Zu diesem Zwecke wurden einige Versuche an Hunden ausgeführt, wobei das Calciumsalz der unterphosphorigen Säure in Anwendung kam.

Ein 10,1 kg schwerer, männlicher Hund erhielt am 17. April um 11 Uhr vormittags 1 g Calciumhypophosphit per os. Der im Verlaufe der folgenden 6 Stunden entleerte Harn (etwa 100 ccm) wurde gesammelt, in einen Wasserstoffentwickelungsapparat gebracht, in welchem verdünnte Schwefelsäure auf Zink wirkte, und das entweichende Gas durch Silbernitratlösung geleitet. Der in dieser Lösung entstandene schwarzbraune Niederschlag wurde durch Dekantation mit Wasser gewaschen und in einem zweiten, ganz kleinen Wasserstoffentwickelungsapparat geprüft, der so eingerichtet war, dass das entweichende Gas über mit Kalilauge getränkte Bimssteinstückchen strich und dann aus einem Porzellanröhrchen entwich, woselbst es angezündet wurde. Es brannte mit stark

[1]) Compt. rend. **43**, 1126.
[2]) Compt. rend. **52**, 1197.
[3]) Compt. rend. **86**, 1505.

Von den Leichentheilen wurden wieder Blut, Leber, beide Nieren, Gehirn und Magendarmkanal verarbeitet, doch wurde letzterer zur vollständigen Entfernung seines Inhaltes durch einen kräftigen Wasserstrahl ausgespült. Ferner wurden die zerkleinerten Organe mit Wasser auf dem Wasserbade erwärmt.

Diesmal konnte in keinem der Untersuchungsstücke unterphosphorige Säure nachgewiesen werden. Bei der Untersuchung der Nieren und des Blutes wurde eine ganz schwache Grünfärbung der Wasserstoff-Flamme beobachtet, aber die Erscheinung war doch nicht deutlich genug, um einen sicheren Schluss zuzulassen.

Um auch einige Aufschlüsse über die Ausscheidungsverhältnisse dieses Salzes beim Menschen zu erhalten, nahm ich selbst 1 g Calciumhypophosphit, das ich, nebenbei bemerkt, ohne Beschwerden vertrug. Nach einer Viertelstunde fand sich noch keine unterphosphorige Säure im Harne, wohl aber nach einer halben Stunde. Der Harn des folgenden Tages enthielt noch Hypophosphit, erst am zweitfolgenden Tage war der Harn frei davon. Die Ausscheidung scheint also beim Menschen etwas langsamer vor sich zu gehen.

Aus den beschriebenen Versuchen ergiebt sich also, dass einem Hunde einverleibtes unterphosphorigsaures Calcium rasch und recht vollständig resorbirt wird, den Organismus durchwandert, ohne irgendwo zurückgehalten zu werden, und sehr rasch wieder ausgeschieden wird. Die Ausscheidung dürfte jedenfalls innerhalb 24 Stunden beendet sein. Die Thatsache, dass in den Organen kein Hypophosphit gefunden wurde, möchte ich nicht so deuten, als ob wirklich keines darin vorhanden gewesen wäre, sondern ich möchte meinen, dass das Dusart-Blondlot'sche Verfahren zu wenig empfindlich ist, um die geringen, in den Organen enthaltenen Spuren von unterphosphoriger Säure nachzuweisen. Die Wasserstoff-Flamme zeigt allerdings die kleinsten Spuren von beigemengtem Phosphorwasserstoff an, es muss also die geringe Empfindlichkeit des Verfahrens in der Gewinnung des Phosphorsilberniederschlages zu suchen sein. Dass beim ersten Versuche im Magen und Darme unterphosphorige Säure gefunden wurde, scheint darauf zurückzuführen zu sein, dass Reste von Darminhalt mitverarbeitet wurden.

Für die forensische Praxis lassen sich nun aus diesen Versuchen folgende Regeln ableiten:

Kommt eine Phosphorvergiftung in Frage, und lässt es sich nachweisen dass die angeblich vergiftete Person auch nur einige Tage vor ihrem Tode kein Hypophosphit genommen hat, so braucht auf die unterphosphorige Säure überhaupt keine Rücksicht genommen zu werden.

Lässt sich dies aber nicht mit Sicherheit feststellen, so sind von der Untersuchung auf phosphorige Säure der Inhalt des Magendarmkanales und der Harn auszuschliessen, dann wird, wenn wirklich ein Hypophosphit genommen wurde, dies das Ergebniss der Untersuchung nicht beeinträchtigen; denn es dürfte wohl der Schluss gerechtfertigt sein, dass, wenn die in den Organen eines so kleinen Thieres, wie die beiden Versuchshunde, nach Einverleibung der ver-

Von den Leichentheilen wurden wieder Blut, Leber, beide Nieren, Gehirn und Magendarmkanal verarbeitet, doch wurde letzterer zur vollständigen Entfernung seines Inhaltes durch einen kräftigen Wasserstrahl ausgespült. Ferner wurden die zerkleinerten Organe mit Wasser auf dem Wasserbade erwärmt.

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hältnissmässig grossen Menge von 1 g vorhandenen Spuren von unterphosphoriger Säure nicht mehr nachgewiesen werden konnten, dies noch weniger der Fall sein wird, wenn dem bedeutend grösseren Organismus eines Menschen die weitaus geringeren medicinalen Gaben dieser Salze zugeführt werden.

J. Pharm. Chim. 28:314-316. 1878.

The Physiological Role of the Hypophosphites

by MM Paquelin and Joly

For a long time the pyrophosphates and the hypophosphites have been used therapeutically as reconstituants. We have demonstrated that the pyrophosphates leave the organism the same as they entered it, without undergoing any transformation; that they are totally recovered in the urine as pyrophosphates; that the ingestion of these agents disturbs economy of function only to the extent of the effort required to eliminate them; that, in sum, the pyrophosphates, far from being reconstituants as we supposed for 30 years, only function as diuretics.

Our research into the physiological action of the hypophosphites has led us to conclusions which are entirely analogous to our findings with the pyrophosphates.

Methodology.

Chemical analysis.-- When they are exposed to the oxidizing action of a mixture of potassium chlorate and hydrochloric acid, the hypophosphites, when in solution, are transformed into phosphates. Our method of analysis rests on this fundamental reaction.

With this fundamental fact in mind, if a liquid contains phosphates and hypophosphites in solution, how can the two salts be measured separately?

To achieve this end: divide the liquid into two equal parts; determine, by means of a uranic titrate, the phosphoric acid contained in one sample; treat the other sample with an oxidizing mixture of potassium chlorate and hydrochloric acid; heat in order to eliminate the excess of chlorine which forms during the operation; then, as above, titrate the phosphoric acid with the uranium solution.

The difference between the two resultant values will yield the sum of phosphoric acid resulting from the transformation of the hypophosphite into phosphates.

When we double the first value and this difference, we learn on the one hand how much of the phosphoric acid was contained in the liquid in the form of phosphates and on the other hand the sum of phosphoric acid resulting from the transformation of the hypophosphite into phosphates.

These operations completed, having determined by weight the relation (mean) that exists between the two elements that constitute a phosphate; having also determined the relation that exists between a known quantity of hypophosphite and the quantity of phosphoric acid produced by peroxidation of this amount of hypophosphite, it becomes easy to determine both the sum of phosphate and the sum of hypophosphite contained in the total liquid sample.

Physiological experimentation.-- For 15 days Mme. N followed a uniform dietary regime. From the 6th day through the 10th day inclusively, she took 0.5 g of hypophosphite of soda at each principal meal; this amounted to 5 g of the salt in 5 days. One gram of the hypophosphite produced by peroxidation 0.45 g of phosphoric acid or, for 5 g of hypophosphite, a total of 2.25 g of phosphoric acid.

Analyses conducted during the first 5 days of the dietary regime found normal composition of the urine. For the final 10 days of the regime, analyses showed changes brought about in urine composition by the ingestion of 1 gram per day of hypophosphite of soda for the 5-day period described above.

Due to the effects of ingesting one gram of hypophosphite soda in 24 hours our analyses revealed: that the average quantity of urine was raised from 1.135 to 1.205 in the same time span; that the density of the liquid had changed from 1.024 to 1.029; that the excretion of urea was increased by 698 milligrams and that of phosphoric acid by 335 milligrams. Moreover, our analyses demonstrated that the hypophosphites traverse the organism without undergoing any transformation and that they can be totally recovered in the urine.

Conclusions.-- The hypophosphites are not reconstituants. They are diuretics.

Paquelin-1878
Bib No. 33

J. Pharm. Chim. 28:314-316. 1878.

Du rôle physiologique des hypophosphites;
par MM. PAQUELIN et JOLY.

Les pyrophosphates et les hypophosphites sont depuis très-longtemps employés en thérapeutique comme reconstituants. Nous avons démontré que les pyrophosphates sortent de l'organisme tels qu'ils y entrent, sans subir aucune transformation; qu'on les retrouve en totalité dans les urines à l'état de pyrophosphates; que l'ingestion de ces produits ne fait qu'augmenter les dépenses de l'économie en raison du travail d'élimination qu'y nécessite leur présence; que les pyrophosphates en somme, bien loin d'être des reconstituants, ainsi qu'on le suppose depuis bientôt trente ans, ne sont que des diurétiques.

Nos recherches sur l'action physiologique des hypophosphites nous ont conduits à des conclusions entièrement analogues.

En voici la démonstration :

Analyse chimique. — Soumis à l'action oxydante d'un mélange de chlorate de potasse et d'acide chlorhydrique, les hypophosphites, quand ils sont en solution, se transforment en phosphates. Tel est le fait principal sur lequel [illegible] notre méthode d'analyse.

Cette donnée acquise, si un liquide contient en solution phosphates et hypophosphites, comment y doser séparément ces deux sortes de sels?

A cet effet, diviser le liquide en deux parties égales; doser

miner, au moyen d'une solution titrée d'urane, l'acide phosphorique contenu dans l'une, traiter l'autre par un mélange oxydant de chlorate de potasse et d'acide chlorhydrique, et chauffer pour chasser l'excès de chlore qui se forme pendant cette opération, puis y doser comme précédemment l'acide phosphorique avec la solution d'urane.

La différence entre les deux résultats obtenus donnera la somme d'acide phosphorique résultant de la transformation de l'hypophosphite en phosphates.

En doublant le premier résultat et cette différence, on saura d'une part ce que le liquide contenait d'acide phosphorique à l'état de phosphates, d'autre part la somme d'acide phosphorique résultant de la transformation de l'hypophosphite en phosphates.

Ces opérations faites, étant connue en poids la relation (moyenne) qui existe entre les deux éléments constituants d'un phosphate, étant connue également la relation qui existe entre un poids donné d'hypophosphite et la quantité d'acide phosphorique que donne ce poids d'hypophosphite par suroxydation, il sera facile de déterminer et la somme de phosphate et la somme d'hypophosphite contenues dans la totalité du liquide.

Expérimentation physiologique. — Mme N... a suivi pendant quinze jours un régime alimentaire uniforme. Du sixième jour inclusivement au onzième jour exclusivement, elle a pris à chaque repas principal 0gr,50 d'hypophosphite de soude, soit 5 grammes de ce sel en cinq jours. Un gramme de cet hypophosphite produit par suroxydation 0gr,450 d'acide phosphorique, soit pour 5 grammes d'hypophosphite 2gr,250 d'acide phosphorique.

Les analyses des cinq premiers jours ont donné la composition normale des urines. Celle des dix derniers jours a montré les changements apportés dans cette composition par l'ingestion d'un gramme par jour d'hypophosphite de soude pendant cinq jours.

De nos analyses, il ressort que, sous l'influence de l'ingestion d'un gramme d'hypophosphite de soude en vingt-quatre heures, dans le même temps, la quantité moyenne des urines s'est

élevée de 1,135 à 1,205; que la densité de ce liquide a été portée de 1,024 à 1,029; que la dépense de l'urée a augmenté de 598 milligrammes et celle de l'acide phosphorique de 335 milligrammes. De plus nos analyses démontrent que les hypophosphites traversent l'organisme sans subir aucune transformation et qu'on les retrouve en totalité dans les urines.

Conclusions. — Les hypophosphites ne sont pas des reconstituants. Les hypophosphites sont des diurétiques.

Polk-1874
Bib No. 36

Pharm. J. 5.425-426. 1874.

THE HYPOPHOSPHITES.*

BY CHARLES C. POLK, M.D.

The hypophosphites, introduced to the medical profession by Dr. Churchill on the erroneous theory that they supply phosphorus to the system, and thereby restore the normal amount of that element which he considered to be deficient in phthisis, and from which deficiency he supposed the tubercule was caused, have outlived the excitement attendant on novelty, and have attained an official position in our pharmacopœia. Without being a panacea for phthisis, hypophosphorous acid and its salts have proven to be valuable companions for cod-liver oil in this and other wasting diseases. Further research has demonstrated that hypophosphorous acid exists in the brain and nerve structure in combination with glycerin and fat, and that it is a deficiency of this, and not uncombined phosphorus, which lies at the foundation of this frequent and fatal malady. Churchill erred in the selection of neutral salts instead of free acid. Therapeutically, nitrate of potassium is not the same as nitric acid; neither is muriate of ammonium muriatic acid. The alkali salts are not always indicated, and if continued through the protracted treatment required in chronic diseases they impair the *crasis* of the Blood, and do a detriment uncompensated for by any [illegible] which can accrue from their use. With the free acid and with its combinations with iron and manganese, these objections do not obtain. Time and again I have tested them with marked advantage, and have learn[illegible] them second only to cod-liver oil in staying the development and progress of tuberculosis. As is well known,

* From the *Tennessee Pharmacal Gazette*, for November.

there are two syrups—the ferric syrup and the ferrous syrup. The former is obtained by decomposing sodic or calcium hypophosphite with ferric tersulphate. The following is a formula I formerly followed for a physician who used the syrup of the ferric hypophosphite considerably :—

℞. Sodic Hypophosphite	256 grs.
Sol. Ferric Tersulph.	q. s.
Acid. Hypophosph. Sol.*	1 oz.
Syrup q.s. ad	12 oz.

Decompose the sodic hypophosphite with sufficient of the iron solution, wash the ferric hypophosphite, dissolve it in the acid, and add the syrup.

The ferrous hypophosphite, owing to its extreme solubility, requires a different mode of manipulation. It has been proposed to form it by decomposing ferrous sulphate with sodic hypophosphite. By making highly concentrated solutions of the ferrous and sodic salts the greater part of the ferrous hypophosphite will be precipitated, and can then be dissolved in syrup acidulated with hypophosphorous acid, so that each drachm will represent about five grains of the ferrous salt. The proportions for forming ferrous hypophosphite thus are five parts of iron to eight of sodium. I do not deem it the most economical or the most satisfactory process. I have found the following to give a valuable syrup, although the process does not come under the laws of double decomposition :—

℞. Ferrous Sulphate.	15 drs.
Potassic Hypophosphite	1 oz.
Hypophosphorous Acid.	11 oz.
Sugar	14 oz.
Water, sufficient.	

Dissolve the iron in five ounces of boiling water, and the potassic hypophosphite in ten ounces of boiling water, mix the solutions; let it stand in a closely-covered vessel two hours, filter on the sugar, and add the hypophosphorous acid solution. No doubt there exists some sulphuric acid and sulphate of iron, and also some hypophosphite of potassium; nevertheless, therapeutically, I have found it a fine combination, and the most economical of any I have tried. The following is my choice for making syrup ferrous hypophosphite :—

℞. Fresh Ferrous Oxide	q. s.
Hypophosphorous Acid	5 oz.
Sugar	8 oz.

Saturate three ounces of the acid with the ferrous oxide, add the remainder of the acid and the sugar, and dissolve without heat. Each drachm will contain about six grains of ferrous hypophosphite almost entirely free from any impurities. This is the formula I give the preference. I am indebted to my friend Mr. Creuse, of New York, for it.

Another formula I have used for a dozen years is to decompose ferrous sulphate with calcium hypophosphite, pour off the supernatant solution from the sulphate of calcium, evaporate with gentle heat, filter on sugar, add hypophosphorous acid, and dissolve. I am confident, however, that I have derived better results from manganese than from iron, and from both than from either alone. The satisfactory formula for a combination for these agents is yet a desideratum. I use the following :—

℞. Ferrous Oxide	256 gr.
Manganese Hypophosphite . . .	256 gr.
Hyphosphorous Acid	10 oz.
Sugar	10 oz.

Dissolve the iron and manganese in the acid, add the sugar, and dissolve. To this I add hypophosphite of ammonium, when disease does not contra indicate, in the proportion of three grains to each drachm. It preserves the syrup, and also enhances, in a very positive manner, its therapeutical properties. If it be desirable, one grain of quinia and one-fortieth of a grain of strychnia may be given with each dose. Thus administered, I have found it, in conjunction with cod-liver oil, to arrest the progress of phthisis in a decidedly positive degree. I can recall many cases in which the cure seems permanent. In the wasting diseases of children in which there seems to be a deficiency of lime in the system, with general impairment of the nutritive functions, the following combination has done very well in my hands :—

℞. Calcii Hypophosph. recent. . . .	256 gr.
Manganesii Hypophosph. recent. .	64 gr.
Acidi Hypophosph. Sol.	2 dr.
Syrupi q.s. ad.	16 oz.
M.	

Teaspoonful thrice a day to a child of two or three years of age. I usually combine this with an aromatic elixir of calisaya, which makes it agreeably tasted and therapeutically more efficient.

* The strength of this solution is not indicated in the original, but we presume it is to be understood that only sufficient is to be used to dissolve the respective precipitates of hypophosphites.

Rev. Stiint. Med. 18: 722-730. 1929.

CONTRIBUTIONS TO THE ACTION OF HYPOPHOSPHITES ON HUMAN RICKETS

Docent Dr. Gh. Popoviciu and Dr. Liviu Dariu

The action and the utility of phosphorus and its derivative products (phosphates, hypophosphites, organic compounds) are widely discussed. These products are prescribed, partially combined with calcium in cases of malnutrition, nervous breakdown, anemia and in general when a poor Ca and P assimilation occurs and especially in diseases of the skeleton. The results obtained following their administration vary and differ according to the product used, and quite often are questionable.

Generally nowadays, the problem discussed is in view of finding out if calcium and phosphorus administered as any unspecific medication could be retained for a longer time in the organism and if in consequence they could produce a more durable therapeutical effect.(Debre, Achard, Dubreuil, M. Labbe, Manoussaki, Cunaut, Weill, Guilleaumin, Blum-Looft, Sichny-Kessler etc.). We do not intend to give in this paper all the experiments and the pro and con arguments concerning the possibility to obtain a therapeutical result in this way. The results obtained in recent years regarding the action of ultraviolet rays and of the antirachitic vitamin have shown indeed that these are the main factors which induce the retention of Ca and parallelly of P in organisms. By this retention they cure the illnesses caused by lack of Ca and P,as are the rickets, tetany, partially tuberculosis etc., ailments, which some time ago,were treated with unspecific medicine containing these elements. Healing would be due to the intensification of the retarded metabolism, characteristic in these ailments,healing,which is possible in case of an excess of vitamins even when a deficiency in Ca and P is present(Hes and his collaborators Huldschinsky, Shipley, Park-Simmonds, Kramer, Casparis-Howland,Freudenberg-Gyorgy, Falkenheim-Gyorgy, Woringer, Lesne, Saidman, Gernes, Varfan, Drumonds, Gyorgy-Popoviciu, Nitescu-Popoviciu, Rosentheim-Webster, Mouriquand, Popoviciu).

Nevertheless these studies are far from excluding the role played by phosphorus or by the phosphate-ions ; on

the contrary, it was recently admitted that their action is due to a similar mechanism, in othe words, also by an intesification of the nutritional process, increasing the activity of specific factors or helping to the formation of vitamin D (Freudenberg-Gyorgy, Pritchard, Hodgson, Embden, Meyerhof, Lunberg, Warburg, Phemister, Miller-Boaar, Bernhardt-Rabl etc.), point of view accepted, as regards the part the phosphoric acid plays, also by Joulie, Desprez, Fissinger, Martinet, Debre, Pouchet, Drouet, Loeper, etc. Similarly Remond-Boulicaud shows that an etheric derivative of phosphoric acid, the mono-ethyl phosphoric acid—ether (phosoforme) , would increase very much the number of red cells , partially the number of the white ones and would also normalize the hepatic fonctions.

On the other hand, even for the treatment of rickets, pathological condition, which seems to be caused specifically by absence of ultra-violet rays or lack of anti-rachititic vitamin, some more conservative authors, led by Marfan, Cozzolino, although they acknowledge the role of ultraviolet rays and of irradiated substances, recommend at the same time or even in the first place, other treatments, some unspecific, as well as the administration of P and of its derivatives. One of the best connoisseurs of the rachitism problems, Prof. Gyorgy, does not either deny the role of these latter factors, and as regards the oral administration of a plus of Ca and P salts, he admits that some organisms suffer from a particular lack of salts and in this case a regimen short in these salts would favor the occurrence of rachitism. Similarly a supplement of these salts together with other artirachitic factors would accelerate the healing. Thus, the conclusion to be drawn is that even according to the more recent trends, the so-called specific medication does not exclude the unspecific one. The advantage presented by the latter is that it is easier to carry out. A proof that the unspecific medication has not been forsaken is shown by the number of P and Ca products , used in various metabolism troubles, which increases daily. Nevertheless these unspecific methods must be justified by accurate and serious experimental research work. One of us (Popovicliu) found during his research work at Sovata spa (Romania), one of the most intensive activities of the salted baths there, on P and Ca retention, an increase of their blood level, facts that proove the high efficacy of this "unspecific" method in rickets treatment, as well as in other cases of deficient P and Ca metabolism. We also observed the obvious effect of P products when administered in similar cases.

In order to control in a scientific manner their action, we studied besides their clinical action , their chemical one, namely their action on blood Ca and P, at the same time, on account of the frequent associations

between P and Ca deficient states and hematopoiesis. (Gyorgy, Czerny, Rosenbaum, Wieland, Hottinger et al.) and their healing with return to normality of the blood picture, we sought to check parallelly, the changes intervening in the number of the globules and hemoglobine, which we had some time ago observed in the action of yellow P, and determine as already mentioned, the effects of a more recent product, namely of the phosforme. As deficiency in P and Ca and their return to normality is more evident and characteristic in rickets, we studied these elements, in the first place, on children having this illness.

In our first series of experiments, we used an old phosphor product, the hypophosphorite syrup , provided by the Egger Comp. (Syrupus hypophosfhit comp.- Egger), we had already used in previous clinical cases and of the clinical effects of which we were by now convinced.

Hypophosphites were introduced in the healing practice by Francis Churchill (1858) who preconized them in treatment of tuberculosis, because they increase the appetite, restore strength and activate hematosis. Hypophosphites were also considered as having the property to increase cell nutrition and enhance organic combustion. Besides these actions, accepted more or less on ground of theoretical considerations or clinical observations, older laboratory research works showed that the hypophosphites pass through the organism, are only oxidized in a unimportant proportion and are almost completely eliminated with urine(Paquelin, Massol, Gamel).

This is the reason why, more modern methods seemed to be necessary especially for the determination of P and Ca blood level, for establishing their increase separately, determination of the Ca×P product, as well as the decrease of the quotient Ca/P (Howlland-Kramer, Hess-Lundagen, Gyorgy) after the treatment, as it would proove a P and Ca retention in the organism, thus, a therapeutical effect. Incidentally nowadays it is known that, the increase of blood level Ca and P, an increase of Ca×P, and decrease of Ca/P, especially characteristic in the cure of rickets and the action of the antirachitic vitamin , allow for much more extensive conclusions to be drawn, as they are also a proof of the increased resistance of the organism to infections (Gyorgy-Popoviciu, Woringer, Mouriquand, Bieling, Heymans, Dowell-Hill-Clark, Kreitmair-Eicholtz, Popoviciu, etc.). From this point of view, our research studies should have brought us indications if this increase of the resistance towards the noxious infections could be obtained with unspecific medication, in the first place by using phosphorus products. The hypophosphite syrup contains beside the potassium, sodium and calcium hypophosphites, phosphorous acid and iron salts (pyrophosphates, citrates) as well as quinine and [illegible] tentative research work could have drawn our a[illegible] an eventual efficacity of several of the medicat[illegible]

Our research studies have been made with 7 children 6 of them had rickets, and one TB peritonitis and pleurisy. The age of the children with rickets was 4-7 months and 1,1/4, years. They were treated with hypophosphite syrup during 4-6 1/2 weeks. The determinations regarding Ca and P blood level, red globules, white ones and hemoglobine were carried out at the beginning of the treatment, at the end of the treatment and 8-20 days after termination. Calcium has been determined by the Kramer-Tisdall method, and Phosphorus after Bell-Doisy-Briggs. Our results are given in the annexed table.

Generally speaking the clinical condition of the children indicates amelioration during the treatment with hypophosphites; these ameliorations continued after the treatment was over, and were partially shown by reduction of craniotabes, gradual decrease of perspiration, eruption of teeth, fontanel decrease and improvement of static functions and general condition. We shall not insist on these clinical improvements because the observation time was relatively short, insufficient for more evident signs of clinical healing and because we could not check the results by X-rays on account of technical difficulties. We focussed our studies on the blood chemical modifications which can show much sooner than the clinical ones the amelioration of the rachitic process, of the Ca and P retention, and the modifications that intervened in the globule numbers and in the hemoglobine.

As regards the Ca and the P, the mean shows some ameliorations which in any case are inferior to those obtained by specific treatments, known to fix the Ca and P as are the ultra violet rays and vitamin D. The Ca and the P increased obviously only in one case (No.[illegible] especially noticeable in the product CaXP, and relative increase of P, reduced Ca/P. In the pleuro-peritonitis case (No.7) we obtained important increase of P and even of Ca which became evident after the treatment was over. These increases as well as the product CaXP, and the factor Ca/P show an obvious P and Ca retention. However in this latter case we could not completely exclude the possibility of a spontaneous amelioration , because it is known that in acute pathological conditions as pneumonia, shivers, fever etc., (Gerastenberer, Urechia-Popoviciu, Birk) hypophospatemia and blood Ca troubles are present.

In general, the increase of the Ca level is maintained and even raises more after the treatment is over. As regards the P, in 4 of the 7 cases its level decreased even during the treatment and dropped more after its interruption.

As regards the modifications in the number of red, white, cells and of the hemoglobine, generally, increases have been observed; these increases were important during the treatment but after it was over, some were [illegible] some reduced and some increased further. In case [illegible]

decrease was due to acute flue and suppurative otitis.

The ameliorations obtained, cannot be credited without discussion, to a mechanism identical to the usual one, in the healing of rickets. Compared to the constant and accentuated increase of P under the action of ultraviolet rays and vitamin D, in the treatment with hypophosphites, P shows an inconstant increase or even decreases. The P decrease in cases 1, 3, 5 as well as in the total mean of the cases, also the decrease of the product Ca×P and the increase of the quotient Ca/P during or after the interruption of the treatment, when administration of hypophosphites was stopped, calls for fighting hypophosphatemia by means of hypophosphites, even in the cases in which the blood level during treatment, indicated only an insignificant plus or even a minus ; we think that the hypophosphites during their struggle against the rachitic process should produce at least a temporary increase of P, even if less significant. The raise of blood Ca [1] is also in support of the increase of blood P as due to the hypophosphites, being admissible at least for a short time, in a first phase of action, after their oral administration, phase which would be followed by amplified hypophosphatemia, noticed in cases 2, 3, 4, 5. We believe, supported by our present knowledge, that the Ca raise cannot occur during rickets without a parallel increase of P, even if only relative and temporary. Moreover the formula given by Rona-Takahashi-Gyorgy:

$$Ca = \frac{H}{HCO_4 \cdot HPO_4}$$

admits an increase of ionized Ca and perhaps even of the total one, in case of minus P. Such an antagonism between total Ca and blood P has been met with, also in other cases: action of adrenalin, ergotinine, hyoscine in hyperventilation tetany, in the spasm of idiopathic parathyroprive tetany (Wolmer-Grant, Goldman, Gyorgy-Vollmer, Popoviciu-Poenaru, Urechia-Popoviciu and Popoviciu-Popescu).

In any case, the increase of Ca, beside a minus of P can only be temporary, because after hypophosphatemia, according to the pathogenic mechanism in rickets, an increased elimination of Ca from the organism occurs and thus we have as result a decrease of blood Ca. But the increase of blood Ca and P , resulting from our experiments with hypophosphites, will have a healing action, even if only temporary and will help the organism to resist in the critical periods until the specific treatment for Ca and P fixation is at hand (antirachitic vitamin , ultraviolet rays etc).

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1) As regards the usual higher values, even in the untreated rickets of our studies, see work of Popoviciu published in Clujul Medical, 1928; it could also be the case of restoring the level of Ca in rickets as shown in older research work.

As regards the increase of the globules and of the hemoglobine it could be due at least partially, to the hypophosphites (similarly to the action observed for yellow phosphorus by Gowers, Taussig and for phosoform by Redond and Boulicaud). On the other hand it may possible that the iron salts , contained by the hypophosphite syrup, (pyrophosphates, citrates), have also a role.

The role of these factors will have to be determined in further research studies in which only the action of the hypophosphites will be considered. Due to the discussions and controversial opinions regarding the therapeutical actions of the hematopoietic products (arsenic), in general the roborant treatments, stimulants of the metabolism on rachitic skeleton modifications as well as the healing of diseases of hematopoietic organs, in the first place of the function of the spine marrow (Marfan-Baudouin , Aschenheimer-Benjamin, Hutinel-Tixier, Ziegler, Oehmer, Schmorl, Christeller, Nitescu-Popoviciu-Ungureanu, Fuchs-Priesel, Seel etc), it would be too soon to decide if the clinical ameliorations observed in these rickets cases should not be considered, at least partially, also due to the other substances contained in the syrup, beside the hypophosphites.

Conclusions: During the therapeutical action of the hypophosphites (Syr. Hypophosph. comp. Egger) on rickets, we observed clinical ameliorations as well as modifications in Ca and P which are more inconstant and less evident, sometimes seeming opposed to the action of ultraviolet rays and of antirachitic vitamin D; nevertheless a similarity, ever if partial, seems to exist between the mechanism of the hypophosphites action and the "specific" antirachitic treatment. At the same time we observed almost without exception, an obvious increase of white and red cells and of hemoglobine in the cases we treated. If these increases are due to the hypophosphites or to the iron contained in the hypophosphite syrup, will have to be elucidated by further research. In any case, the hypophosphites effect on the blood globules and on hemoglobine could partially explain the ameliorations obtained by this treatment. All these actions justify the use of the hypophophites syrup, especially as a temporary or adjuvant treatment , beside the specific treatment , in rickets and other troubles of the Ca and P metabolism as well as a wide utilization even by itself in case of anemia and malnutrition.

[illegible]	Data	Ca mgr. %	% creşt sau scade	P mgr. %	%	Ca × P	%	Ca/P	%	Globule roşii	
Pop Ion	1 I 1929	12,4		3,0		37,2		4,1		3,[illegible]	
	7 III cu ipotsf.	11,[illegible]	— 10,4	3,3	+ 10,0	36,6	— 1,5	3,3	— 19,0	4,000,000	+ [illegible]
	27 III fără ipotsf.	13,5	+ 8,8	2,8	— 6,6	45,8	+ 23,0	4,8	+ 17,0	4,600,000	+ [illegible]
Eisenberger Regina	4 II	11,4		3,0		34,2		3,8		4,300,000	
	16 III cu	13,4	+ 17,5	2,5	— 16,6	23,5	— 31,5	5,3	+ 41,6	4,600,000	+ [illegible]
	28 III fără	12,6	+ 10,5	2,1	— 30,0	26,5	— 22,9	6,0	+ 64,4	4,700,000	+ [illegible]
Brumar Ion	6 II	13,3		3,2		42,6		4,1		5,000,000	
	25 III cu	10,4	— [illegible]	2,8	— 12,5	29,1	— 31,3	3,7	— 9,7	4,400,000	— [illegible]
	2 IV fără	11,6	— 12,7	2,3	— 27,1	26,7	— 37,3	5	+ 21,9	5,200,000	+ [illegible]
Pleden Zoe	18 III	10,7		2,7		28,9		3,9		3,600,000	
	26 III cu	12,5	+ 16,8	2,1	— 22,2	26,3	— 9,8	5,9	+ 51,2	5,300,000	[illegible]
	10 IV fără	12,7	+ 18,6	2,7	0	34,3	+ 18,6	4,7	+ 20,5	4,800,000	+ [illegible]
Anghi Rafila	28 II	12,2		3,0		36,6		4,1		4,200,000	
	28 III cu	13,2	+ 8,1	2,6	— 13,3	34,3	— 6,2	5,1	+ 24,3	5,300,000	[illegible]
	10 IV fără	14,8	+ 21,3	1,7	— 43,3	25,2	— 31,2	8,7	+ 112,1	4,800,000	+ 14,0
Sârbu Emil	5 III	10,5		2,0		21,0		5,2		4,090,000	
	8 III cu	11,7	+ 11,4	4,5	+ 12,5	52,7	+ 150,7	2,6	— 50,0	4,800,000	[illegible]
Raţ Elisaveta	31 I	12,4		2.3		28,52		5,3		4,270,000	
	17 III cu	12,3	— 0,8	5,0	+ 117	61,2	+ 115	2,4	— 36	5,600,000	[illegible]
	2 IV fără	13,9	+ 12,1	5,4	+ 134,7	75,1	+ 136,1	2,5	— 52,8	5,600,000	[illegible]
Media	Caz 1—7 cu ipotsf.		+ 3,0		+ 26,9		+ 16,0		+ 0,3		[illegible]
	Caz 1—5, 7 fără ipotsf.		+ 9,8		+ 4,6		+ 14,4		+ 30,5		+ [illegible]
	Caz 1—6 cu		+ 3,6		+ 11,7		+ 11,7		+ 6,4		
	Caz 1—5 fără		+ 9,3		— 21,4		— 9,9		+ 47,2		+ [illegible]

					OBSERVAȚIUNI CLINICE
					Spasm piloric. Gripă, otită. Distrofie. Rachitism, transpirațiuni. [illegible] Mătănii costale. Alopecie occipitală.
				+ 1,3	Splina palpabilă. Caput quadratum. Bose frontale. Fontanela deschisă [illegible]
2[illegible]	[illegible]	[illegible]	[illegible],0	0	Nu șade. Fontanela mai redusă. Splină puțin palpabilă. Transpirațiunile mai reduse.
	[illegible]		57,0		Distrofie Rachitism. Torace în formă de pâlnie, mătănii costale foarte pronunțate. Br[illegible] Fontanela deschisă pentru 2 degete.
6[illegible]	[illegible]	+ 7,6	65,0	+ 14,0	Transpirațiuni intense. Funcțiunile statice întârziate. Aceeaș stare, dar transpiră mai puțin
8[illegible]	[illegible]	[illegible]	65,0	+ 14,0	Starea generală mai bună, se ridică spontan.
	[illegible]		70,0		Distrofie, rachitism. Otită medie supur, Mătănii costale. Transpirațiuni Vegetații ade[illegible]
[illegible]	[illegible]	- 23,5	70,0	0	St. idem.
[illegible]	[illegible]	[illegible]	[illegible]	+ 22,0	Starea generală mai bună. Crește în greutate 350 gr. Funcțiunile statice întârziate nu se [illegible] săptămână fenomene de meningită tbc. + la 13 IV. Autopsie: tbc. mil.
	[illegible]		75,0		Distrofie, rachitism. Lipsa dentiției, mătănii costale, transpirațiuni, abdomen balonat [illegible] Splina palpabilă. Funcțiuni statice întârziate.
4[illegible]	[illegible]	[illegible]	[illegible]	+ 20,0	Starea generală mai bună, se ridică spontan. Transpirațiunile reduse. Splina se palpează
30,8	[illegible]	+ 23,1	86,0	+ 14,6	Starea se menține bună. Dinții încă nu apar.
	[illegible]		+ 75,0		Distrofie, rachitism, transpirațiuni, mătănii costale, Dinții lipsesc. Nu se ridică.
[illegible]	[illegible]	[illegible]	80,0	+ 6,6	Aceeaș stare. Apar incisivii inf.
[illegible]	[illegible]	[illegible]	85,0	+ 13,3	Aceeaș stare, nu crește în greutate.
			70,0		Rachitism, diateză exudativă. Abdomen balonat. Fontanela de 3 degete. Ușor craniotabes [illegible] Transpirațiuni abondente
+ 15,[illegible]	[illegible]	[illegible]	75,0	+ 7,1	Craniotabesul dispărut, transpirațiunile diminuate. Nu șade. Crește în greutate 500 gr.
	[illegible]		60,0		Kerato-conjunctivită eczematoasă. Peritonită și pleuresie.
+ 25,[illegible]	[illegible]	[illegible]	78,0	+ 30,0	Starea generală mai bună.
+ 35,1	[illegible]	3,6	82,0	+ 36,6	Transpirațiunile reduse
+ 21,4		+ 18,5	+ 10,8		
+ 18,8		+ 9,5	+ 16,4		
		[illegible]	+ 7,3		
+ 17,5		+ 12,1	+ 12,4		

EFFECTS OF HYPHOPHOSPHITES

TABLE

a= name and age, b= date, c= Ca mg%, d= [illegible] [illegible] sc, f= red globules, g= born h= with [illegible] [illegible] without hypophosphites, j= with, k= without, l= [illegible] [illegible] leukocytes, n= hemoglobine, o= clinical observations

p= case no. 1

Pyloric spasm. Flue, otitis. Dystrophie, Rickets, perspiration, especially on head. Slight craniotabes. Rachitic rosary. Occipetal alopecia. Palpable spleen. Square head. Frontal bossings. 2 finger wide fontanel. Does not sit. Reduced fontanel. Less palpable spleen. Reduced perspiration.

q= case no2.

Dystrophie. Rickets. Thoracic deformities., very accentuated rachitic rosary. Rachitic bracelets. 2 finger open fontanel. Intense perspiration. Static functi[illegible] delayed. S[illegible] condition, but reduced perspiration. Better general condition, gets up spontaneously.

r= case no. 3

Dystrophie, rickets. Suppurated medium otitis. Rachiti[illegible] rosary. Perspiration. Adenoid vegetations.
Same state.
Better general condition. 350 gr. weight increase.
Static functions delayed. Does not get up. After a [illegible] meningitis phenomena ,+the 13 April. Autopsy: TB.

s= case no. 4

Dystrophie. Rickets. No teeth. Rachitic rosary. Perspir[illegible] Abdominal distension, thorax base enlarged. Palpable spleen. Static functions delayed. General condition i[illegible] ed., gets up spontaneously. Reduced perspiration. Palpable spleen. Good general condition, but no teeth.

t= case no.5

Dystrophie, rickets, perspiration, rachitic rosary. No teeth. Does not get up. Same general condition. Lower incisive teeth erupt. Same state, weight stati[illegible]

u= case no.6

Rickets, exudative diathesis. Abdominal distention. 3 finger wide fontanel. Slight craniotabes. Rachitic rosary. Excessive perspiration. Dissapearance of craniotabes, decreased perspiration. Does not sit. 300 g. weight increase.

v= case no.7

Eczematous keratoconjunctivitis. Peritonitis and pleurisy . Better general condition. Reduced perspiration.

Popoviciu- '29
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Institutul de farmacologie și
Centrul pentru ocrotirea copiilor din Cluj.

CONTRIBUȚIUNI LA ACȚIUNEA IPOFOSFIȚILOR IN RACHITISMUL UMAN

de

Docent Dr. GHEORGHE POPOVICIU și Dr. LIVIU DARIU

Acțiunea și utilitatea preparațiunilor fosforului și a derivaților săi (fosfați, ipofosfiți, combinațiuni organ... sunt mult discutate. Aceste preparațiuni se prescri... parte, combinate cu calciu, în stările de nutriție de... tuoasă, epuizări nervoase, anemii și în genere în ca... asimilare vicioasă a P-lui și a Ca-lui, cu deosebire în ... burările sistemului osos. Rezultatele ce s'au obținut p... administrarea lor, sunt diferite variind și după prepa... țiuni, fiind adesea problematice.

In genere astăzi se discută mult dacă fosforul ... calciul administrați în orice formă medicamentoasă p... să fie reținuți pe timp mai îndelungat în organism ... consecință dacă ar putea să producă o acțiune terap... mai durabilă (Debré, Achard, Dubreuil, M. Labb... Manoussaki, Cunaut, Weill, Guillemo... Blum-Looft, Sichny-Kessler etc.). Nu este ... tențiunea noastră să arătăm aici toate experiențele și ar... mentele ridicate pentru sau contra posibilității unui re... tat terapeutic pe această cale. Intr'adevăr rezultatele ul... milor ani în ce privește acțiunea razelor ultraviolete ... vitaminei antirachitice, au arătat că acestea din urmă ... factorii principali cari dau o retenție de P și paralel ... de Ca în organism. Prin această retenție ele vindecă st... cari sunt condiționate de o lipsă de Ca și P, stări ce re...

tismul, tetania, în parte tuberculoza etc., cari înainte erau tratate printr'un aport medicamentos al acestor elemente. Vindecarea s'ar produce printr'o intensificare a metabolismului întârziat în aceste stări, vindecare posibilă în cazul unui plus de vitamină chiar și pe lângă un aport defectuos de Ca și P (Hes și colaboratorii săi Huldschinsky, Shipley, Park-Simmonds, Kramer, Casparis-Howland, Freudenberg-György, Falkenheim-György, Woringer, Lesné, Saidman, Gennes, Marfan, Drumonds, György-Popoviciu, Nițescu-Popoviciu, Rosentheim-Webster, Mouriquand, Popoviciu).

Totuși aceste cercetări sunt departe de a exclude rolul P-lui sau a fosfat-ionilor, din potrivă se admite mai nou că și aceștia ar acționa printr'un mecanism analog adecă tot printr'o intensificare a proceselor nutritive, mărind acțiunea factorilor specifici sau ajutând formarea vitaminei D (Freudenberg-György, Pritchard, Hodgson, Embden, Meyerhof, Lunberg, Warburg, Phemister, Miller-Bonar, Bernhardt-Rabl etc.), din punct de vedere împărtășit, în ce privește rolul acidului fosforic, de către Joulie, Desprez, Fissinger, Martinet, Debré, Pouchet, Drouet, Loeper, etc. In aceeași ordine de idei Remond-Boulicaud, arată că un derivat eteric al acidului fosforic, acid-eterul monoetil-fosforic (phosoforme), ar produce o mărire însemnată a globulelor roșii, în parte și a celor albe, cât și o normalizare a funcțiunilor hepatice.

Pe de altă parte chiar și în tratamentul rachitismului, o stare patologică, care pare a fi cauzată în mod specific de lipsa razelor ultraviolete sau a vitaminei antirachitice, unii autori mai conservatori, în frunte cu Marfan, Cozzolino, deși recunosc un rol însemnat razelor ultraviolete și substanțelor iradiate, recomandă în acelaș timp sau chiar în primul loc alte tratamente, parte nespecifice, cât și administrarea de P și a derivaților lui. Unul dintre cei mai buni cunoscători ai problemei rachitismului, Prof. György, deasemenea nu neagă rolul acestor factori din urmă, și în ce privește administrarea pe cale bucală a

unui plus de săruri de P și Ca, admite că unele organisme ar suferi de o lipsă deosebită de săruri și aci un regim sărat în ele ar favoriza producerea rachitismului. In mod analog un aport al acestor săruri, împreună cu alți factori antirachitici, ar accelera vindecarea. Concluzia ar fi deci că nici după orientările mai noui așa zisa medicațiune specifică nu exclude pe cea medicamentoasă. Dealtcum aceasta din urmă adesea este mai ușor de executat. Că tratamentul medicamentos nu este nici decât abandonat, ne-o dovedește numărul de preparațiuni fosforate, întrebuințat în diferite turburări ale metabolismului, de Ca și P, și care crește din zi în zi. Dar aceste metode nespecifice sau medicamentoase trebuesc justificate prin cercetări experimentale serioase și exacte. Unul dintre noi (Popoviciu) a găsit în cercetările făcute la băile Sovata o acțiune din cele mai intensive a băilor sărate de acolo, asupra retenției de Ca și P, o ridicare a nivelului lor sanguin, ceeace pledează pentru deosebita eficacitate a acestei metode „nespecifice" în rachitism cât și în alte stări cu metabolism de Ca și P defectuos. Pe de altă parte am putut observa în mod evident efectul unor preparațiuni de P, administrate în stări similare.

Pentru a putea controla în mod științific acțiunea lor am crezut de necesar să studiem alături de acțiunea clinică, cu deosebire pe cea chimică și anume asupra Ca-lui și P-lui sanghin. In acelaș timp, date fiind asocierile frecvente ale stărilor de deficit în Ca și P cu hematopoeza (György, Czerny, Rosenbaum, Wieland, Hottinger etc.) și vindecarea lor cu restabilirea tabloului sanghin spre normal, am căutat să cercetăm în mod paralel modificările în numărul globulelor și ale hemoglobinei, observate de mai de mult în acțiunea P-lui galben, și cum am mai accentuat, și în efectul unei preparațiuni mai noui, ca phosoformul. Deficitul în Ca și P și restabilirea lor la normal fiind mai accentuate și caracteristice în rachitism, am cercetat elementele de mai sus în primul rând la copiii suferind de această boală.

Am întrebuințat în prima serie de cercetări pe care am întreprins-o în scopul amintit, un vechiu preparat

fosforat, siropul de ipofosfiți pe care ni l-a pus la dispoziție casa Egger (Syrupus hypophosfhit comp. — Egger). și de a cărui eficacitate clinică am avut ocazia să ne convingem în numeroase observațiuni anterioare clinice.

Ipofosfiții au fost introduși în practica curativă de Francis Churchill (1858) care i-a preconizat în tratamentul tuberculozei. Aici ei ar mări pofta de mâncare, ar crește forțele și ar activa hematoza. Deasemenea s'a atribuit ipofosfiților calitatea de a mări nutriațiunea celulară și proprietatea de a augmenta combustiunile organice. Față de aceste acțiuni, admise mai mult la baza unor considerațiuni teoretice, sau observațiuni clinice, cercetările de laborator mai vechi au arătat că ipofosfiții străbat organismul, oxidându-se numai într'o proporție neînsemnată și că se elimină, aproape complet prin urină (Paquelin, Massol, Gamel).

Se impunea în consecință în mod imperios, cercetări cu metodele mai noui, dintre cari cu deosebire determinarea nivelului sanghin al Ca-lui și P-lui, stabilirea creșterii separate a lor și și a produsului Ca, P, cât și a descreșterii cvoțientului $\frac{Ca}{P}$ (Howlland-Kramer. Hess-Lundagen, György) în urma tratamentului. pot să dovedească o retenție de Ca și P în organism, adică un efect terapeutic. In acelaș timp azi se știe că o ridicare a Ca-lui și P-lui sanghin, o creștere a Ca×P-lui și o scădere a $\frac{Ca}{P}$ caracteristice cu deosebire pentru vindecarea rachitismului și acțiunea vitaminei antirachitice, îngăduie concluziuni mai largi, dovedind în acelaș timp mărirea rezistenței organismului față de infecțiuni (György-Popoviciu, Woringer, Mouriquand, Bieling, Heymans, Dowell-Hill-Clark, Kreitmair-Eicholtz, Popoviciu etc.). Din acest punct de vedere cercetările noastre trebuiau să ne aducă indicii dacă această mărire a rezistenței față de noxele infecțioase ar putea să fie obținută și pe calea medicamentoasă în primul rând prin medicațiunea fosforică. siropul de ipofosfiți, conținând alături de ipofosfiți de calciu, sodiu și potasiu, acid ipo-

fosforos și săruri de fier (pirofosfați, citrați) cât și chiniua în cercetările noastre de tatonare putea să ne atragă atențiunea asupra eventualei eficacități a mai multor factori medicamentoși.

Cercetările noastre au fost făcute la 8 copii, dintre cari 6 rachitici, iar unul suferind de pleuresie și peritonită tbc. Vârsta copiilor rachitici a variat între 4—7 luni — 1½ ani. Siropul de ipofosfiți le-a fost administrat, timp de 4—6½ săptămâni. Determinările de Ca și P sanghin, globule roșii, albe și hemoglobină, au fost făcute la începutul tratamentului, la sfârșitul lui și 8—20 zile după terminarea lui. Calciul a fost determinat după metoda Kramer-Tisdall, fosforul după Bell-Doisy-Briggs. Rezultatele noastre sunt redate în tabela următoare.

In genere starea clinică arată unele ameliorări în timpul tratamentului cu ipofosfiți, continuată și după întreruperea lui, ameliorări manifestate, în parte, prin reducerea craniotabesului, diminuarea transpirațiunilor, apariția dinților, reducerea fontanelei, ameliorarea funcțiunilor statice și a stării generale. N'am dori totuși să insistăm prea mult asupra acestor ameliorări clinice fiind timpul de observațiune relativ scurt, insuficient pentru a da semne mai evidente de vindecare clinică și lipsindu-ne din cauze technice posibilitatea unui control mai sigur prin razele X. Ceeace am cercetat noi au fost modificările chimice sanghine cari pot arăta mult înaintea celor clinice o ameliorare a procesului rachitic, a retenției, de Ca și P, cât și modificările în numărul globulelor și a hemoglobinei.

In ce privește Ca-l și P-l, media ne arată oareșicari ameliorări, în tot cazul inferioare acelora pe cari le dau tratamentele specifice calci- și fosfofixatoare, ca razele ultraviolete și vitamina D. Ca-l și P-l au crescut în mod însemnat într'un singur caz de rachitism (No. 6), evidențiat cu deosebire prin produsul lor Ca×P, deasemenea și prin creșterea relativă a P-lui ($\frac{Ca}{P}$ scăzut). In cazuri de pleuro-peritonită (No. 7) deasemenea s'au obținut creșteri însemnate de P și chiar de Ca manifestat după întreruperea

tratamentului. Ele cât și produsul Ca P, și factorul $\frac{Ca}{P}$ arată o accentuată retenție de P și Ca la acest caz. Totuși în acest caz din urmă n'am putea să exludem cu desăvârșire și posibilitatea unei ameliorări spontane, cunoscut fiind că la stările patologice acute, ca pneumonia, frison, febră etc. (Gerastenberer, Urechia-Popoviciu, Birk) există o ipofosfatemie, cât și tulburări ale Ca-lui sanghin.

In genere ridicarea nivelului de calciu se menține sau se accentuează chiar, după întreruperea tratamentului. In privința P-lui în 1 din cele 7 cazuri se observă scăderi chiar și în timpul tratamentului, scăderi cari se accentuează după întreruperea lui.

In ce privește modificările globulelor roșii, albe și ale hemoglobinei, ele prezintă în genere creșteri, uneori considerabile în timpul tratamentului, cari se mențin, reduc sau se accentuează în parte după tratament. In cazul No. 3 diminuarea trebue datorită procesului acut gripal cu otită supurată.

Ameliorările obținute nu pot fi atribuite, fără discuție, unui mecanism identic cu cel obișnuit în vindecarea rachitismului. Față de creșterea stabilă și accentuată a P-lui din acțiunea razelor ultraviolete și a vitaminei D, la tratamentul cu ipofosfiți P-l crește inconstant sau chiar scade. Scăderea P-lui din cazurile 1, 3, 5 cât și în media totală a cazurilor, deasemenea scăderea produsului Ca×P și creșterea cvoțientului $\frac{Ca}{P}$ apărută sau exagerată după întreruperea tratamentului, după sistarea administrării ipofosfiților, pledează pentru o combatere a ipofosfatemiei prin ipofosfiți, chiar și în cazurile unde nivelul sanghin din timpul tratamentului s'a înregistrat decât un plus neînsemnat sau chiar un minus, datorit însă luptei dintre procesul rachitogen, ipofosfatemizant și dintre ipofosfiți, cari cel puțin temporar credem că pot produce o creștere, fie chiar mai puțin însemnată, de P. Pentru o ridicare cel puțin temporară a P-lui sanghin prin ipofosfiți, admisibilă cel puțin pe scurt timp într'o primă fază de acțiune

după introducerea lor pe cale bucală, cărei faze i-ar urma chiar o exagerare a ipofosfatemiei, aceea adecă din cazurile 2, 3, 4, 5, — pledează și ridicările în nivelul Ca-lui sanghin[1]). Intr'adevăr o creștere a calciului sanghin în rachitism, conform cunoștințelor noastre de azi, nu se poate produce fără o creștere paralelă — chiar și numai temporară sau relativă, adăugăm noi — de P. De altcum formula lui Rona-Takahashi-György: $Ca = f \frac{H}{HCO_4 . HPO_4}$ admite o mărire a calciului ionizat și poate și a celui total, în caz de minus de P. Un asemenea antagonism între Ca-l total și P-ul sanghin s'a văzut și în alte acțiuni, așa în acțiunea adrenalinei, ergotaminei, hioscinei, în tetania de iperventilație, în spasmul din tetania paratireoprivă, idiopatică (Wolmer-Grant, Goldman, György-Vollmer, Popoviciu-Poercanu, Urechia-Popoviciu și Popoviciu-Popescu).

In tot cazul creșterea de Ca, alături de un minus de P, nu poate fi decât trecătoare, urmând ipofosfatemiei, conform mecanismului patogenetic din rachitism, o eliminare mărită de Ca din organism, deci în cele din urmă o scădere a nivelului de Ca sanghin. Dar creșterea, cel puțin temporară, a P-lui sanghin și a calciului, rezultând din cercetările noastre cu ipofosfiți, trebue să aibă un efect curativ cel puțin temporar, să ajute organismul să treacă prin perioade mai critice, și până la un aport de factor specific calci- și fosfofixator (vitamina antirachitică, raze ultraviolete etc.).

In ce privește creșterea globulelor și a hemoglobinei, ea ar putea să fie datorată, în parte cel puțin și ipofosfiților (analog unei acțiuni văzute, la P-ul galben, și de Gowers, Taussig, la phosoform de Remond și Boulicaud). Pe de altă parte însă ar putea să joace un rol sărurile de fer (pirofosfați, citrați), pe cari îi conține siropul de ipofosfiți.

1) In ce privește valorile în genere mai înalte, chiar și în rachitismul netratat din cercetările noastre, v. lucr. *Popoviciu*. Cluj Med. 1. 1928, sau apoi ar putea fi vorba de vindecarea nivelului la Ca din rachitism acumulată de cercetările mai vechi.

No.	Numele și vârsta	Data	Ca mgr. %	% crește sau scade	P mgr. %	%	Ca × P	%	Ca/P	%	Globule roșii	%
1	Pop Ion născut Octombrie 1928	1 II 1929	12,4		3,0		37,2		4,1		3,600,000	
		7 III cu ipotsf.	11,1	— 10,4	3,3	+ 10,0	36,6	— 1,5	3,3	— 19,0	4,000,000	+ [illegible]
		27 III fără ipotsf.	13,5	+ 8,8	2,8	— 6,6	45,8	+ 23,0	4,8	+ 17,0	4,600,000	+ 27
2	Eisenberger Regina născut 21 Sept. 1926	4 II	11,4		3,0		34,2		3,8		4,300,000	
		16 III cu	13,4	+ 17,5	2,5	— 16,6	23,5	— 31,5	5,3	+ 41,6	4,600,000	+ 6,9
		28 III fără	12,6	+ 10,5	2,1	— 30,0	26,5	— 22,9	6,0	+ 64,4	4,700,000	+ [illegible]
3	Brumar Ion născut 3 August 1928	6 II	13,3		3,2		42,6		4,1		5,000,000	
		25 III cu	10,4	— 21,9	2,8	— 12,5	29,1	— 31,3	3,7	— 9,7	4,400,000	— 12
		2 IV fără	11,6	— 12,7	2,3	— 27,1	26,7	— 37,3	5	+ 21,9	5,200,000	+ [illegible]
4	Pledea Zoe născut Decembrie 1927	18 III	10,7		2,7		28,9		3,9		3,600,000	
		26 III cu	12,5	+ 16,8	2,1	— 22,2	26,3	— 9,8	5,9	+ 51,2	5,300,000	+ 47,2
		10 IV fără	12,7	+ 18,6	2,7	0	34,3	+ 18,6	4,7	+ 20,5	4,800,000	+ 33,3
5	Anghi Rafila născut 31 Iulie 1928	28 II	12,2		3,0		36,6		4,1		4,200,000	
		28 III cu	13,2	+ 8,1	2,6	— 13,3	34,3	— 6,2	5,1	+ 24,3	5,300,000	+ 28,6
		10 IV fără	14,8	+ 21,3	1,7	— 43,3	25,2	31,2	8,7	+ 112,1	4,800,000	+ 14,0
6	Sârbu Emil născut 22 Oct. 1928	5 III	10,5		2,0		21,0		5,2		4,000,000	
		8 III cu	11,7	+ 11,4	4,5	+ 12,5	52,7	+ 150,7	2,6	— 50,0	4,800,000	+ 17,5
7	Rat Elisaveta născut 26 Martie 1924	31 I	12,4		2,3		28,52		5,3		4,270,000	
		17 III cu	12,3	— 0,8	5,0	+ 117	61,2	+ 115	2,4	— 36	5,600,000	+ 31,1
		2 IV fără	13,9	+ 12,1	5,4	+ 134,7	75,1	+ 135,1	2,5	— 52,8	5,600,000	+ 31,1
Media	Caz 1—7 cu ipotsf.			+ 3,0		+ 26,0		+ 16,0		+ 0,3		+ 21,4
	Caz 1—5, 7 fără ipotsf.			+ 9,8		+ 4,6		+ [illegible]		+ 30,5		+ 10,0
	Caz 1—6 cu			+ 3,6		+ 11,7		+ 11,7		+ 6,4		+ 19,8
	Caz 1—5 fără			+ 9,3		— 21,4		— 9,9		+ 47,2		+ 17,5

	Leucocite	%	Hemoglobina	%	OBSERVAȚIUNI CLINICE
	8,000		75,0		Spasm piloric. Gripă, otită. Distrofie. Rachitism, transpirațiuni, îndeosebi la cap. Ușor craniotabes. Mătănii costale. Alopecie occipitală.
	13,000	+ 22	76,0	+ 1,3	Splina palpabilă. Caput quadratum. Bose frontale. Fontanela deschisă pentru 2 degete.
	12,000	+ 3	57,0	0	Nu șade. Fontanela mai redusă. Splină puțin palpabilă. Transpirațiunile mai reduse.
	[illegible]		57,0		Distrofie Rachitism. Torace în formă de pâlnie, mătănii costale foarte pronunțate. Brațe[illegible] Fontanela deschisă pentru 2 degete.
	[illegible]	+ 7,3	65,0	+ 14,0	Transpirațiuni intense. Funcțiunile statice întârziate. Aceeaș stare, dar transpiră mai puț[illegible]
0,3	14,000	+ 7,6	65,0	+ 14,0	Starea generală mai bună, se ridică spontan.
	17,000		70,0		Distrofie, rachitism. Otită medie supur, Mătănii costale. Transpirațiuni Vegetații adenoide. Pirque[illegible]
- 12	13,000	— 23,5	70,0	0	St. idem.
+ 4	12,000	— 29,4	85,0	+ 22,0	Starea generală mai bună. Crește în greutate 350 gr. Funcțiunile statice întârziate nu se ridică. Du[illegible] săptămână fenomene de meningită tbc. + la 13 IV. Autopsie: tbc. mil.
	6,500		75,0		Distrofie, rachitism. Lipsa dentiției, mătănii costale, transpirațiuni, abdomen balonat torace lărgit la ba[illegible] Splina palpabilă. Funcțiuni statice întârziate.
47,2	9,700	+ 49,3	90,0	+ 20,0	Starea generală mai bună, se ridică spontan. Transpirațiunile reduse. Splina se palpează.
33,3	8,000	+ 23,1	86,0	+ 14,6	Starea se menține bună. Dinții încă nu apar.
	9,500		+ 75,0		Distrofie, rachitism, transpirațiuni, mătănii costale, Dinții lipsesc. Nu se ridică.
- 26,9	15,000	+ 57,8	80,0	+ 6,6	Aceeaș stare. Apar incisivii inf.
- 14,0	92,000	+ 26,1	85,0	+ 13,3	Aceeaș stare, nu crește în greutate.
	10,500		70,0		Rachitism, diateză exudativă. Abdomen balonat. Fontanela de 3 degete. Ușor craniotabes. Mătănii [illegible] Transpirațiuni abondente
- 17,3	12,000	+ 14,2	75,0	+ 7,1	Craniotabesul dispărut, transpirațiunile diminuate. Nu șade. Crește în greutate 500 gr.
	8,300		60,0		Kerato-conjunctivită eczematoasă. Peritonită și pleuresie.
+ 31,1	8,500	+ 2,4	78,0	+ 30,0	Starea generală mai buă.
+ 31,1	8,000	— 3,6	82,0	+ 36,6	Transpirațiunile reduse
+ 21,4		+ 18,5	+ 10,6		
+ 19,6		+ 9,5	+ 16,4		
+ 10,8		+ 21,2	+ 7,3		
+ 17,5		+ 12,1	+ 12,4		

Rolul acestor factori aparte, va trebui lămurit în cercetări viitoare cari vor trebui să studieze exclusiv numai acțiunea sărurilor de ipofosfiți. Date fiind discuțiunile și părerile contrazicătoare asupra acțiunilor terapeutice pe cari le au preparațiunile hematopoetice, (arsenical) în genere tratamentele roborante, stimulante ale metabolismului asupra modificărilor osoase rachitice, cât și îndreptarea turburărilor organelor hematopoetice, în primul rând a funcțiunei măduvei osoase (Marfan-Baudouin, Aschenheimer-Benjamin, Hutinel-Tixier, Ziegler, Oehmer, Schmorl, Christeller, Nipescu, Popoviciu-Ungureanu, Fuchs-Priesel, Seel etc.), ar fi pripit să decidem de acum dacă ameliorările clinice pe cari le-am văzut în aceste cazuri de rachitism, nu cumva ar trebui să le atribuim, în parte cel puțin, și celorlalte substanțe pe cari le conține siropul, în afară de ipofosfiți.

Concluziuni: In decursul acțiunei terapeutice a ipofosfiților (Syr. Hypophosph. comp. Egger) din rachitism, am observat ameliorări clinice cât și modificări în Ca și P, cari ce-i drept sunt mai puțin accentuate și mai inconstante, uneori în parte și în aparență chiar contrare acțiunei terapeutice a razelor ultraviolete și a vitaminei antirachitice D; totuși par să existe, cel puțin în parte, oarecari analogii între mecanismul acțiunii ipofosfiților și a tratamentului antirachitic „specific". In același timp am putut să observăm aproape fără excepție, o creștere evidentă a globulelor roșii, albe și hemoglobinei din cazurile tratate. Dacă aceasta se datorează ipofosfiților sau terului din siropul de ipofosfiți, va trebui elucidat în cercetări viitoare. In tot cazul efectele asupra globulelor din sânge și asupra hemoglobinei ar putea să explice în parte ameliorările obținute prin acest tratament. Toate aceste acțiuni justifică întrebuințarea siropului de ipofosfiți ca deosebire ca tratament temporar, sau adjuvant, alături de tratamentul specific, în rachitism și în alte turburări ale metabolismului de Ca și P, cât și o larga utilizare, chiar și fără alte tratamente, în anemii și stări de denutriție.

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THE EFFECT OF CERTAIN SULFUR COMPOUNDS ON THE COAGULATION OF BLOOD

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In the course of some metabolism experiments with various sulfur-containing compounds, carried out in the laboratory of the Lankenau Hospital Research Institute, confirmation of the inhibiting effect of cysteine on coagulation reported by Mueller and Sturgis (1932) was obtained. A similar effect of methionine was observed in *in vivo* but not in *in vitro* experiments. In an attempt to explain the mechanism of this inhibiting action, the following series of experiments was undertaken.

I. *In vitro experiments with whole blood.* Cysteine hydrochloride, taurine, methionine, glycine, alanine and cysteic acid, neutralized to pH 7.0 ± 0.1, were added in graded amounts to whole blood giving final concentrations of from 4.4×10^{-5} to 0.18 *M*, and the coagulation time was determined by the 8 mm. tube method. The results are summarized in figure 1. The abscissae represent molar concentrations of the substances, 0.4 cc. of which was added to 1 cc. of whole blood. It may be seen that a marked inhibition of coagulation occurred when taurocholic acid, taurine and cysteine were employed, whereas methionine had no effect to the limit of its solubility.

II. *In vitro experiments with the isolated components of the blood clotting system.* The components of the coagulation system were isolated by the following methods: Prothrombin was prepared from horse, rabbit and human plasmas by the method of Mellanby (1931). Fibrinogen was repeatedly salted out from horse plasma with sodium chloride (Eagle, 1934–35). The tissue factor was obtained from two sources: a, platelet suspension (Eagle, 1934–35), and b, desiccated rabbit lung (Eagle, personal communication), by drying in the Flosdorf-Mudd desiccator.[1] Calcium was added as 1 per cent $CaCl_2$. The pH was controlled at 7.0 ± 0.1 with bromthymol blue as indicator. From a stock solution of cysteine hydrochloride, freshly prepared immediately before using and adjusted to pH

[1] The authors are indebted to Sharp and Dohme for generous supplies of horse blood and also to Doctor Flosdorf of the Department of Bacteriology of the University of Pennsylvania for use of their Flosdorf desiccator.

7.0 ± 0.1 with NaOH, a series of dilutions was made. In a typical experiment the following procedure and amounts of constituents were used:

Into a carefully cleaned test tube (70 mm. × 8 mm. inside diameter) were placed 0.01 cc. of $CaCl_2$ solution and approximately 2 mgm. of desiccated rabbit lung or 0.02 cc. of platelet suspension. Two-tenths cubic centimeter of prothrombin solution was added, the material thoroughly mixed by shaking the tube and allowed to stand for 10 minutes (which time was found to be adequate for maximum formation of thrombin). Eight-tenths cubic centimeter of fibrinogen solution was added, the

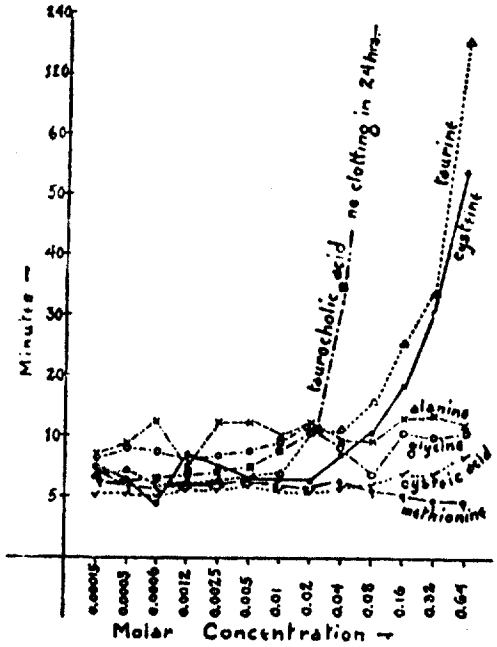

Fig. 1

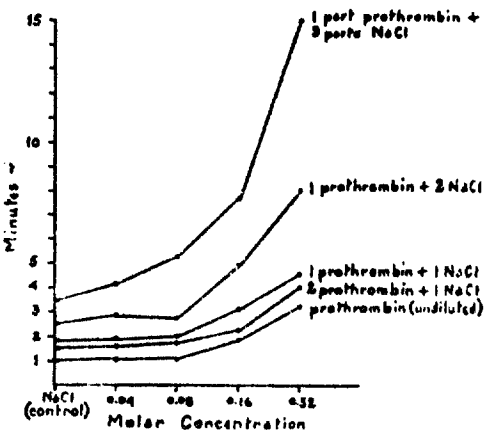

Fig. 2

Fig. 1. Effect of various compounds on the coagulation of whole blood. Abscissae, molar concentrations added, 0.4 cc. to 1 cc. of whole blood. Ordinates, time in minutes.

Fig. 2. Effect of cysteine on coagulation time with varying concentrations of prothrombin. Abscissae, molar concentrations of cysteine. Ordinates, coagulation time in minutes.

materials mixed by a few quick shakes, and the tube examined for coagulation at 10 to 15 second intervals until it could be inverted without appreciable change in the level of the contents. The coagulation time, the time elapsing between the addition of fibrinogen and the clotting, ranged with different batches of the preparation from 30 seconds to 4 minutes, with the majority around 1 minute. The addition of cysteine at different steps in the procedure will be described below. As a control, physiological salt solution (0.85 per cent NaCl) was added in similar amounts at the corresponding time.

a. *Effect of cysteine on coagulation with varying dilutions of prothrombin.* From a solution of prothrombin which gave a coagulation time of 1 minute,

varying dilutions with physiological salt solution were made. To tubes containing calcium and tissue factor, 0.4 cc. of cysteine in concentrations from 0.32 *M* to 0.04 *M* followed by 0.2 cc. of the prothrombin in a series of dilutions in physiological salt solution were added and allowed to stand for 10 minutes before the addition of fibrinogen. The coagulation times, given in figure 2, are increasingly prolonged in the tubes containing 0.16 and 0.32 *M* cysteine, with an accentuation of the curve in those tubes with high concentrations of cysteine and more dilute prothrombin solutions.

TABLE 1

Effect on coagulation time of cysteine added before and after thrombin formation

Calcium chloride sol. (1 per cent) 0.01 cc.; platelet suspension, 0.02 cc.; prothrombin sol., 0.2 cc. cysteine hydrochloride (neutralized) in varying concentrations and 0.8 cc. fibrinogen solution were employed. In *a* cysteine was added before the prothrombin, in *b* cysteine was added 10 minutes after the prothrombin.

	CONTROL	MOLAR CONC. CYSTEINE					
		0.02	0.04	0.08	0.16	0.32	0.64
a. Cysteine added before thrombin formation. Coag. time (mins.)	4	4	7	24	77	167	600
b. Cysteine added after thrombin formation. Coag. time (mins.)		4	4	4	6	7	17

TABLE 2

Effect on coagulation time of varying periods of thrombin formation in the presence of cysteine

	COAGULATION TIME									
	Period of thrombin formation (min.)									
	5	10	15	20	30	60	120	180	240	300
Control (sec.)	60	60	60	55	60	55	60			
0.04 *M* cysteine (sec.)	60	55	60	60	65	60	60			
0.16 *M* cysteine (sec.)	85	80	105	110	140	150	140			
0.32 *M* cysteine (sec.)	370	320	310	425	425	410	355			
0.64 *M* cysteine (min.)	117	216	246	180	526	180	100	155	190	215

b. *Effect of cysteine on coagulation when added at different phases of the coagulation process.* Table 1 shows the marked difference in the effect of cysteine when added at different phases of the coagulation process. If thrombin is allowed to form for 10 minutes before cysteine is added, the inhibition of coagulation is relatively slight compared with the considerable delay in clotting occasioned by the addition of cysteine before thrombin formation, i.e., when cysteine in 0.32 and 0.64 molar concentrations was added before thrombin formation, the coagulation times were 167 and 600

minutes, respectively, as against 7 and 17 minutes when the cysteine in the same concentrations was added after thrombin formation.

c. *Effect on coagulation time of varying periods of thrombin formation in the presence of cysteine.* Calcium, tissue factor, cysteine and prothrombin were allowed to react for varying intervals of time before the fibrinogen was added. The results, given in table 2 show that the maximal inhibition of coagulation was obtained within 5 minutes and that no additional thrombin was formed during the following 2 to 5 hours.

TABLE 3

Effect of cysteine on thrombin

Two-hundredths cubic centimeter 1 per cent $CaCl_2$, 0.4 cc. prothrombin solution and approximately 2 mgm. desiccated rabbit lung, allowed to stand 10 minutes. Four-tenths cubic centimeter of 0.64 *M* cysteine was added followed by fibrinogen after varying periods. The control tube, in which the cysteine solutions were replaced with physiological salt solution, clotted in 60 seconds.

	TIME BETWEEN ADDITION OF CYSTEINE AND FIBRINOGEN (MIN.)						
	5	10	15	20	30	60	90
Coagulation time (sec.)	210	305	335	515	570	705	645

TABLE 4

Effect of various substances on coagulation time when added to the system before and after thrombin formation

	CONTROL NaCl	MOLAR CONC.			
		0.04	0.08	0.16	0.32
a. Added before thrombin formation:					
Cysteine (sec.)	75	95	160	230	360
Ascorbic acid (sec.)	95	95	95	95	95
Phenosafranine (sec.)	120	120	125	120	120
Taurine (sec.)	90	95	95	100	95
Taurocholic acid (sec.)	85	3 hrs.			
Taurocholic acid (sec.)	85	3 hrs.			
b. Added after thrombin formation:					
Taurocholic acid (sec.)	100	450	20 min.	43 min.	76 min.

d. *Effect of cysteine on thrombin for varying periods, as indicated by coagulation time.* Thrombin was allowed to form during the usual 10 minute period. Then 0.4 cc. of 0.64 *M* cysteine was added, followed by fibrinogen solution at varying intervals from 5 to 90 minutes (table 3). The slight inhibiting effect on coagulation became constant after about 30 to 60 minutes.

e. *Effect of various other substances on coagulation before and after thrombin formation.* Ascorbic acid and phenosaphranine, two compounds with reducing properties comparable to those of cysteine, produced no effect on rate of coagulation, as seen in table 4. Taurine, added to the coagulation system set up with isolated components, had no effect, contrasting with the marked effect produced when added to whole blood. Taurocholic acid, however, showed a more powerful inhibiting action than cysteine when added before thrombin formation, no clotting taking place when 0.08 *M* or

TABLE 5

Effect of cysteine on platelets (tissue factor)

Platelets obtained from horse plasma was subjected to cysteine or cysteine and $CaCl_2$ according to the procedure below. Control tests were made similarly with physiological salt replacing the cysteine.

(a) 0.5 cc. platelets + 1.0 cc. 0.64 *M* cysteine.
(b) 0.5 cc. platelets + 0.25 cc. of 1 per cent $CaCl_2$ + 1.0 cc. 0.64 *M* cysteine.
(c) 0.5 cc. platelets + 1 cc. physiological salt solution.
(d) 0.5 cc. platelets + 0.25 cc. 1 per cent $CaCl_2$ + 1 cc. physiological salt solution.

The platelets were recovered free from cysteine and 0.02 cc. used in coagulation tests as in the routine procedure.

	PLATELETS RECOVERED FROM			
	a	b	c	d
Coagulation time (sec.)	70	75	75	65

TABLE 6

Effect of cysteine on fibrinogen and prothrombin as indicated by the coagulation time

(a) Fibrinogen and (b) prothrombin treated with cysteine and recovered. Coagulation time by the 8 mm. tube method.

	COAGULATION TIME			
	CONTROL NaCl	Molar conc. cysteine		
		0.16	0.32	0.64
Fibrinogen (sec.)	40	40	50	50
Prothrombin (sec.)	80	305	28 min.	540 min.

greater concentrations were added. It also affected the rate of clotting when added after the thrombin was formed, though the effect was far less than before its formation.

f. *Effect of cysteine on the individual components of the coagulation system.* Five-tenths cubic centimeter of platelet suspension and 1 cc. of 0.64 *M* cysteine were placed together for 15 minutes and centrifuged to recover the platelets. The latter were repeatedly washed with physiological salt solution and centrifuged until free from traces of SH. In a similar manner,

platelet suspension plus $CaCl_2$ solution was treated with cysteine and the platelets recovered. In table 5 it may be seen that platelets so treated were as effective as control platelets in producing coagulation.

Samples of fibrinogen were subjected to concentrations of cysteine from 0.16 to 0.64 *M* for 10 minutes, reprecipitated, washed free from traces of SH, and taken up in buffered physiological salt solution. The results (table 6) show no effect on the fibrinogen as indicated by the coagulation rate. No appreciable gross difference in the precipitate was detected. When prothrombin was similarly treated, however, the delay in coagulation was marked and was directly proportional to the concentrations of cysteine (table 6). There was a slightly greater yield in the control and in the lower concentrations of cysteine, but there was no apparent variance in the character of the precipitate.

A sample of rabbit plasma (citrated (0.5 per cent) blood) was added to twice the amount of 0.64 cysteine, after which the prothrombin was extracted in the manner described above. This prothrombin failed to cause coagulation in 6 hours, although the control, treated similarly with physiological salt, gave no greater yield of precipitate, but caused coagulation in the normal range of time.

As a further control prothrombin was subjected to a 0.64 *M* concentration of sodium hypophosphite, with no inhibition of the coagulation time over the control of 1 minute.

III. *In vivo experiments in human subjects.* a. *Methionine.* The effects produced by the ingestion and intravenous injection of methionine in 2 human subjects are shown in figure 3. For the intravenous administration, 1.31 gram of methionine was dissolved in 50 cc. of water, buffered to pH 7.0 + 0.1 and injected into the median basilic or cephalic veins during 2 minutes. The bleeding time was determined by the method of Ivy (Ivy, Shapiro and Melnick, 1935) and the coagulation time by the 8 mm. tube method.

In both intravenous tests, depicted for the two subjects in curves *A* and *B*, the coagulation and bleeding times were definitely prolonged, although the latter was less marked in the case of *B*. The effect on the coagulation time continued almost twice as long as on the bleeding time. Curves *C* represent the effects of ingestion of 1.31 gram of methionine and curve *D*, the effects of ingestion of 3.35 grams. Following ingestion of the smaller amount, the duration of the effect was about the same for the bleeding time as for the coagulation time. After ingestion of the 3.35 grams, the curve rose higher and was more prolonged than after the ingestion of 1.31 gram.

b. *Cysteine.* One and thirty-one hundredths gram of cysteine hydrochloride in 50 cc. of water and neutralized to about pH 7.0, was injected intravenously in one fasting subject and a similar amount ingested by a second. The results are shown in figure 4. The Ivy bleeding time was increased in

both cases, rising more abruptly and falling more quickly following injection than following ingestion. The coagulation time remained within normal limits in both instances.

At intervals during the experiment, the coagulation time was determined in the presence of varying concentrations of cysteine (table 7). The usual inhibiting effect on coagulation of added cysteine was much less marked in those cases in which blood was taken at 27 minutes after ingestion and 20 minutes after injection as shown in the table, than when taken at the beginning of the experiment or at a later period. The "buffering effect"

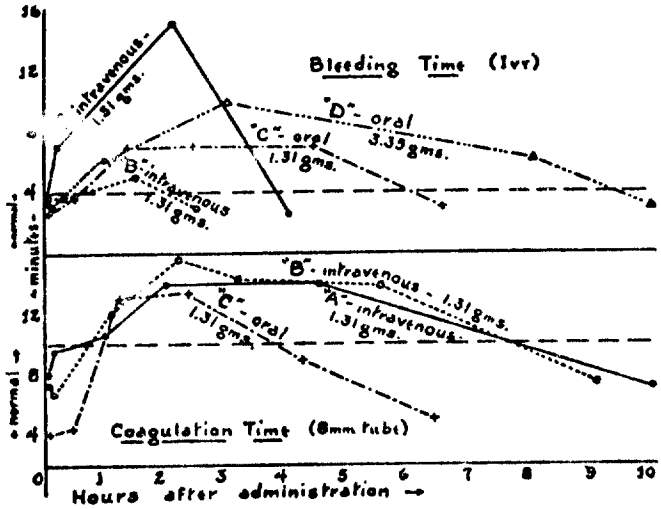

Fig. 3. Effect of ingestion and intravenous injection of methionine on bleeding time and coagulation time. Upper set of curves, Ivy bleeding time, lower set, coagulation time by the 8 mm. tube method. Curves *A* and *B*, intravenous injection of 1.31 grams methionine; *C*, ingestion of 1.31 grams; *D*, ingestion of 3.35 grams. *A* and *D* on the same subject, *B* and *C* on a second subject. Abscissae, hours after administration. Ordinates, bleeding and coagulation time, respectively.

coincided with the period of greatest prolongation of the bleeding time. (See fig. 4.)

IV. Discussion. The experimental work shows that cysteine and methionine *in vivo* and cysteine *in vitro* exert a marked effect upon the mechanism of coagulation. The effect is mainly upon only one factor of the coagulation system, prothrombin. The change in the nature of this protein is proportional to the amount of cysteine added, although no loss of SH could be detected in the supernatant liquid after reprecipitating out the prothrombin. Moreover, the effect appears to be a qualitative one, for the quantity of prothrombin recovered after addition of cysteine to a prothrombin solution, is grossly equal to that of the control precipitate. The presence or absence of the cysteine, once the nature of the prothrombin

has been affected, has little or no action on the mechanism of coagulation, since fibrinogen is unaffected and thrombin only slightly inactivated.

The nature of the action on prothrombin has not been determined. Sodium hypophosphite did not inactivate prothrombin, and ascorbic acid and phenosafranine, the latter especially having oxidation-reduction potentials closely similar to that of cysteine, produced no effect when added to the reassembled isolated components. It seems hardly probable, therefore, that the effect of cysteine is due to its reducing action. Its mode of attack is certainly different from that of taurine, which caused no delay

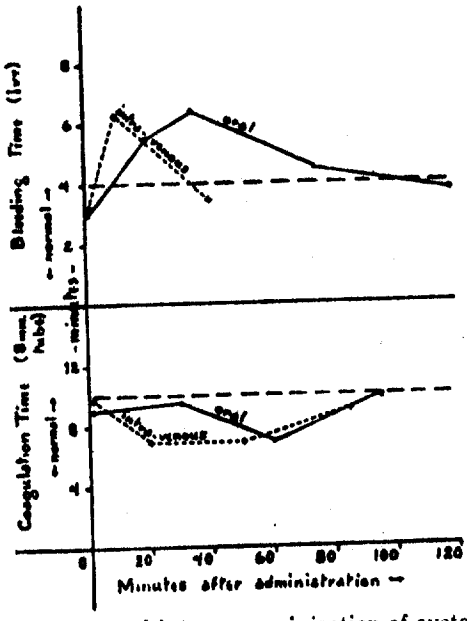

Fig. 4. Effect of ingestion and intravenous injection of cysteine on bleeding time and coagulation time. One and twenty-one hundredths grams cysteine hydrochloride (neutralized) ingested and injected in 2 different subjects. Abscissae, time in minutes after administration. Ordinates, bleeding and coagulation times, respectively.

when added similarly to the reassembled system. Taurocholic acid, on the other hand, though far more powerful in its inhibiting action, resembled cysteine in that its effect, when introduced before thrombin formation, greatly exceeded that produced when added afterwards. It is difficult to visualize any chemical action which could be possessed in common by cysteine and taurocholic acid. In view of the widespread interest recently developed in the relation of sulfhydryl to various enzyme systems and in the denaturing of proteins (Mirsky and Anson, 1934) it would be of interest to determine if a change in the number of sulfhydryl groups has occurred in the prothrombin.

These studies would confirm those of Carr and Foote suggesting that cysteine may be a responsible agent in the production of the hemorrhagic tendency in jaundice, but would indicate the defect in the formation of thrombin from prothrombin rather than in the fibrinogen factor suggested by them. In a preliminary experiment with a jaundiced patient the isolated prothrombin gave a prolonged coagulation time over a control prothrombin. But in view of the powerful inhibiting action of taurocholic acid, under conditions similar to that shown by cysteine, it would be difficult to separate these factors in jaundice.

While both the bleeding time and the coagulation time were prolonged abnormally in most instances following the administration of cysteine and methionine these deviations from normal did not always correspond.

TABLE 7

Buffering effect of normal blood toward coagulation-inhibiting action of cysteine: a. After ingestion of 1.31 grams of cysteine, and b, after intravenous injection of 1.31 grams cysteine

Coagulation time expressed in minutes

	CONTROL	MOLAR CONC. CYSTEINE		
		0.08	0.16	0.32
(a) After ingestion				
1 fasting	9	31	47	100
2 27 min.	9	12	16	41
3 1 hr.	7	11	21	75
4 1 hr. 36 min.	10	29	38	7[illegible]
(b) After injection				
1 fasting	10	15	23	[illegible]
2 20 min.	7	8	9	[illegible]
3 50 min.	7	10	14	40
4 1 hr. 25 min.	9	15	26	54

In figure 3, experiment *B*, the bleeding time following the intravenous injection of methionine is but slightly increased, in contrast to the marked variation from normal as seen in *A* under similar conditions. The corresponding coagulation time curves in these two experiments were almost identical. The results, in figure 4, show an abnormally prolonged bleeding time. In other similar experiments, not shown, the bleeding time was similarly high, and there was also a considerable increase in the coagulation time. Ivy, Shapiro and Melnick (1935) suggest that "prolonged coagulation time in jaundice is not so much an index of bleeding tendency as of liver damage." The work reported above seems to indicate that disturbance in sulfur-amino acid metabolism, without necessarily involving liver damage, may be responsible for a delayed coagulation time as well as prolonged bleeding time.

The effect of methionine *in vivo* is in sharp contrast to its behavior *in vitro*. In experiments of the latter type, there was no delay in coagulation with final concentrations of methionine up to 0.09 *M*, whereas following the administration of methionine to the human subject, with an estimated final concentration in the blood stream of 0.0016 *M*, there was a delay in the coagulation time to 12 to 16 minutes. A bleeding time, taken 10 minutes after the intravenous injection of 1.31 gram, was almost twice the value of the upper normal limit; the coagulation time, done about the same time, was normal but reached definitely elevated values after an hour. Was there later a demethylation of the S-CH_3 grouping (i.e., production of homocysteine) to bring about the coagulation delay, or has the methionine stimulated the release or production of some other substance (i.e., cysteine) to effect the change?

SUMMARY

1. Cysteine, taurine and taurocholic acid, added to whole blood, delay coagulation.

2. The action of cysteine in inhibiting coagulation is on prothrombin, preventing activation to thrombin. Tissue factor, calcium, thrombin and fibrinogen are little or not affected by it.

3. Ascorbic acid, phenosaphranine and sodium hypophosphite do not show any inhibiting effect under the conditions of these experiments.

4. Cysteine and methionine, administered orally and intravenously in the human subject, prolong both the bleeding time and the coagulation time.

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Comparative research on the elimination of drugs administered intravenously and subcutaneously.

I

A) Much is known about the elimination of drugs. For example, it is known that volatile substances leave the organism through the lungs, that non-volatile substances are mostly eliminated through the kidneys; that some, like heavy metals, are electively eliminated through the mucous membrane of the large intestine. Not a few drugs are eliminated through the bile, others through the saliva; the lachrymal glands, the skin, the mammary glands are channels for elimination.

But while this knowledge is now certain, while we know precisely the elimination of various drugs through channels that may be called elective, there is a lack of comparative researches on the influence of the method of administration upon the elimination of drugs.

This is a wide field that deserves deep study because of its importance.

I limited my study to the influence of intravenous or subcutaneous administration on the quantitative elimination of drugs.

The importance of this problem today is very great, considering that intravenous administration of drugs is becoming more and more widespread. The reason why current therapy follows this direction is clear if we consider that the intravenous injection of a drug brings a really conspicuous quantity into circulation rapidly, so that the concentration of the drug circulating in the organism is much greater than that obtained when the drug is administered by any other method. When, for example, a drug is administered subcutaneously, or gastrically, it reaches the blood a little at a time, as it is gradually absorbed, but, as soon as it is in circulation, the organism tends to expel it through the elimination channels. The concentration of the drug able to produce the pharmacological or therapeutic action is given by the difference between the quantity of the drug that enters the blood and the quantity that is eliminated. Instead, by intravenous injection, the whole mass of the drug that must act is brought into circulation, and the elimination, that begins immediately, cannot be such as to reduce, in most cases, the concentration of the drug as it happens in the case of subcutaneous or gastric administration.

There is an exception to this rule when we inject into the veins drugs that, as soon as they arrive in the blood, are rapidly transformed into volatile products. In such case, we may obtain two types of consequences: either the volatile product released by the drug has little toxicity and is rapidly eliminated through the respiratory organs (such as sodium cacodylate, transformed into cacodyl, rapidly eliminated by the lungs), or the volatile product is highly toxic and rapidly kills by acting upon the vital centres (such as colloidal sulphur, rapidly transformed into sulfureted hydrogen, of extremely high toxicity).

B) From these preliminary remarks there arises a problem that has not yet been taken into consideration: does the elimination of drugs administered intravenously take place more rapidly than when the subcutaneous method is adopted, or the gastric method? Is the amount of the drug that is eliminated the same or different?

Before undertaking a study of the literature on this problem, let us solve the question theoretically, on the basis of what present knowledge teaches us. When we inject a drug into the veins, the drug, unless it is a substance that exerts its action upon the blood, is fixed in all the tissues of the organism, and probably better and in a larger amount in those organs upon which it acts electively. It is probable that the drug is also fixed in the organs that eliminate it, inhibits their action and then its elimination does not take place as rapidly as when it is introduced by other methods of administration. It may be that, in practice, both cases occur: that is, that certain drugs of little toxicity actually stimulate the organs charged with their elimination, so that a very rapid elimination follows, but the opposite may also be true. It is clear that only experiments on animals can provide the solution of this problem.

C) Before beginning the study of the subject, it is useful to examine the literature concerning it.

I do not know of researches carried out for the purpose that I had in my experiments, but I have seen some works that may be considered close to them.

Thus, for example, JENSELME (1) found arsenic in the urine one hour after an intravenous injection of "606" and demonstrated its presence for 4 more days, while following an intramuscular injection of the same drug, he instead found arsenic in the urine in the 3-6 days after the administration.

MOREL obtained similar results.

GALONNIER (2), following administration of "selfo-arsenolo", found that, if the intravenous method is used, the elimination of the arsenic takes place in the first hours after the introduction of the drug, while, if the method of administration was intramuscular, the elimination of the arsenic lasts for 2 days. I also recall the experimental researches on some bismuth preparations carried out by PERANTONI, who found that in man bismuth begins to be eliminated in a period ranging between 4 and 20 hours after injection of the drug, if the endomuscular method is used, while the presence of metal is found in the urine even after one and a half hour when usin the intravenous method. Absorption and elimination therefore proceed in step, very rapidly with intravenous injection, less rapidly with intramuscular injection.

All these researches are qualitative. The most important experiments, from my viewpoint, are those conducted by TESTONI, (4) (5), who undertook a comparative examination of the quantitative elimination of "aspirochyl" and "solarson", administering both drugs intravenously and subcutaneously.

In the experiments with "aspirochyl", he observed that when the method of administration is intravenous, arsenic is eliminated in greater amount and more rapidly than when the method of administration is subcutaneous: in experiments with "solarson", on the contrary, he observed that arsenic passes through the renal filter in greater amount and more rapidly when it is introduced subcutaneously than when it is injected into the veins.

These few data obtained so far on this question show such inconsistent results that they clearly demonstrate that it is not yet possible to determine the rule governing the relationship between absorption and elimination of drugs depending on the method of intro-

duction and that only experiments covering a large number of drugs will be able to clarify this interesting problem.

II. - EXPERIMENTAL RESEARCHES

As I had to conduct accurate comparative researches, I chose three drugs of little toxicity, having a limited local effect, that are rapidly absorbed even after subcutaneous administration. They are: sodium hypophosphite, potassium iodide, sodium salicylate.

A. - Sodium hypophosphite

This drug, as is known from old and recent researches, passes through the organism without modifications and is nearly entirely found in the urine (6-7-8).

In order to dose it in the urine, I transformed it by oxidation into phosphates. Several methods are appropriate:

1) Oxidation by means of HNO_2;
2) Oxidation by means of $KClO_2$ and HCl.

The best results are obtained with the second method. I used highly pure hypophosphite and, because of its hygroscopicity, I prepared a solution of it and titrated it every time before using it.

After administration of sodium hypophosphite to rabbits, I collected the urine, measured its volume and then took two equal amounts.

In the first, I dosed phosphoric anhydride directly by means of a titrated solution of uranyl acetate, according to the well-known rules; I oxidised the second portion and then dried it in a double boiler; after dissolving the residue in distilled water, I acidified the mixture with acetic acid and potassium acetate, then determined the phosphoric anhydride that was present with the uranyl solution. The first determination showed the quantity of phosphoric anhydride due to the phosphates pre-existing in the urine; the second showed the phosphoric anhydride of the pre-existing phosphates plus that due to the oxidation of hypophosphites. The difference between the figures obtained in the two determinations represented the phosphoric anhydride of the phosphates produced by the oxidation of the hypophosphites, that were eliminated without alteration.

Two blank tests conducted before the experiments on

animals yielded the following results:

1) out of g. 1 of hypophosphites, I found g. 0.984, that is 98.4%.
2) out of g. 2 of hypophosphites, I found g. 1.966, that is 98.3%.

After I had a good method for measuring hypophosphites in the urine, I proceeded to the experiments on rabbits. The results of the experiments are reported in table I.

B. - Experiments with potassium iodide

a) Iodides are very well-known drugs, in common use in the practice of medicine (9-10). From the numerous researches conducted on the absorption and elimination of iodides, it appeared certain that these easily soluble salts, are rapidly absorbed by the gastrointestinal organs, dispersed in the organism and then very rapidly appear in its various secretions. Thus, iodides were found in saliva 7-12 minutes after ingestion; in urine, 3-13 minutes later.

Experimental researches demonstrate that they are mostly eliminated through the renal emunctory; but it is also very well known that a certain part remains in the organism.

In past years, the question of absorption and elimination of iodides became of great practical interest, especially because of the studies and researches by SCHLAZERS and TAKAZASUS, in order to determine a clinical test of renal functions.

SCHLAZERS and TAKAZASUS believe that, while the elimination of g. 0.50 of potassium iodide normally takes a constant time of 54-60 hours, the time of elimination is greatly prolonged by renal insufficiency;

With respect to the amount of iodide that is eliminated, it appears from SALAZAR's (11) experiments that, depending on the dose that was administered, between 83 and 96 percent of the salt that was introduced is found in the urine, and specifically that the elimination is weaker with a smaller dose, while with larger doses the elimination is greater; this is also the result of experiments conducted by BURHHOLTZ (12).

Table I

Number of the Experiment and Method of Administration	Weight of the Rabbit in Kg	Date of Injection	cc. of Solution Injected	Date of Collection of the Urine	$NaH_2PO_2H_2O$ in cc. Injected: Total	$NaH_2PO_2H_2O$ in cc. Injected: Per Kg	Urine Expelled in 24 Hours: In cc.	Urine Expelled in 24 Hours: Reaction	P_2O_5 due to Pre-existing Phosphates	P_2O_5 due to Hypophosphites	Eliminated Hypophosphite	Percentage of Hypophosphite Eliminated
I (Intravenous)	2.00	17-3-28	cc. 10 of a 10% solution of Sodium Hypophosphite	—	1.00	0.50	—	Alkaline	—	—	—	—
				18-3-28	—	—	240		0.080	0.526	0.78.50	78.50
				19-3-28	—	—	290		0.085	0.014	0.0208	2.08
				20-3-28	—	—	180		0.090	0.005	0.0071	0.71
				21-3-28	—	—	190		0.090	—	Traccie ind.	—
												81.29
II (Intravenous)	2.00	28-3-28	cc. 20 of a 10% solution of Sodium Hypophosphite	—	2.00	1.00	—	Alkaline	—	—	—	—
				29-3-28	—	—	135		0.065	1.0965	1.6265	81.43
				30-3-28	—	—	140		0.075	0.0205	0.0390	1.91
				31-3-28	—	—	160		0.070	0.0080	0.0116	0.58
				1-4-28	—	—	153		0.070	—	Traccie ind.	—
												83.85
III (Subcutaneous)	1.500	26-3-28	cc. 75 of a 10% solution of Sodium Hypophosphite	—	0.75	0.50	—	Alkaline	—	—	—	—
				27-3-28	—	—	150		0.0785	0.4025	0.6008	80.10
				28-3-28	—	—	75		0.075	0.0015	0.0082	1.09
				29-3-28	—	—	130		0.086	0.0036	0.0051	0.68
												81.87
IV (Subcutaneous)	1.600	30-3-28	cc. 16 of a 10% solution of Sodium Hypophosphite	—	1.50	1.00	—	Alkaline	—	—	—	—
				31-3-28	—	—	185		0.065	0.855	1.2764	79.77
				1-4-28	—	—	180		0.075	0.0167	0.0157	0.98
				2-4-28	—	—	200		0.080	0.0085	0.0126	0.78
												81.53

The latter dealt in particular, in a series of researches, with the iodide content of the blood, following theis ingestion, and reached the following conclusions:

a) After the administration of a greater quantity of potassium iodide, the iodine percentage in the blood rises sharply in the first hour and reaches its peak 2-3 hours after the administration.

b) The blood iodine concentration is directly and constantly related to the quantity of iodine contained in the organism.

c) If fractional doses of KI were administered during the whole day, the blood iodine concentration would rise after each administration, then slowly decrease. When a constant concentration is required, it is thus necessary to administer very small fractional doses during the whole day.

d) Most of the administered iodide is eliminated through the urine, where 9/10 of the quantity that was administered are often found.

I am not considering other researches on this subject because they are not closely related to my work.

B. - METHODOLOGY

The method that I followed in the quantitative research of iodides in the urine of rabbits, following administration of highly pure KI both intravenously and subcutaneously, is that of BESNIER and PERON, who describe it as follows (13): 50 cc. of urine are alkalized with 5 cc. of a saturated solution of sodium carbonate, then evaporated, the dry residue is carbonized, then exhausted with boiling water. Crystals of potassium permanganate are slowly added to the resulting solution, that is brought to and kept boiling for some minutes, until a violet color remains. By boiling the alkaline solution together with potassium permanganate, the iodides are oxidized into iodate according to the equation:

$$KI + KMnO_4 + H_2O = KIO_3 + 2MnO_2 + 2KOH$$

Destroy the premanganate in excess by adding a few cc. of alcohol to the hot liquid. Thus the permanganate in alkaline solution is first reduced to manganate and

later to manganese dioxide, that is deposited in the form of a brown powder. The iodates are not influenced by this reduction.

After filtering, the precipitate of manganese dioxide is separated and accurately washed with hot water.

5 cc. of HCl and 5 cc. of a 10% solution of KI without iodates are poured, after cooling, into the filtrate and the washing water. Then the released iodine is titrated with N/10 hyposulfite, using starch water as indicator:

$$KIO_3 + KI + 2HCl = HIO_3 + HI + 2KCl$$
$$5HI + HIO_3 = 3H_2O + 3I_2$$
$$I_2 + 2Na_2S_2O_3 = 2NaI + Na_2S_4O_6$$

It is clear that "this method essentially consists in transforming iodide into iodate by oxidation with permanganate in an alkaline environment, and then in releasing the iodine from the iodate, by adding KI solution after acidifying the liquid".

As it appears from the equation reproduced above, only one of the six iodine atoms that are released is due to the iodate originating from the oxidation of the iodide; therefore the results obtained in the titration must be divided by six.

The results, however, are excellnt, as shown by the blank tests that I conducted, That I briefly summarize:

1st test: g. 0.10 of KI added to cc. 50 of urine: found g. 0.0986, that is 98.6%;
2nd test: g. 0.20 of KI added to c. s.: found g. 0.1992, that is 99.6%.

C. - <u>Experiments with sodium salicylate</u>.

a) Salicylic preparations, as is well known, have been widely used in therapy for many years because of their antipyretic and antineuralgic influence, their specific influence in rheumatic diseases, their action upon metabolism and so on.

These preparations, if administered in excessive

TABLE II — EXPERIMENTS WITH POTASSIUM IODIDE

Number of the experiment and method of administration	Weight of the rabbit in Kg.	Date of injection	cc. of solution injected	Date of collection of the urine	KI injected in g.		Urine excreted in 24 hours				KI (in g.) eliminated in the urine in 24 hours	Percentage of KI eliminated
					Total	Per Kg.	In cc.	Reaction	Albumin	Specific gravity		
V intravenous	1.500	2-3-28	cc. 7.5 of 1% solution of KI	—	0.075	0.05	—				—	—
				3-3-28	—	—	100	Alkaline	Absent	—	0.06142	81.89
				4-3-28	—	—	118			22.2	0.00498	6.64
				5-3-28	—	—	45			1.8	0.001382	1.84
				6-3-28	—	—	200			0.5	0.001382	1.84
				7-3-28	—	—	75			0.5	Ind traces	—
										—		92.21
VI intravenous	0.865	25-2-28	cc. 5.65 of 1% solution of KI	—	0.0865	0.10	—					—
				26-2-28	—	—	105	Alkaline	Absent	—	0.0644	74.45
				27-2-28	—	—	145			23.3	0.0103	11.9
				28-2-28	—	—	140			3.75	0.00303	3.50
				29-2-28	—	—	35			1.1	0.002208	2.55
				1-3-28	—	—	32			0.8	Ind traces	—
										—		92.40
VII subcutaneous	0.865	6-3-28	cc. 4.3 of 1% solution of KI	—	0.043	0.05	—					—
				7-3-28	—	—	165	Alkaline	Absent	—	0.02483	57.74
				8-3-28	—	—	77			9	0.00166	3.86
				9-3-28	—	—	37			0.60	0.00069	1.60
				10-3-28	—	—	45			0.25	0.00083	1.93
				11-3-28	—	—	50			0.30	0.000548	1.27
				12-3-28	—	—	65			0.20	Traces	—
				13-3-28	—	—	120			—	Min traces	—
										—		66.40
VIII subcutaneous	1.159	11-3-28	cc. 14.5 of 1% solution of KI	—	0.145	0.10	—					—
				12-3-28	—	—	90	Alkaline	Absent	42	0.1163	80.20
				13-3-28	—	—	50			0.50	0.001382	0.95
				14-3-28	—	—	160			0.40	0.001105	0.76
				15-3-28	—	—	110			0.30	0.00083	0.57
				16-3-28	—	—	140			—	Ind. traces	—
										—		82.48

doses or over too long a period, may cause the occurrence of toxic phenomena, such as headache, ear buzzing, cardiopalmus, vertigo, delirium, and may even reach, with toxic doses, a comatose phase.

Fortunately, poisoning is very rare because these drugs are rapidly eleminated through the kidneys, as demonstrated by the experiments by Brouardel, who noted that elimination is more rapid in the early youth, slower in adults, and considerably reduced in old people, because of a more or less advanced renal sclerosis.

A quantitative determination of some salicylic preparations in the urine was carried out by Magi (14), who found that, after administration of sodium salicylate, between 80 and 90 percent of the ingested acid passes into the urine.

Many other Authors dealt instead with the transformations that salicylic acid undergoes inside the organism, and maintained that this acid in the human body is mostly combined with glycocoll, forming salicyluric acid, that as such is eliminated through the urine, together with the part of salicylic acid that does not undergo this transformation.

Three experiments that U. MOSSO (15) carried out on himself showed that, after ingestion of sodium salicylate, precisely in the amounts of g. 1.914, g. 3 taken in 3 doses, and g. 3 taken in one dose, g. 0.3775, g. 0.3850 and g. 0.5280 of salicylic acid respectively were found in the urine, as compared to g. 2.3550, g. 3.5650 and g. 3.4320 of salicyluric acid.

The same Author, after oral administration of g. 4 of sodium salicylate, divided among 4 doses over 24 hours, to dogs, extracted from the urine g. 2.7750 of salicylic acid and g. 1.8250 of salicyluric acid.

In contrast with MOSSO, whose results are after all little different, because the salicylic acid that he extracted from the urine was 1/5 (1st exp.), 1/7 (2nd exp.), 1/5 (3rd exp.) of that introduced, STOCKMAN (16) obtained widely fluctuating values: once he was able to extract one half of the administered salicylic acid, in other cases quantities ranging between 1/2, 1/8 and 1/20 of

the ingested acid. He was unable to identify which conditions cause the combination of salicylic acid with glycocoll to vary; he was only able to convince himself that normally the quantity of salicyluric acid is much greater than that of salicylic acid.

G. POUCHET (17) reports that, after administration of salicylic acid and sodium salicylate, salicyluric acid represents 2/3 of the acid that is eliminated, the remainder being constituted by sodium salicylate, potassium salicylate, salicylic aldehyde, etc.

These discordant data demonstrate that nothing is known concerning the causes that influence and regulate the formation of salicyluric acid.

Examining the results obtained in his experiments, BALDONI (18) arr. the conclusion that, when salicylic acid and sodium salicylate are administered orally, the formation of salicyluric acid in the organism is facilitated. The elimination of this acid is also considerable. Moreover, part of the salicylic acid is not combined with glycocoll and is expelled through the urine in the form of alkaline salicylate; there is no constant ratio between salicyluric acid and salicylic acid that are eliminated; however, salicyluric acid appears constantly in a much greater quantity.

I omitted all research concerning the form of elimination and only determined the quantity of salicylic acid eliminatad through the urine.

I used LAGRANGE's method (19) that can be briefly summarized as follows:

"If bromine water in excess is added to a liquid containing salicylic acid, there is formed a precipitate constituted by tribromophenol bromide.

"By titrating the excess bromine that remains free after the precipitation of the bromated compound, we can calculate how much bromine was consumed in the formation of this tribromophenol bromide; from the resulting quantity, by a simple calculation, the corresponding quantity of salicylic acid is found".

The following solutions are needed:

1) Bromated water, whose titer must be determined every time.
2) An N/10 solution of sodium hyposulfite.

3) A 10% solution of KI.
4) Recently prepared starch water.

Before each experiment it is necessary to titrate the bromated water. For this purpose, KI solution in excess is taken and put in a bowl with a frosted glass stopper. A certain quantity of bromated water is then taken with a pipette and, with caution, is poured into the bowl; the bowl is closed with the stopper and vigorously shaken. The well-known reaction then takes place and a quantity of iodine corresponding to the bromine that was poured is released.

$$2KI + Br_2 = 2KBr + I_2$$

An N/10 hyposulfite solution, 1 cc. of which is equivalent to g. 0.01127 of iodine and g. 0.0028 of bromine, is poured from a graduated burette, drop by drop, into the liquid, until the liquid maintains a weak yellow color. A few drops of recently prepared starch water is then added and hyposulfite poured again until the blue color disappears. From the number of cc. of N/10 hyposulfite that were used the quantity of iodine, and therefore of bromine, contained in the bowl is immediately deduced.

After titrating the bromated water, some accurately measured excess bromated water, of which the titer is known, is added to the liquid in which salicylic acid must be measured, that is placed in a beaker; a more or less abundant precipitate will be obtained. When no more precipitate is formed and the liquid above the precipitate assumes a yellowish color, an excess 10% solution of pure KI is poured, shaken as described above, and then N/10 hyposulfite solution is poured very slowly from a graduated burette, until the liquid maintains a weak yellow color. A few cc. of starch water are then added and hyposulfite is poured again until the blue color disappears.

Then the quantity of tribromophenol bromide is deduced from the quantity of bromine, and hence the quantity of salicylic acid, keeping in mind that:

a) 1 g. of tribromophenol contains g. 0.7804 of bromine;
b) 1 g. of salicylic acid corresponds to g. 2.971 of tribromophenol bromide.

After many completely successful blank tests, that persuaded me of the soundness of the method, I proceeded to the researches on urine. I did not operate on it directly, because that produces more that negligible sources of error: instead, I performed repeated extractions from the urine, acidified with sulfuric acid, by means of ethyl ether, taking care after each extraction to wait for some time so that the ether would be completely separated from the urine.

I collected the various ether extracts, evaporated the ether in a double boiler, took the residue with water acidified with sulfuric acid and on that I titrated salicylic acid according to the method described above.

I reported the results of the experiments carried out with sodium salicylate in table II.

CHAPTER III

EXAMINATION OF RESULTS.

A. - If we consider the experiments carried out with sodium hypophosphite (Table I), we see that they are composed of two groups that differ with respect to the dose. I injected two animals with the dose of g. 0.5 per kg., one intravenously, the other subcutaneously. Two other animals were instead subjected to the injection of g. 1 per kg., one intravenously, the other subcutaneously.

The conclusion that can be drawn by the two series of experiments is the following:

a) The amounts of drug that are eliminated, after intravenous and subcutaneous administration, do not greatly differ.

b) By injecting 50 cg. of hypophosphite per kg. either intravenously or subcutaneously, in both cases a little more that 81% of the preparation that was administered is found in the urine and the elimination lasts three days.

c)By injecting 1 g. of hypophosphite per kg. with both methods the amount of the drug eliminated after intravenous injection (1) is slightly greater than that eliminated after subcutaneous injection (2); but the differences are not such as to be especially noteworthy. In both cases the elimination lasts three days.

TABLE II

EXPERIMENTS WITH SODIUM SALICYLATE

NUMBER OF THE EXPERIMENT AND METHOD OF ADMINISTRATION	WEIGHT OF THE RABBIT IN KG.	DATE OF INJECTION	CC. OF SOLUTION INJECTED	DATE OF COLLECTION OF URINE	SODIUM SALICYLATE ELIMINATED IN G		URINE COLLECTED IN 24 HOURS		EXCRETING DUE TO THE DISEASE	SALICYLATE ELIMINATED IN G	PERCENTAGE OF THE SALICYLATE ELIMINATED
					TOTAL	PER KG.	IN CC.	REACTION			
X (INTRAVENOUS)	1.200	30-4-28	CC. 6 OF 3% SOLUTION OF SODIUM SALICYLATE	—	0.18	0.15	—		—	0.14768	—
				1-5-28	—	—	40	ALKALINE	0.29536	0.003937	82.04
				2-5-28	—	—	60		0.00792		2.19
				3-5-28	—	—	60		—	TRACES	—
											84.23
X (INTRAVENOUS)	1.650	24-4-28	CC. 16.5 OF 3% SOLUTION OF SODIUM SALICYLATE	—	0.495	0.30	—		—	0.405225	—
				25-4-28	—	—	150	ALKALINE	0.81045	0.012348	81.86
				26-4-28	—	—	140		0.02471		2.49
				27-4-28	—	—	120		—	TRACES	—
											84.35
XI (SUBCUTANEOUS)	1.250	30-4-28	CC. 6.25 OF 3% SOLUTION OF SODIUM SALICYLATE	—	0.1875	0.15	—		—	0.146416	—
				1-5-28	—	—	40	ALKALINE	0.2928	0.003607	78.08
				2-5-28	—	—	43		0.007396		1.97
				3-5-28	—	—	50		—	TRACES	—
											80.05
XII (SUBCUTANEOUS)	1.400	24-4-28	CC. 14 OF 3% SOLUTION OF SODIUM SALICYLATE	—	0.42	0.30	—		—	0.332918	—
				25-4-28	—	—	160	ALKALINE	0.66576	0.008499	79.26
				26-4-28	—	—	170		0.0170		2.02
				27-4-28	—	—	150			NO TRACES	81.28

B - Proceeding to the examination of the tables concerning the experiments carried out with potassium iodide (Table II), we immediately notice much greater differences in the elimination of the drug introduced by the two methods, namely we notice:

a) The amount of drug that was eliminated was always greater when I used the intravenous method.

b) When I administered cg. 5 of KI per kg. the amount of iodide eliminated after intravenous administration was 92.21% of that introduced and the elimination lasted four days; when I administered the same dose per kg. subcutaneously, the amount eliminated was 66.40% as compared to that administered and iodine could be dosed in the urine until the fifth day.

c) When I administered 10 cg. per kg. the intravenous elimination was 92.40% of the injected drug. After subcutaneous administration, it was only 82.48%; in both cases, the preparation could be dosed in the urine for four days.

Therefore, it appears that, when the drug is administered intravenously, a greater amount is eliminated through the urine.

C. - Finally, the experiments with sodium salicylate (Table II) show a behavior that might perhaps be defined as intermediate between that of hypophosphites and that of iodides. Concerning this drug, it appears that:

a) By injecting either 15 or 30 cg. per kg. intravenously, the percentage of salicylate that was eliminated was approximately 84%.

b) By injecting instead the same amounts of salicylate subcutaneously, the elimination was approximately 80-81% of the amount that was administered. This actually means that after intravenous injection a greater elimination was obtained, but the increase was not exceedingly high and at any rate very far from that obtained with potassium iodide.

The elimination of amounts that can be dosed lasts two days in both cases.

CHAPTER IV

CONCLUSION

It would be unjustified to draw from these experiments general conclusion that could have the value of a law.

However, these experiments point out a series of really important facts, because, besides establishing with certainty some points, they open the way to new researches.

The facts that were established are the following:

a) There are drugs, such as sodium hypophosphite, that do not show constant differences, to which any particular value could be attributed, in their elimination, when they are administered intravenously or subcutaneously in equal doses.

b) Other drugs, such as potassium iodide, are actually eliminated in much greater amounts when an equal dose is administered intravenously that when it is administered subcutaneously. The difference between the two cases, that is always considerable, may be important even with small doses.

c) Finally, other drugs, such as sodium salicylate, are actually always eliminated in greater amount when they are administered intravenously, but the difference, while constant, is small.

It is difficult to explain these variations in elimination. The first idea that comes to mind is that, when a drug is eliminated in smaller quantity after subcutaneous administration, it produces a local effect at the place of application, whereby a greater amount is fixed in the tissues, retained inside the organism and later leiminated extremely slowly. Actually, a local action on the part of iodide, that can even reach the point of seriously damaging the tissues (20), and also, although through a different mechanism, on the part of sodium salicylate, cannot be denied.

Another explanation would consist in admitting that, when the drugs introduced subcutaneously are eliminated in a smaller amount than when administered intravenously, there occurs a greater transformation of them inside the organism, while such transformation would not take place at all if the drugs administered by different methods are eliminated in equal amounts, and would take place to a lesser extent when there is a small difference, such as in the case of sodium salicylate.

Now we lack most of the elements that may make possible to support this interpretation. It is true that,

in the case of hypophosphite, it is admitted that the drug is not transformed inside the organism and is eliminated without alterations, but, concerning the behavior of salicylate, we know that it is combined with glycocoll, although we do not know how much of the preparation is eliminated without change, nor do we know in which proportion this occurs in relation to the dose that is administered.

It is clear that, as long as we do not know precisely the behavior of salicylate, our reasoning lacks a foundation.

Finally, concerning potassium iodide, for a number of considerations and experimentally demonstrated facts, it is now admitted that this drug is split inside the organism. The following facts are submitted in support of the scission thesis:

1) If we pass carbon dioxide through an iodide solution in contact with vegetable protoplasm that is contused, iodine is immediately released, as can be demonstrated by common chemical methods (BINZ).

2) This phenomenon no longer occurs if the vegetable protoplasm is boiled before the experiment (BINZ), thus demonstrating that the presence of protoplasm is not sufficient, but that it is necessary that the protoplasm be alive.

3) CLAUDE BERNARD observed that after administration of iodides to animals, when traces of iodine were no longer found in the urine, the elimination of iodides through the saliva continued for many days, thus inducing him to believe that iodides are decomposed inside the organism and become part of organic molecules that are broken up a little at a time, so that the released iodine formed a saline compound that could be eliminated through saliva secretion.

4) It was observed that after administration of iodides the quantity of iodine organically fixed in the thyroid increases.(in the rabbit, total thyroid iodine content - mg. 0.12).

Therefore, it may be thought that, when iodides are administered subcutaneously, they are fixed in a much greater amount that after intravenous administration, although we have no direct proof in this respect. But it may also be thought that, because of the slow and gradual absorption that occurs with the subcutaneous method, the tissues that have the function of decomposing iodides, being in the presence of a smaller quantity of the drug,

may better perform their decomposing function. I repeat that these are hypotheses, that must be confirmed by further experiments.

What I believe I demonstrated is that drugs do not all behave in the same manner, when they are administered intravenously or subcutaneously in equal doses. After all, also TESTONI's experiments with "aspirochyl" and "solarson", although they had a different experimental purpose and were therefore incomplete from my viewpoint, lead to similar conclusions and assume a new value after my experiments.

It is certain that some drugs are eliminated in the same amount, regardless of the method of introduction; others in slightly dofferent amounts, others again in extremely different amounts. It is certain that the problem is a very complex one, and that it includes the local action of the drug on the tissues and its transformations inside the organism. Only many researches on this subject, with the most diverse drugs, taking into account these complex factors and attempting to solve them in the best possible way, will lead to the discovery of a general law.

I am satisfied with having stated the problem and brought a modest contribution to its solution.

TABLE IV (SUMMARY)

Drug administered	Dose per kg.	Elimination during the day	Elimination percentage intravenously	Elimination percentage subcutaneously
SODIUM HYPOPHOSPHITE	0,50	1°	78.50	80.10
	—	2°	2.08	1.09
	—	3°	0.71	0.68
			Tot. 81.29	Tot. 81.87
	1.00	1°	81.32	79.77
		2°	1.91	0.98
		3°	0.58	0.78
			Tot. 83.85	Tot. 81.53
POTASSIUM IODIDE	0.05	1°	81.89	57.74
		2°	6.64	3.86
		3°	1.84	1.60
		4°	1.84	1.93
		5°	—	1.27
			Tot. 92.21	Tot. 66.40
	0.10	1°	74.45	80.20
		2°	11.9	0.95
		3°	3.50	0.76
		4°	2.55	0.57
			Tot. 92.40	Tot. 82.48
SODIUM SALICYLATE	0.15	1°	82.04	78.08
		2°	2.19	1.97
			Tot. 84.23	Tot. 80.05
	0.30	1°	81.86	79.26
		2°	2.49	2.02
			Tot. 84.35	Tot. 81.28

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ISTITUTO DI FARMACOLOGIA SPERIMENTALE
DELLA R. UNIVERSITA' DI PAVIA
Diretto dal Prof. I. SIMON

Dott.ssa LINA STRADA

Ricerca comparata sull'eliminazione dei farmaci introdotti per via endovenosa e via sottocutanea.

I.

A) Circa l'eliminazione dei farmaci molti fatti sono noti. Si sa ad esempio che le sostanze volatili abbandonano l'organismo per la via polmonare, che le sostanze non volatili vengono eliminate in massima parte attraverso il rene; che alcune, come i metalli pesanti, si eliminano elettivamente attraverso la mucosa dell'intestino crasso. Non pochi farmaci sono eliminati colla bile, altri per mezzo della saliva; vie di eliminazione sono le ghiandole lacrimali, la cute, le mammelle.

Ma se queste cognizioni sono ormai sicure, se noi conosciamo in modo esatto la eliminazione di svariati farmaci attraverso vie che si possono dire elettive, mancano ricerche comparative intorno all'influenza della via di somministrazione sull'eliminazione dei farmaci.

E' un vasto campo che merita di essere studiato a fondo per la sua importanza.

Io mi sono limitata a studiare in quale modo influisca sulla eliminazione quantitativa dei farmaci l'introduzione per via endovenosa o per via sottocutanea.

L'importanza del problema oggi è grandissima se si considera che l'applicazione endovenosa dei farmaci tende a diffondersi sempre più. La ragione per la quale oggi in terapia si segue questo indirizzo è chiara quando si consideri che l'iniezione endovenose di un farmaco ne porta rapidamente in circolo una massa veramente cospicua, sicchè la concentrazione di farmaco che circola nell'organismo è molto superiore a quella che si ottiene quando il rimedio venga somministrato per qualsiasi altra via. Infatti quando un farmaco applicato per via sottocutanea, per esempio, o per via gastrica, arriva nel sangue, vi

giunge un po' per volta a mano a mano che viene assorbito, ma non appena si trova in circolo, l'organismo tende ad espellerlo attraverso le vie di eliminazione. La concentrazione del farmaco atta a produrre l'azione farmacologica o terapeutica, è data dalla differenza fra la quantità di farmaco che penetra nel sangue e quella che viene eliminata. Quando invece si fa l'iniezione endovenosa, si porta in circolo tutta la massa di farmaco che deve agire, e l'eliminazione, che si stabilisce subito, non può essere tale da ridurre, nella maggioranza dei casi, la concentrazione del farmaco, così come avviene nella somministrazione per via sottocutanea o per via gastrica.

A questa regola v'è una eccezione allorchè noi iniettiamo nelle vene farmaci i quali, appena giungono nel sangue, sono [illegible] trasformati in prodotti volatili. In questo caso possiamo ottenere due ordini di conseguenze: o il prodotto volatile che si libera dal farmaco è poco tossico e viene eliminato rapidamente attraverso l'albero respiratorio (come il cacodilato di sodio, trasformato in cacodile, rapidamente eliminato dai polmoni), o il prodotto volatile è molto tossico e uccide rapidamente per azione sui centri vitali (come lo zolfo colloidale, rapidamente trasformato in idrogeno solforato, enormemente tossico).

B) Da queste premesse scaturisce un problema finora non preso in considerazione: l'eliminazione dei farmaci somministrati per via endovenosa avviene più rapidamente di quello che si verifica quando si adotti la via sottocutanea o la via gastrica? La quantità di farmaco eliminato è uguale o diversa?

Prima di addentrarci nello studio della letteratura del problema, vediamo di risolvere teoricamente il quesito, colla scorta di quello che le cognizioni attuali ci insegnano. Quando iniettiamo un farmaco nelle vene, il farmaco, a meno che non si tratti di sostanza che svolga la sua azione sul sangue, si fissa su tutti i tessuti dell'organismo e probabilmente meglio ed in quantità maggiore sugli organi sui quali agisce elettivamente. E' probabile che il farmaco si fissi anche sugli organi eliminatorii, ne inibisca la funzione e che allora l'eliminazione sua non avvenga con quella rapidità che si ha introducendolo per le altre vie di somministrazione. Può darsi che, praticamente, entrambi i casi si avverino: che cioè certi farmaci poco tossici realmente stimolino gli organi deputati alla eliminazione sicchè ne consegue una eliminazione assai rapida, come può darsi il

caso opposto. E' chiaro che solamente l'esperimento sull'animale potrà darci la risoluzione del problema.

C) Prima di iniziare lo studio dell'argomento è bene esaminare la letteratura in proposito.

Io non conosco ricerche fatte collo scopo che mi sono proposta come meta delle mie esperienze, ma ho visto qualche lavoro che può realmente essere ravvicinato ad esse.

Così, ad esempio, *Jenselme* (1) trovò arsenico nelle urine un'ora dopo l'iniezione endovenosa di « 606 » e lo dimostrò ancora per 4 giorni, mentre in seguito ad iniezione intramuscolare dello stesso farmaco, invece trovò arsenico nelle urine nei 3-6 giorni dopo la somministrazione.

A risultati analoghi pervenne *Morel*.

Galonnier (2), in seguito a somministrazione di solfo-arsenolo, trovò che se si adopera la via endovenosa, l'eliminazione dell'arsenico avviene nelle prime ore successive all'introduzione del farmaco, mentre se la via di somministrazione fu la intramuscolare, l'eliminazione dell'arsenico dura 2 giorni. Ricordo poi le ricerche sperimentali su alcuni preparati bismutici di *Perantoni* (3) il quale constatò che nell'uomo il bismuto comincia ad essere eliminato in un tempo variabile fra 4 e 20 ore dopo l'iniezione del farmaco, se si segue la via endomuscolare, mentre si rileva la presenza del metallo nelle urine perfino dopo un'ora e mezzo usando la via endovenosa. Assorbimento ed eliminazione vanno quindi di pari passo, rapidissimi con le iniezioni endovenose, meno rapidi con la intramuscolare.

Tutte queste ricerche sono qualitative. Le esperienze più importanti, dal mio punto di vista, sono quelle di *Testoni*, (4) (5), il quale ha preso in esame comparativamente l'eliminazione quantitativa dell'aspirochyl e del solarson, somministrando i due farmaci per via endovenosa e per via sottocutanea.

Egli nelle esperienze con l'aspirochyl osservò che quando la via di somministrazione è l'endovenosa l'arsenico viene eliminato in quantità maggiore e più rapidamente che non quando la via di somministrazione è la sottocutanea; nelle esperienze col solarson osservò al contrario che l'arsenico passa attraverso il filtro renale in quantità maggiore e più rapidamente quando venga introdotto sotto la cute che non iniettato nelle vene.

Questi pochi dati sinora ottenuti su questo argomento pre-

sentano risultati così opposti, che dimostrano chiaramente che non si può stabilire per ora la regola che governa i rapporti fra assorbimento ed eliminazione dei farmaci a seconda la via di introduzione e che solo l'esperimento esteso ad un largo numero di farmaci potrà far luce su questo interessante problema.

II. — RICERCHE SPERIMENTALI

Dovendo fare ricerche comparative esatte, scelsi tre farmaci poco tossici, dotati di scarsa azione locale, che si assorbono rapidamente anche in seguito ad applicazione sottocutanea. [illegible] ipofosfito di sodio, l'ioduro di potassio, il salicilato di sodio.

A. — *Ipofosfito di sodio.*

Questo farmaco, com'è noto in seguito a ricerche antiche e recenti, attraverso l'organismo immodificato e si trova quasi nella totalità nelle urine (6-7-8).

Per dosarlo nelle urine lo trasformai per ossidazione in fosfati. Sono indicati varii metodi:

1° Ossidazione per mezzo di HNO_3;

2° Ossidazione per mezzo di $KClO_3$ e di HCl.

I migliori risultati si ottengono col secondo metodo. Usai ipofosfito purissimo e per la sua ingroscopicità ne preparai una soluzione che titolai ogni volta prima di usarla.

Dopo la somministrazione di ipofosfito sodico ai conigli raccoglievo le urine, ne misuravo il volume e quindi prendevo due porzioni uguali.

Dosavo nella prima direttamente l'anidride fosforica per mezzo di una soluzione titolata di acetato di uranile, con le ben note regole; ossidavo la seconda porzione e poi la portavo a secco a b. m.; sciolto il residuo in acqua distillata acidificavo il miscuglio con acido acetico e con acetato potassico, indi determinavo l'anidride fosforica presente con la soluzione uranica. La prima determinazione mi dava la quantità di anidride fosforica dovuta ai fosfati presistenti nell'urina; la seconda l'anidride fosforica dei fosfati preesistenti più quella dovuta all'ossidazione degli ipofosfiti. La differenza fra le cifre ottenute nelle due determinazioni rappresentava l'anidride fosforica dei fosfati prodotti dall'ossidazione degli ipofosfiti, che venivano eliminati inalterati.

Due prove in bianco che feci precedere alle esperienze sull'animale mi diedero i seguenti risultati:

1ª di gr. 1 di ipofosfiti ne trovai gr. 0,984, cioè il 98,4 %.

2ª di gr. 2 di ipofosfiti ne trovai gr. 1,966, cioè il 98,3 %.

Una volta in possesso di un buon metodo di dosaggio degli ipofosfiti nelle urine, passai alle esperienze sui conigli. Nella tabella I sono riportati i risultati delle esperienze.

Numero dell'esperienza e via di somministrazione	Peso del coniglio in kg.	Data della iniezione	Cc. di soluzione iniettata	Data della raccolta dell'urina	$NaH_2PO_2 \cdot H_2O$ in gr. iniettati: in tutto	$NaH_2PO_2 \cdot H_2O$ in gr. iniettati: per kgr.	Urina emessa nelle 24 ore: in Cc.	Urina emessa nelle 24 ore: Reazion	P_2O_5 dovuta ai fostati preesistenti	P_2O_5 dovuta agli ipofosfiti	Ipofosfito in gr. eliminato	Per centuale dell'ipofosfito eliminato
I (endovenosa)	2.00	17-3-28	cc. 10 di una soluz. di ipofosfito sodico al 10 %	—	1.00	0.50	—	—	—	—	—	—
				18-3-28	—	—	240	alcalina	0.080	0.526	0.7850	78.50
				19-3-28	—	—	200	«	0.085	0.014	0.0208	2.08
				20-3-28	—	—	180	«	0.090	0.005	0.0071	0.71
				21-3-28	—	—	190	«	0.090	—	Traccie ind.	—
												81.29
II (endovenosa)	2.00	28-3-28	cc. 20 di una soluz. di ipofosfito sodico al 10 %	—	2.00	1.00	—	—	—	—	—	—
				29-3-28	—	—	135	alcalina	0.065	1.0065	1.6265	81.43
				30-3-28	—	—	140	«	0.075	0.0265	0.0390	1.91
				31-3-28	—	—	160	«	0.070	0.0080	0.0116	0.58
				1-4-28	—	—	153	«	0.070	—	Traccie ind.	—
												83.85
III (sottocutanea)	1.500	26-3-28	cc. 75 di soluz. di ipof. sod. al 10 %	—	0.75	0.50	—	—	—	—	—	—
				27-3-28	—	—	150	alcalina	0.0785	0.4025	0.6068	80.10
				28-3-28	—	—	75	«	0.075	0.0055	0.0082	1.09
				29-3-28	—	—	130	«	0.086	0.0036	0.0051	0.68
												81.87
IV (sottocutanea)	1.600	30-3-28	cc. 16 di soluz. di ipof. sod. al 10 %	—	1.50	1.00	—	—	—	—	—	—
				31-3-28	—	—	185	alcalina	0.065	0.855	1.2764	79.77
				1-4-28	—	—	180	«	0.075	0.0167	0.0157	0.98
				2-4-28	—	—	200	«	0.080	0.0085	0.0126	0.78
												81.53

B. — *Esperienze con ioduro potassico*

a) Gli ioduri sono farmaci molto noti e di uso corrente nella pratica medica (9-10). Dalle numerose ricerche eseguite sull'assorbimento e sulla eliminazione degli ioduri, risultò certamente che questi sali, facilmente solubili, sono rapidamente assorbiti per la via gastro-intestinale, diffondono nell'organismo e poi appaiono molto rapidamente nei vari secreti di questo. Così 7-12 minuti primi dopo l'ingestione degli ioduri furono ritrovati nella saliva; nell'urina 3-15 minuti dopo.

Le ricerche sperimentali dimostrano che essi si eliminano in massima parte attraverso l'emuntorio renale; ma si sa anche assai bene che una certa parte rimane nell'organismo.

Negli ultimi tempi passati la questione dell'assorbimento e delle eliminazione degli ioduri acquistò un grande interesse pratico, specialmente per gli studi e per le ricerche di *Schlazers* e di *Takazasus*, per stabilire una prova clinica della funzionalità renale.

Schlazers e *Takazasus* credono che, mentre l'eliminazione di gr. 0.50 di ioduro di potassio richiede normalmente un tempo costante di 54-60 ore, per cause d'insufficienza renale il tempo dell'eliminazione viene di molto prolungato.

Circa la quantità di iodoro eliminato risulta dalle esperienze di *Salazár* (11) che nelle urine si trova, a seconda della dose somministrata, dall'83 al 96 per cento del sale introdotto, e precisamente con dose minore l'eliminazione è più debole, con dosi maggiori l'eliminazione è più forte; il che pure risulta ugualmente da esperienze fatte dal *Barkholtz* (12).

Quest'ultimo si occupò particolarmente, in una serie di ricerche, del contenuto in ioduri del sangue, dopo l'ingestione di essi, arrivando alle seguenti conclusioni:

(*a*) Dopo la somministrazione di una maggiore quantità di ioduro potassico, la percentuale in iodio del sangue aumenta rapidamente nella prima ora e raggiunge il massimo 2-3 ore dopo la simministrazione.

b) La concentrazione di iodio nel sangue sta in rapporto diretto e costante colla quantità di iodio contenuto nell'organismo.

c) Se si somministrassero dosi frazionate di KI durante tutta la giornata, la concentrazione in iodio del sangue cresce dopo ogni somministrazione, per diminuire poi lentamen-

te. E' necessario quindi, quando si vuole una concentrazione costante, somministrare durante tutto il giorno delle dosi frazionate molto piccole.

d) La maggior parte dello ioduro somministrato viene eliminato coll'urina, nella quale si ritrovano spesso i 9/10 della quantità somministrata.

Tralascio le altre ricerche su questo argomento, perchè non hanno col mio lavoro stretto rapporto.

B. — *Metodo.*

Il metodo da me usato nella ricerca quantitativa degli ioduri nelle urine dei conigli, in seguito a somministrazione per via endovenosa e per via sottocutanea di KI purissimo, è quello di [illegible] PERON che lo descrivono così (13); 50 cc. di urina [illegible] alcalinizzata [illegible] cc. di soluzione satura di carbonato sodico, si evapora, si carbonizza il residuo secco, si esaurisce con acqua bollente. Alla soluzione ottenuta, portata e mantenuta all'ebollizione per qualche minuto, si aggiungono poco a poco cristalli di permanganato potassico, finchè permane colorazione violetta. Coll'ebollizione della soluzione alcalina assieme al permanganato potassico, gli ioduri vengono ossidati a iodati secondo l'equazione:

$$KI + 2KMnO_4 + H_2O = KIO_3 + 2MnO_2 + 2KOH$$

Si distrugga l'eccesso di permanganato coll'aggiunta di qualche cc. di alcool al liquido caldo. In tal modo il permanganato in soluzione alcalina viene ridotto prima a manganato e poi in biossidio di manganese, che si deposita sotto forma di polvere bruna. Gli iodati non vengono influenzati da questa riduzione.

Si filtra, si separa il precipitato di biossido di manganese, che si lava accuratamente con acqua calda.

Nel filtrato, unito alle acque di lavaggio, dopo raffreddamento, si versano 5 cc. di HCl e 5 di soluzione al 10 % di KI esente da iodati. Poi si titola con soluzione N/10 di iposolfito, indicatore la salda d'amido, lo iodio messo in libertà:

$$KIO_3 + KI + 2HCl = HIO_3 + HI + 2KCl$$
$$5HI + HIO_3 = 3H_2O + 3I_2$$
$$I_2 + 2Na_2S_2O_3 = 2NaI + Na_2S_4O_6$$

Come si vede, « questo metodo consiste essenzialmente nel trasformare lo ioduro in iodato mediante ossidazione con permanganato in ambiente alcalino, e poi mettere in libertà lo iodio dello iodato, aggiungendo soluzione di KI dopo acidificazione del liquido ».

Come risulta dall'equazione sopra riportata dei sei atomi di iodio che si mettono in libertà, soltanto uno è dovuto allo iodato proveniente dalla ossidazione dello ioduro: quindi i risultati che nella titolazione si ottengono devono essere divisi per sei.

I risultati però sono ottimi come risulta dalle prove in bianco fatte da me, che riporto brevemente:

1ª prova: gr. 0,10 di KI aggiunti a cmc. 50 di urina; trovati gr. 0,0986, cioè il 98,6 %;

2ª prova: gr. 0,20 di KI aggiunti c. s.; trovati gr. 0,1992, vale a dire il 99,6 %.

TABELLA II. — Esperienze con ioduro di potassio.

Numero dell'esperienza e via di somministrazione	Peso del coniglio in kgr.	Data della iniezione	cc. di soluzione iniettata	Data della raccolta dell'urina	KI iniettato in gr. in tutto	KI iniettato in gr. per Kgr.	Urina e [illegible] nelle 24 ore in cc.	Urina e [illegible] nelle 24 ore Reazione	Urina e [illegible] nelle 24 ore Albumina	Nº di soluz. N/10 di iposolfito impiegato nella titolazione	KI in gr. eliminato nelle urine delle 24 ore	Percentuale del KI eliminato
V (endovenosa)	1.500	2-3-28	cc. 7.5 di soluzione di KJ all' 1 %	—	0.075	0.05	—	—	—	—	—	—
				3-3-28		—	100	alcalina	assente	22.2	0.06142	81.89
				4-3-28	—	—	118	»	»	1.8	0.00498	6.64
				5-3-28		—	45	»	»	0.5	0.001382	1.84
				6-3-28	—	—	200	»	»	0.5	0.001382	1.84
				7-3-28	—	—	75	»	»	—	traccie ind.	—
												92.21
VI (endovenosa)	0.865	25-2-28	cc. 5.65 di soluzione di KJ all'1 %	—	0.0865	0.10			—	—	—	
				26-2-28	—	—	105	alcalina	assente	23.3	0.0644	74.45
				27-2-28	—	—	145	»	»	3.75	0.0103	11.9
				28-2-28	—	—	140	»	»	1.1	0.00303	3.50
				29-2-28	—	—	35	»	»	0.8	0.002208	2.55
				1-3-28	—	—	32	»	»	—	traccie ind.	—
												92.49
VII (sottocutanea)	0.865	6-3-28	cc. 4.3 di soluzione di KJ all'1 %.	—	0.043	0.05	—	—	—	—	—	—
				7-3-28	—	—	165	alcalina	assente	9	0.02483	57.74
				8-3-28	—	—	77	»	»	0.60	0.00166	3.86
				9-3-28	—	—	37	»	»	0.25	0.00069	1.60
				10-3-28	—	—	45	»	»	0.30	0.00083	1.93
				11-3-28	—	—	50	»	»	0.20	0.000548	1.27
				12-3-28	—	—	65	»	»	—	traccie	—
				13-3-28	—	—	120	»	»	—	traccie min.	—
												66.49
VIII (sottocutanea)	1.450	11-3-28	cc. 14.5 di soluzione di KJ all'1 %		0.145	0.10	—	—	—	42	—	—
				12-3-28		—	90	alcalina	assente	0.50	0.1163	80.20
				13-3-28	—	—	50	»	»	0.40	0.001382	0.95
				14-3-28		—	160	»	»	0.30	0.001105	0.76
				15-3-28		—	140	»	»	—	0.00083	0.57
				16-3-28	—	—	140	»	»	—	traccie ind.	
												48

C. — *Esperienze con salicilato sodico.*

Gli preparati salicilici da molti anni sono, come tutti sanno, largamente usati in terapia per l'influenza antipiretica ed antinevralgica, per quella specifica nelle forme morbose reumatiche per l'azione sul ricambio e via dicendo.

Questi preparati, per somministrazione di dosi esagerate o troppo prolungate, possono determinare l'insorgenza di fenomeni tossici, come cefalea, ronzio agli orecchi, cardiopalmo, vertigini, delirio, fino a raggiungere, per dosi tossiche, una fase comatosa.

Per fortuna gli avvelenamenti sono molto rari per la rapida eliminazione di essi attraverso il rene, come dimostrano le e[illegible] del Brouardel il quale constatò come l'eliminazione sia più rapida nella prima giovinezza, più lenta nell'uomo adulto, notevolmente ridotta nei vecchi per una più o meno avanzata sclerosi renale.

Una determinazione quantitativa di alcuni preparati salicilici nelle urine fu fatta dal Magi (14) il quale trovò che in seguito a somministrazione di salicilato sodico, fra l'80 ed il 90 per cento dell'acido ingerito passa nelle urine.

Molti altri Autori si occuparono piuttosto delle trasformazioni che l'acido salicilico subisce nell'organismo sostenendo che detto acido, in massima parte, nell'organismo umano si combina colla glicocolla, dando luogo all'acido salicilurico, che come tale viene eliminato attraverso le urine, insieme con quella parte di acido salicilico che non subisce questa trasformazione.

Da tre esperienze eseguite da *U. Mosso* (15) su sè stesso, risultò, che in seguito ad ingestione di salicilato sodico e precisamente di gr. 1.914, gr. 3 presi in tre volte, gr. 3 presi in una volta sola, nelle urine si riscontrarono rispettivamente gr. 0.3775, gr. 0.3850, gr. 0.5280 di acido salicilico di fronte a gr. 2.3550, gr. 3.5650 e gr. 3.4320 di acido salicilurico.

Lo stesso Autore sul cane in seguito a somministrazione per bocca di gr. 4 di salicilato sodico in 4 volte nelle 24 ore, estrasse dalle urine gr. 2.7750 di acido salicilico e gr. 1.8250 di acido salicilurico.

A differenza di *Mosso*, i cui risultati in fondo sono poco discordanti, perchè l'acido salicilico da lui estratto dalle urine fu 1/5 (1. esp.), 1/7 (2. esp.), 1/5 (3. esp.) dell'introdotto, *Sto-*

ckman (16) ha ottenuto cifre molto oscillanti, avendo potuto estrarre una volta metà dell'acido salicilico somministrato, ed altre volte quantità che oscillano fra 1/2, 1/8 ed 1/20 dell'acido ingerito. Nè ha potuto riconoscere quali condizioni facciano variare la combinazione dell'acido salicilico colla glicocolla; solo si è potuto convincere che ordinariamente la quantità dell'acido salicilurico è molto superiore a quella dell'acido salicilico.

G. Pouchet (17) riferisce che nella somministrazione di acido salicilico e di salicilato sodico, l'acido salicilurico rappresenta i 2/3 dell'acido eliminato, essendo il resto salicilato di sodio, salicilato di potassio, aldeide salicilica, ecc.

Questi dati così discordanti dimostrano che sulle cause che influiscono e regolano la formazione dell'acido salicilurica si è completamente all'oscuro.

Esaminando i risultati ottenuti nelle sue esperienze *Baldoni* (18) trae la conclusione che, quando si somministra acido salicilico e salicilato sodico per bocca, facile è nell'organismo la formazione di acido salicilurico. Notevole del pari è l'eliminazione di detto acido. Una parte poi dell'acido salicilico non si accoppia colla glicocolla e fuoriesce per le urine allo stato di salicilato alcalino; non esiste un rapporto costante fra acido salicilurico e acido salicilico eliminato; ma tuttavia l'acido salicilurico è costantemente in quantità molto maggiore.

Io trascurai ogni ricerca intorno alla forma di eliminazione e determinai solamente la quantità di acidi salicilico eliminato con le urine.

Usai il metodo di *Lagrange* (19) che si può descrivere brevemente nel modo seguente:

« Se ad un liquido, contenente acido salicilico, si aggiunge acqua di bromo in eccesso, si forma un precipitato costituito da bromuro di tribromofenolo.

« Titolando l'eccesso di bromo che resta libero dopo la precipitazione del composto bromato, possiamo calcolare quanto bromo fu consumato per la formazione di questo bromuro di tribromofenolo; dalla quantità trovata si risale, con facile calcolo, all'acido salicilico corrispondente ».

Occorrono le seguenti soluzioni.

1° Acqua bromata di cui ogni volta va determinato il titolo.
2° Una soluzione N/10 di iposolfito sodico.
3° Una soluzione di KI al 10 %.
4° Salda d'amido di recente preparata.

Prima di ogni esperienza occorre titolare l'acqua di bromo. A

tale scopo si prende un eccesso della soluzione di KI e si pone in una boccia a tappo smerigliato. Si preleva poi con una pipetta una determinata quantità di acqua bromata e, con precauzione, si fa cadere nella boccia; si chiude questa col tappo e si agita fortemente. Avviene allora la nota reazione e si mette in libertà una quantità di iodio corrispondente al bromo versato.

$$2KI + Br_2 = 2KBr + I_2$$

Si fa quindi cadere nel liquido, da una buretta graduata, goccia a goccia, una soluzione di N/10 di iposolfito, di cui 1 cmc. è equivalente a gr. 0,01127 di iodio e a gr. 0,3018 di bromo, finchè il liquido conserva debole colorazione gialla. Si aggiunge allora qualche [illegible] di soluzione d'amido recente e si continua a versare iposolfito fino a [illegible] della colorazione azzurra. Dal numero dei cc. di soluzione N/10 di iposolfito impiegato si deduce immediatamente la quantità di iodio e quindi di bromo contenuto nella boccia.

Fatta la titolazione all'acqua bromata, aggiungiamo al liquido del quale si vuol dosare l'acido salicilico, posto in una bevuta, un eccesso esattamente misurato di questa acqua di bromo, della quale si conosce il titolo; otterremo un precipitato più o meno abbondante. Dopo che non si forma più precipitato e che il liquido soprastante al precipitato ha assunto un colorito giallastro, si versa un eccesso di soluzione al 10% di KI puro, si agita come sopra, e quindi si fa cadere da una buretta graduata, a poco a poco, soluzione N/10 di iposolfito, finchè il liquido conserva debole colorazione gialla. Si aggiunge allora qualche cc. di salda d'amido e si torna a versare iposolfito fino a scomparsa della colorazione azzurra.

Allora dalla quantità di bromo si risale alla quantità di bromuro di tribromofenolo e quindi alla quantità di acido salicilico, ricordando che:

a) in 1 gr. di bromuro di tribromofenolo sono contenuti gr. 0,7894 di bromo;

b) 1 gr. di acido salicilico corrisponde a gr. 2,971 di bromuro di tribromofenolo.

Dopo numerose prove in bianco, perfettamente riuscite, che mi assicurarono intorno alla bontà del metodo, passai alle ricerche sulle orine. Non operai direttamente su esse, perchè in tal caso si hanno cause di errore non indifferenti; ma sulle urine, acidificate con acido solforico, feci ripetute estrazioni con etere etilico, avendo cura dopo ogni estrazione di lasciare a sè per qualche tempo affinchè l'etere si separasse completamente dall'urina.

Riunii i vari estratti eterei; evaporai l'etere a bagno-maria, ripresi il residuo con acqua acida per acido solforico e su di essa dosai l'acido salicilico col metodo più sopra descritto.

Nella tabella II ho riportato i risultati delle esperienze fatte con salicilato sodico.

TABELLA III

Esperienze con salicilato di sod[illegible]

Numero dell'esperienza e via di somministrazione	Peso del coniglio in kgr.	Data della iniezione	Cc. di soluzione iniettati	Data della raccolta dell'urina	Salicilato sodico eliminato in gr.		Urina emessa nelle 24 ore		Bromo immagazzinato nel dosaggio	Salicilato eliminato in gr.	Percentuale di salic. eliminato
					[illegible]	per kgr.	[illegible]	Reazione			
IX (endovenosa)	1.200	30-4-28	cc. 6 di soluz. di salicilato sodico al 3 %	—	0.18	0.15	—	—	—	—	—
				1-5-28	—		40	alcalina	0.29[illegible]	[illegible]	82.04
				2-5-28	—	—	60	»	0.00732	[illegible]	2.19
				3-5-28	—		60	»		tracce m.	—
											84.23
X (endovenosa)	1.650	24-4-28	cc. 16.5 di soluz. di salicilato sodico al 3 %	—	0.495	0.30	—	—	—	—	—
				25-4-28	—	—	150	alcalina	0.81945	0.[illegible]5225	81.86
				26-4-28	—	—	140	»	0.02471	[illegible]2348	2.49
				27-4-28	—	—	120	»	—	tracce m.	—
											84.35
XI (sottocutanea)	1.250	30-4-28	cc. 6.25 di soluz. di salicilato sodico al 3 %.	—	0.1875	0.15	—	—	—	—	—
				1-5-28	—	—	40	alcalina	0.2928	0.14[illegible]416	78.08
				2-5-28	—	—	43	»	0.007396	[illegible]2697	1.97
				3-5-28	—	—	50	»	—	tracce m.	—
											80.05
XII (sottocutanea)	1.400	24-4-28	cc. 14 di soluz. di salicilato sodico al 3 %		0.42	0.30	—	—	—	—	—
				25-4-28	—	—	160	alcalina	0.66576	0.332918	79.26
				26-4-28	—	—	170	»	0.0170	[illegible]	2.02
				27-4-28	—	—	150	»		tracce ind.	81.28

CAPITOLO III.

Esame dei risultati.

Se noi guardiamo le esperienze fatte con ipofosfito sodico (Tab. I), vediamo che esse sono costituite da due gruppi diversi a seconda della dose. A due animali ho iniettato la dose di gr. 0.5 per kg., ad uno per via endovenosa, all'altro per via sottocutanea. Altri due animali invece subirono l'iniezione di gr. 1 per kg., l'uno nelle vene, l'altro sotto cute.

La conclusione che può trarre dalle due serie di esperimenti è questa:

a) Le quantità di farmaco eliminato dopo la somministrazione endovenosa e sottocutanea, non differiscono molto.

b) Iniettando 50 ctgr. di ipofosfito per kg. nelle vene o sottocute, nelle urine si trova in tutte e due i casi poco più dell'81 % del preparato somministrato e l'eliminazione dura per tre giorni.

(*Continua*).

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DELLA R. UNIVERSITA' DI PAVIA
Diretto dal Prof. I. SIMON

Dott.ssa LINA STRADA

Ricerca comparata sull'eliminazione dei farmaci introdotti per via endovenosa e via sottocutanea.

I.

(Continuazione, vedi num. precedente)

c) Iniettando 1 gr. di ipofosfito per kg. per le due vie la quantità di farmaco eliminata dopo l'iniezione endovenosa (1) è lievemente superiore a quella eliminata in seguito ad injezione sottocutanea (2); ma le differenze non sono tali da venire particolarmente notate. L'eliminazione in entrambi i casi dura per tre giorni.

B — Passando all'esame delle tabelle relative alle esperienze fatte con ioduro potassico (Tab. II) notiamo subito differenze ben maggiori nella eliminazione del farmaco introdotto per le due vie, e precisamente notiamo:

a) Sempre la quantità di farmaco eliminata fu maggiore quando mi servii della via endovenosa.

b) Quando somministrai ctgr. 5 per kg. di KI la quantità di ioduro eliminata in seguito a somministrazione endovenosa fu del 92.21 % dell'introdotta e l'eliminazione durò per quattro giorni; quando somministrai la stessa dose per kg. per via sottocutanea, la quantità eliminata fu del 66.40 % rispetto a quella somministrata e lo iodio fu dosabile nelle urine fino al quinto giorno.

c) Quando somministrai 10 ctgr. per kg. l'eliminazione

(1) 83,85 %.
(2) 81,53 %.

endovenoca fu del 92.40 % del farmaco iniettato. Fu solo dell'82,48% in seguito a somministrazione sottocutanea; in entrambi i casi il preparato fu dosabile nelle urine per quattro giorni.

Risulta dunque che allorchè somministriamo il farmaco per via endovenosa, ne viene eliminata colle urine una quantità maggiore.

C. - Da ultimo le esperienze col salicilato sodico (Tabella II) mostrano un comportamento che si potrebbe forse dire intermedio fra quello degli ipofosfiti e quello degli ioduri. Infatti per questo farmaco si rileva:

a) somministrando per via endovenosa sia 15 ctgr. che 30 per kg., la percentuale di salicilato eliminato fu dell'84 % circa.

b) Iniettando invece le stesse quantità di salicilato per via sottocutanea l'eliminazione si aggirò intorno all'80.81 %, del preparato somministrato. Vale a dire realmente dopo l'iniezione endovenosa si ottenne una eliminazione più copiosa, ma l'aumento non fu eccessivamente elevato e ad ogni modo assai lontano da quello ottenuto con ioduro potassico.

L'eliminazione di quantità dosabili dura in entrambi i casi per due giorni.

CAPITOLO IV.

Conclusione.

Trarre da queste esperienze conclusioni generali, che abbiano il valore di una legge, sarebbe ingiustificato. Queste esperienze però valgono a mettere in luce una serie di fatti di importanza veramente grande, in quanto, oltre a stabilire alcuni punti in modo sicuro, aprono la strada a nuove ricerche.

I fatti stabiliti sono i seguenti:

a) Vi sono farmaci i quali come l'ipofosfito sodico, non mostrano, nell'eliminazione, differenze costanti, alle quali si possa attribuire un valore particolare, quando vengono somministrati per via endovenosa o per via sottocutanea in ugual dose.

b) Altri farmaci, come lo ioduro potassico, sono realmente eliminati in quantità molto maggiore, quando si somministrano in ugual dose per via endovenosa che non per via sotto-

cutanea. La differenza fra i due casi, sempre ragguardevole, può essere anche cospicua per le piccole dosi.

c) Altri farmaci infine, tipo salicilato sodico, sono realmente eliminati sempre in quantità maggiore allorchè si somministrino per le vene, ma la differenza, sebbene costante, è piccola.

E' difficile spiegare queste variazioni dell'eliminazione. La prima idea che si affaccia alla mente è quella che, quando un farmaco si elimina meno nella somministrazione sottocutanea; produca un effetto locale nel punto di applicazione in seguito al quale una maggiore quantità di esso viene fissata nei tessuti, trattenuta nell'organismo ed eliminata poi con grandissima lentezza. Ora non si può negare un'azione locale dell'ioduro che può arrivare fino a danneggiare seriamente i tessuti (20), e nemmeno del salicilato di sodio, sebbene con meccanismo diverso.

Un'altra spiegazione consisterebbe nell'ammettere che allorquando i farmaci introdotti per via sottocutanea, sono eliminati in minor quantità che nel caso della somministrazione endovenosa, avvenga una maggiore trasformazione loro nell'organismo, mentre tale trasformazione non si verificherebbe affatto se i due farmaci somministrati per via diversa vengono eliminati nella stessa misura, e si verificherebbe meno quando vi sia una piccola differenza, come nel caso del salicilato sodico.

Ora ci mancano, in massima parte, gli elementi che ci permettano di sostenere una così fatta interpretazione. E' vero che nel caso dell'ipofosfito si ammette che il farmaco non venga trasformato nell'organismo e che si elimini inalterato, ma per quanto riguarda il contegno del salicilato nell'organismo sappiamo che esso si accoppia alla glicocolla, sebbene non conosciamo quanto preparato venga eliminato immodificato, nè in quale proporzione questo fatto si verifichi in rapporto alla dose somministrata.

E' chiaro che, finchè non conosciamo esattamente il comportamento del salicilato, il nostro ragionamento manca di base.

Infine per ciò che riguarda lo ioduro potassico, oggi per una serie di considerazioni e di fatti constatati sperimentalmente, si ammette che questo farmaco venga scisso nell'organismo. A sostegno della scissione si portano i seguenti fatti:

TABELLA IV (RIASSUNTIVA)

Farmaco somministrato	Dose per kg.	Eliminazione nella giornata	Eliminazione percentuale per via endovenosa	Eliminazione percentuale per via sottocutanea
ipo[illegible]fito di sodio	0,50	1°	78.50	80.10
	—	2°	2.08	1.09
	—	3°	0.71	0.68
			Tot. 81.29	Tot. 81.87
	1.00	1°	81.32	79.77
		2°	1.91	0.98
		3°	0.58	0.78
			Tot. 83.85	Tot. 81.53
ioduro di potassio	0.05	1°	81.89	57.74
		2°	6.64	3.86
		3°	1.84	1.60
		4°	1.84	1.93
		5°	-	1.27
			Tot. 92.21	Tot. 66.40
	0.10	1°	74.45	80.20
		2°	11.9	0.95
		3°	3.50	0.76
		4°	2.55	0.57
			Tot. 92.40	Tot. 82.48
salicilato di sodio	0.15	1°	82.04	78.08
		2°	2.19	1.97
			Tot. 84.23	Tot. 80.05
	0.30	1°	81.86	79.26
		2°	2.49	2.02
			Tot. 84.35	Tot. 81.28

1°. Se noi facciamo attraversare da anidride carbonica una soluzione di ioduro posta a contatto con un protoplasma vegetale vivente contuso, si ha immediatamente liberazione di iodio dimostrabile coi comuni metodi chimici (*Binz*).

2°. Il fenomeno non si verifica più se il protoplasma vegetale vien fatto bollire prima dell'esperimento (*Binz*), il che dimostra che non basta la presenza di un protoplasma, ma che è necessario che tale protoplasma sia vivo.

3°. *Claude Bernard* osservava che dopo la somministrazione di ioduri agli animali, quando nell'urina non si trovava più traccia di iodio, per molti giorni continuava l'eliminazione degli ioduri attraverso la saliva, il che lo induceva ad ammettere [illegible] ioduri venissero scomposti nell'organismo ed entrassero a far parte di molecole organiche le quali un pò per volta venivano scisse, sicchè lo iodio rimesso in libertà formava un composto salino eliminabile col secreto salivare.

4°. Fu osservato che dopo la somministrazione di ioduri cresce la quantità di iodio fissato organicamente nella tiroide, (nel coniglio contenuto totale della tiroide in iodio — mmgr. 0.12).

Per questo si può supporre che, quando noi somministriamo ioduri per via sottocutanea, avvenga una fissazione di essi assai maggiore di quella che non si verifichi nella somministrazione endovenosa, sebbene nessuna prova diretta possediamo a questo riguardo. Ma si può anche pensare che, per l'assorbimento lento e graduale che si verifica per via sottocutanea, i tessuti destinati a scomporre gli ioduri, trovandosi in presenza di una massa minore di farmaco, possano meglio attendere alla loro funzione scompositrice. Ripeto che si tratta di ipotesi, le quali hanno bisogno di essere suffragate da esperienze ulteriori.

Quello che io credo di aver dimostrato è che i farmaci non si comportano tutti allo stesso modo, quando vengono somministrati in dosi uguali per via endovenosa e per via sottocutanea. In fondo anche le esperienze di *Testoni* con aspirochyl e con solarson, sebbene volte ad altro scopo sperimentale e perciò incomplete dal mio punto di vista, portano a conclusioni analoghe ed acquistano dopo le mie esperienze un valore nuovo.

E' certo che alcuni farmaci sono eliminati nella stessa quantità, qualunque sia la via di introduzione; altri in modo

reco diverso, altri infine in modo diversissimo. E' certo che il problema è assai complesso, e che in esso entrano in giuoco l'azione locale del farmaco sui tessuti e le sue trasformazioni nell'organismo. Solamente numerose ricerche sull'argomento coi più svariati farmaci, che tengano conto di questi fattori complessi, e che cerchino di risolverli nel modo migliore, potranno portare ad una legge generale.

A me basti l'avere impostato il problema e l'avere portato alla risoluzione sua un modesto contributo.

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INTRODUCTION

It has long been known that phosphoric acids are important constituents of animals and are essential for their growth, but no study has yet been carried out on the difference in nutritive value due to the form of phosphoric acid.

In 1910, E.B. Hart et al. (1) reported that pigs stopped growing when given a feed with poor phosphoric acid content, but began to show normal growth when lime phosphate was added to the feed. Thus, they assumed that organic phosphoric acids were not necessary. In 1918, T.B. Osborne et al. (2) raised white rats on synthetic feeds and determined the nutritive significance of various inorganic components. In that experiment, a group of animals given a feed without phosphoric acid did not grow at all whereas a feed with N-phosphoric acid resulted in perfect growth. Thus, they concluded that protein-bonded phosphoric acid or other organic phosphoric acids were not necessary. The effectiveness of glycerophosphoric acid, phytin, or hexose phosphoric acid as a source of phosphoric acid has been reported by many investigators, but no attempt has yet been made to study them on a comparative basis. Pyrophosphoric acid was found in animal muscle cells by Lohmann et al. (3) in 1928, suggesting its significance in relation to the growth of animal, but no report has yet been made on such aspect of metaphosphoric acid.

Under these circumstances, the author studied various forms of phosphoric acid comparatively with regard to their nutritive value.

1. PHOSPHATE UNDER STUDY

Sodijm salts of orthophosphoric acid (sec.), pyrophosphoric acid, metaphosphoric acid, hypophosphorous acid, glycerophosphoric acid, and phytic acid, and calcium salts of orthophosphoric acid (sec.), and hexosediphosphoric acid.

The chemicals used were :

Orthophosphoric acid (Merk) : recrystallized from warm water.

Pyrophosphoric acid (Merk) : recrystallized from warm water, silver nitrate was used to produce pure white precipitates of silver salt; at normal temperature, no reaction occurred upon addition of ammonium molybdate and nitric acid, which indicates that no N-phosphoric acid was contained.

Metaphosphoric acid (Carlbaum) : Washed with cold water; silver nitrate was added to obtain precipitates of silver salt; neither ammonium molybdate nor nitric acid produced reaction at normal temperature; neither zinc acetate nor acetic acid produced any precipitate, which indicates that neither N-phosphoric acid nor pyrophosphoric acid was contained.

Hypophosphorous acid (Merk).
Glycerophosphoric acid (Merk) : recrystallized from water; theoretical value as P_2O_5, 32.87%; experimental value, 32.84%.
Phytin : obtained from rice bran; converted into an iron salt with iron chloride, then sodium salt with caustic soda, and recrystallized from alcohol; theoretical value as P_2O_5, 46.11%; experimental value, 46.02%.
Hexosediphosphoric acid : Bayer's candiolin was purified according to C. Neuberg and S. Sabetay's method; theoretical value as P_2O_5, 34.13%; experimental value, 34.02%.

II. BASAL FEED

Composition : horse meat protein, 17%; starch, 60%; butter, 20%; phosphoric acid-free inorganic salts (Osborne), 3%; oryzanin, small amount.

The horse meat protein contained 0.46% of lime and 0.32% of total phosphoric acids in the form of P_2O_5. Therefore, the basal feed contained 0.055% of total P_2O_5.

III. EXPERIMENTAL PROCEDURE

Male white rats weighing 30-35 gm were raised with a feed containing 16% of horse meat protein, 60% of starch, 20% of butter, 4% of Macamum's salt (translator's note - unable to verify) No. 185, and a small amount of oryzanin until the body weight reached 60 gm, and raised with the basal feed for approximately 10 days. During the 10 days, no weight gain was noted. Subsequently, 1% of phosphate was added as a free acid to the basal feed and changes in body weight were observed.

IV. PERIOD OF EXPERIMENT

Group A : From February to August, 1930
Group B : From July, 1930 to January, 1931.

V. EXPERIMENTAL RESULTS

The group without phosphoric acid indicated no growth, the amount of body weight gained being approximately 30-40 gm for a period of 4 months. The hypophosphorous acid group indicated no growth, even when the amount of acid added was increased to 0.5%. The increase of acid content to 1.5 - 2% caused death of the animals in several days.

Normal growth was observed in the orthophosphoric, pyrophosphoric, metaphosphoric, glycerophosphoric, phytic, and hexosediphosphoric acids groups.

Skeletal X-ray examination revealed no abnormality in any of the groups.

The author is deeply indebted to Dr. Suzuki for his assistance throughout this study.

TABLE

Group A

	a 動物番號	b 始めの目方	c 最高目方	d 經過日數
Glycerophosphoric	75	54 gm.	[illegible]	[illegible]
"	86	64	[illegible]	[illegible]
Pyrophosphoric	81	59	[illegible]	[illegible]
"	78	69	[illegible]	[illegible]
Phytic	79	54	[illegible]	[illegible]
"	80	58	[illegible]	[illegible]
Glycerophosphoric	87	62.5	[illegible]	[illegible]
"	88	64.5	[illegible]	[illegible]
Metaphosphoric	82	56	[illegible]	[illegible]
"	92	62.5	[illegible]	[illegible]

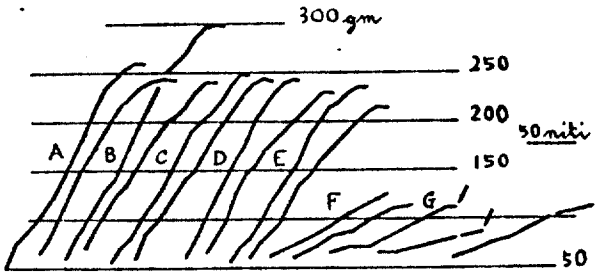
Group B

	a 動物番號	b 始めの目方	c 最高目方	d 經過日數
Orthophosphoric		71 gm.	[illegible]	[illegible]
"	9[illegible]	[illegible]2	[illegible]	[illegible]
Hexosephosphoric	98	69	[illegible]	[illegible]
"	95	69	[illegible]	[illegible]

KEY : a, animal No.; b, initial weight; c, maximum weight; d, number of days

Figure 1

第 一 圖



From left :

A: Orthophosphoric	No. 75 and No. 86
B: Pyrophosphoric	No. 81 and No. 78
C: Phytic	No. 79 and No. 80
D: Glycerophosphoric	No. 87 and No. 88
E: Metaphosphoric	No. 82 and No. 88
F: Hypophosphorous	No. 90 and No. 91

No. 85 : given N-phosphoric acid subsequently.
No. 89 : given 0.5% hypophosphorous, then N-phosphoric acid subsequently.
No. 93 : given 0.5% hypophosphorous acid subsequently.

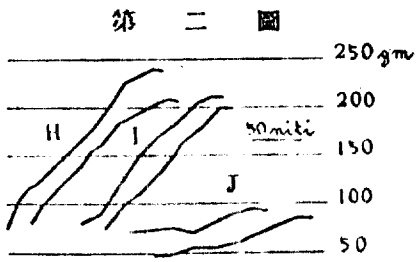
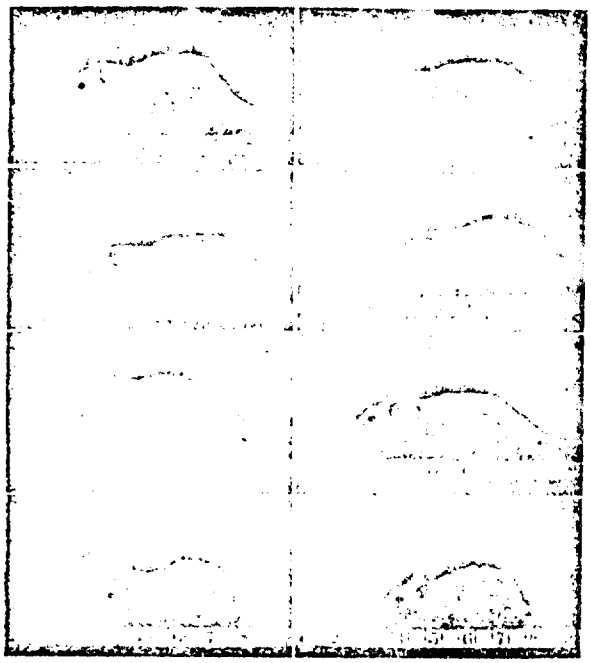


Figure 2

From left :

H: Orthophosphoric	No. 96 and No. 97
I: Hexosephosphoric	No. 98 and No. 95
J: P-free	No. 99 and No. 100

Figure 3



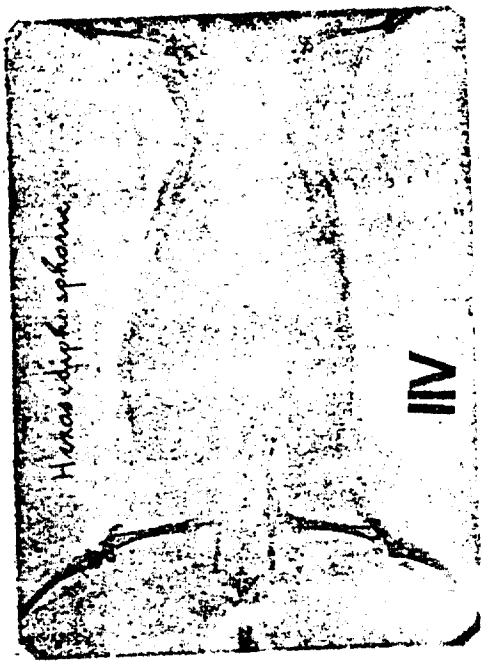
(1): Orthophosphoric	No. 75
(2): Pyrophosphoric	No. 81
(3): Pyrophosphoric	No. 78
(4): Phytic	No. 79
(5): Glycerophosphoric	No. 87
(6): Metophosphoric	No. 82
(7): Hypophosphorous	No. 90
(8): P-free	No. 85

Given various phosphoric acids for approximately 4 months.

Figure 6

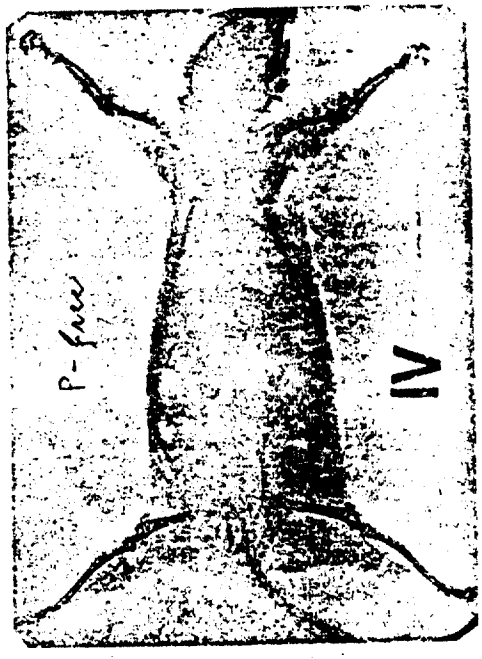
Given various phosphoric acids for 3 months; legs only; photograph ed from top. (orthophosphoric; metaphosphoric; phytin; orthophosphoric-Ca; pyrophosphoric; glycerophosphoric; hexosephosphoric; P-free)

Figure 4



Given hexosephosphoric acid for 3 months; No. 98; reduced to 1/2 of aetual size.

Figure 5



Raised without phosphoric acid for 3 months; reduced to 1/2 of actual size.

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Takahashi-'32
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燐酸の形態による榮養價値比較

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(昭和六年十一月廿六日受理)

序 言

燐酸が動物體の重要なる成分であり從つて動物體の生長發育に必要な事は古くから知られて居るが與へる燐酸の形態による差に付ては殆ど報告がない。

1910年 E. B. Hart 氏等[1]は豚が燐酸に乏しい飼料では生長せず之に燐酸石灰を加へれば完全に發育すから有機態燐酸は必要でないと報告した。 T. B. Osborne 氏等[2]は 1918 年合成飼料で白鼠を飼育し各種無機成分の榮養上の意義を明にした際燐酸に付て無燐酸區は全然生長せず之に正燐酸を與へれば完全な發育をするから蛋白に結合した燐酸其他の有機態燐酸は必要ではないと報告した。グリセロ燐酸，フイチン，ヘキソース燐酸が各燐酸の給源として有効な事は諸氏の報告がある丸も[illegible]したものはない。ピロ燐酸は 1928 年 Lohmann 氏[3]が動物筋肉細胞中に存在する事を報告しその有効な事は想像されるがメタ燐酸に關しては報告がない。

以上によつて各種燐酸の形態について榮養上の比較を行つた。

I. 試驗した燐酸鹽類

Orthophosphoric acid (sec.), Pyrophosphoric acid, Metaphosphoric acid, Hypophosphorous acid, Glycerophosphoric acid, Phytic acid，以上各酸の Na 鹽及び Orthophosphoric acid (sec.), Hexosediphosphoric acid の Ca 鹽。

使用した藥品は次の通り，

Orthophosphoric acid: メルク社製品を溫水から再結す。

Pyrophosphoric acid: メルク社製品を溫水から再結す硝酸銀で純白の銀鹽を沈澱す。 Ammonium molybdate と硝酸とを加へても常溫では反應なく正燐酸の混在を認めない。

Metaphosphoric acid: カールバウム社製品を冷水で洗ふ硝酸銀で白色の銀鹽を沈澱す。 Ammonium molybdate と硝酸とを加へても常溫では反應なく燐酸亞鉛と醋酸とを加へても沈澱なし正燐酸ピロ燐酸の混在を認めない。

Hypophosphorous acid: メルク社製局方品。

Glycerophosphoric acid: メルク社製品を水から再結す。P_2O_5 理論數 32.87%實驗數 32.84%

Phytin: 米糠からとつたフイチンを鹽化鐵で鐵鹽とし苛性曹達で曹達鹽としてアルコールで再結す。P_2O_5 理論數 46.11% 實驗數 46.02%。

Hexosediphosphoric acid: バイエル社製 Candiolin を C. Neuberg 及 S. Sabetay 氏法[4]に從つて製精す P_2O_5 理論數 34.13% 實驗數 34.02%。

II. 基 本 飼 料

馬肉蛋白 17% 澱粉 60% 牛酪油 20% 無燐酸無機鹽類 (Osborne 氏) 3% オリザニン少量。

但し馬肉蛋白中に灰分 0.46% 全燐酸が P_2O_5 として 0.32%あり從つて基本飼料中に P_2O_5

0.055 %あり．

III. 試驗の方法

30～35 gm. の雄白鼠を馬肉蛋白 16%，澱粉 60 %，牛酪油 20%，マカマム氏鹽 185 號 4 %，及びオリザニン少量の飼料で 60 gm. 前後まで育て次で基本飼料で約 10 日間飼育す．この間體重增加は殆どない．次に基本飼料へ各燐酸鹽類を free acid として 1 %づゝ添加し體重の增加を觀測した．

IV. 試驗の期日

A 組　昭和五年二月より八月まで

B 組　同　　七月より昭和六年一月まで

V. 試驗の結果

無燐酸部は殆ど成長せず四ケ月間に約 30～40 gm. 體重增加した．

Hypophosporous acid部も無燐酸部と同樣に殆ど成長しない．酸を 0.5 %にしても同じ酸を 1.5 % 2% [illegible] 死す．

Orth[illegible]phoric acid, [illegible]osph[illegible] acid, Metaphosphoric acid, Glycerophosphoric acid Phytic acid, Hexosediphosphoric acid はいづれも殆ど差はなく順當に生長した．

A　組

	a動物番號	始めの日方 b	最高日方 c	成長日數 d
Glycerophosphoric	75	54 gm.	261 gm.	130日
〃	86	64	244	120
Pyrophosphoric	81	59	298	150
〃	78	6[illegible]	243	13[illegible]
Phytic	79	5[illegible]	2[illegible]9	1[illegible]0
〃	80	58	246	13[illegible]
Glycerophosphoric	87	62.5	243	1[illegible]0
〃	88	64.5	230	13[illegible]
Metaphosphoric	82	56	234	1[illegible]
〃	92	62.5	216	13[illegible]

B　組

	a動物番號	b始めの日方	c最高日方	d成長日數
Orthophosphoric	96	71 gm.	238 gm.	140日
〃	97	72	205	130
Hexosephosphoric	98	69	209	125
〃	95	69	199	120

X 光線寫眞で骨格を驗べたが各燐酸部も無燐酸部も異狀はない．

此試驗について御懇篤な御指導を賜つた鈴木先生へ深謝す．

昭和六年四月　　東京帝國大學農學部農藝化學教室にて

文　　献

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(3) K. Lohmann: Biochem. Z., 202, 466.
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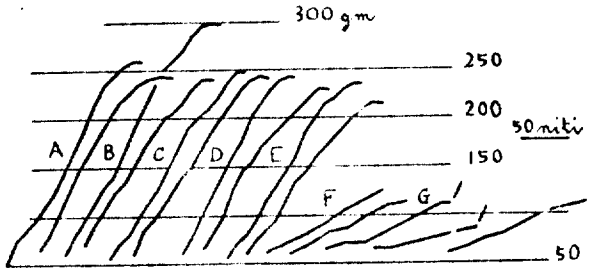
第　一　圖

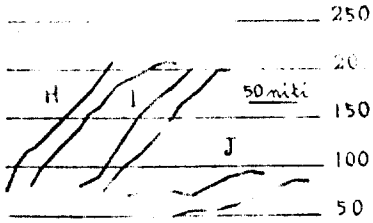


第一圖左から

A：	Orthophosphoric	No. 75	及び	No. 86
B：	Pyrophosphoric	No. 81	及び	No. 78
C：	Phytic	No. 79	及び	No. 80
D：	Glycerophosphoric	No. 87	及び	No. 88
E：	Metaphosphoric	No. 82	及び	No. 92
F：	Hypophosphorous	No. 90	及び	No. 91

No. 85　後正燐酸を與ふ
No. 89　後 0.5% Hypophosphorous, 後更に正燐酸を與ふ
No. 93　後 0.5% Hypophosphorous, を與ふ

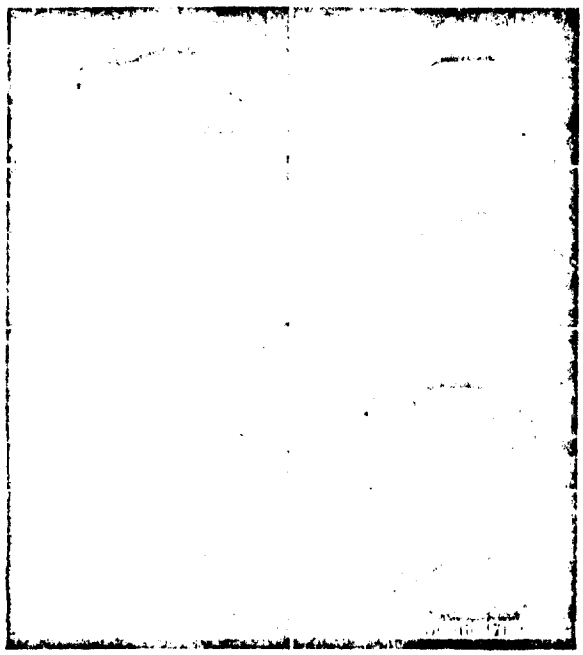
第　二　圖



第二圖左から

H：	Orthophosphoric	No. 96	及び	No. 97
I：	Hexosephosphoric	No. 98	及び	No. 95
J：	P-free	No. 99	及び	No.100

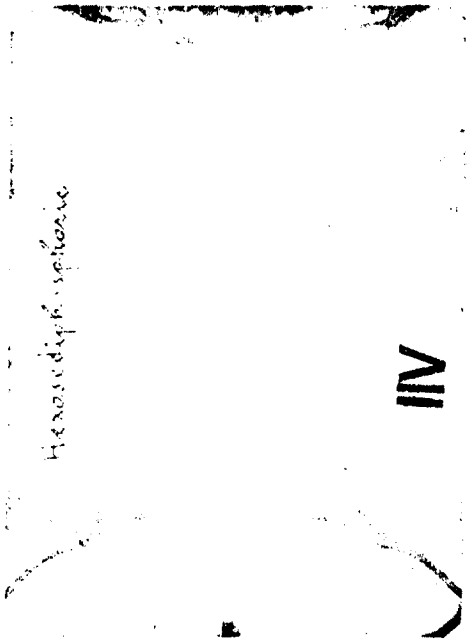
第　三　圖



(1)：Orthophosphoric　No. 75
(2)：Pyrophosphoric　No. 81
(3)：Pyrophosphoric　No. 78
(4)：Phytic　No. 79
(5)：Glycerophosphoric　No. 87
(6)：Metophosphoric　No. 82
(7)：Hypophosphorous　No. 90
(8)：P-free　No. 85

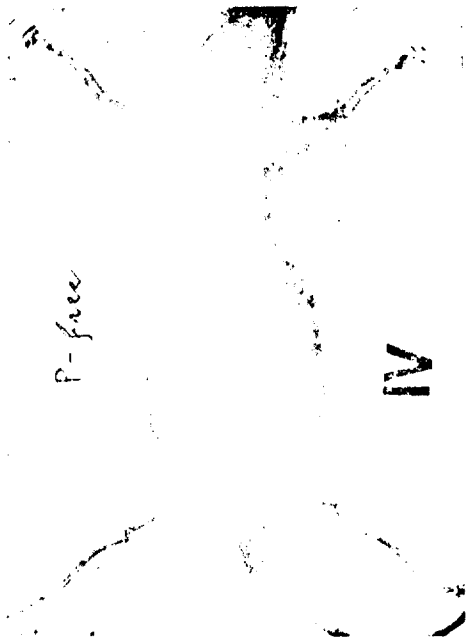
各種燐酸にて約四月飼育せるもの

第四圖



Hexosephosphoric で3月飼つたもの
No. 98.
約二分一實物大

第五圖



無燐酸で3月飼へるもの
No. 99.
約二分一實物大

第六圖

各種燐酸で3月飼へるものの脚部のみ上から

Orthophosphoric	Pyrophosphoric
Metaphosphoric	Glycerophosphoric
Phytin	Hexosephosphoric
Orthophosphoric-Ca	P-free

Vasenius-'64
Bib No. 58

Nord. Vet.-Med. 1964, *16*, 806—812.

From the Department of Physiology, College of Veterinary Medicine, Helsinki.

The Urinary Excretion of Intravenously Administered Hypophosphite

by *L. Vasenius* & *K. Kallela.*

Introduction

Owing to the close metabolic connection between phosphorus and calcium, the element of prominent importance in bovine medicine, there has been a tendency to combine calcium and phosphorus in therapeutical preparations. However, this has met with difficulties caused by the low water solubility of the calcium phosphates, and as a result one has looked for suitable solutions among compounds other than orthophosphate. Calcium hypophosphite, which has a fairly good solubility in water, was to our knowledge introduced in veterinary medical use by W. Hallgren in 1938 (*Hallgren* 1955). It has since been rather generally used, at least in the Scandinavian countries, and from the practitioner's experience it is comparatively favourable (*Hallgren* 1955, *Vuorinen* 1959). On the other hand, however, no studies have been made as to whether the organism is really capable of converting the hypophosphite into orthophosphate. It should be noted that such a conversion involves a change in valence, and that reactions of this type are rare in animal organisms. The experiments reported here were undertaken in order to clarify how rapidly hypophosphite administered by injection is excreted, and to estimate on this basis the degree to which hypophosphite may possess significance for the organism as a source of phosphorus.

Method and Material

Iodometric titration (*Rupp & Finck* 1902, *Rosenheim & Pinskar* 1909) is used for the determination of hypophosphite in the presence of phosphite and phosphate. The method is based on the fact that iodine readily oxidizes hypophosphite to phosphite in a solution containing sulphuric acid, and phosphite to phosphate in a neutral solution. If the solution contains only hypophosphite and no phosphite, the iodine consumption will be equal in magnitude in both reactions. The determinations were made from urine, which in itself contains reducing substances; under the conditions present here about two thirds of these are oxidized in the first and one third in the second stage of the reaction. This circumstance produced some variation in iodine consumption after the hypophosphite injection, but they were slight enough to justify the conclusion that there was no phosphite, or only a negligible quantity, in the urine. The actual determinations, therefore, were carried out by determining only the total iodine consumption according to the recommendation of *Rupp & Finck* (1902). For each determination 10 ml urine were used, and 50 ml 0.1-N iodine solution and 5 ml 10 % sulphuric acid were added. After about 15 hours the solution was neutralized with sodium bicarbonate and left to stand for another two hours, whereupon the excess iodine was titrated with 0.1-N sodium thiosulphate. All determinations were carried out as double determinations.

In all tests the experimental animal received an injection of 0.3 moles hypophosphite, which is approximately equivalent to the usual therapeutic dosage. Since our study concerned only the hypophosphite ion and the subjects were healthy cows, half of the hypophosphite was given as sodium salt and the other half as calcium salt. For the collection of urine the urination was stimulated mechanically.

The experimental animals were two cows, one of which with a good productive capacity (19 kg milk per day), pregnant, estimated live weight 400 kg; the other had a low productive capacity (5 kg milk per day), non-pregnant, estimated live weight 320 kg. The experiment was repeated three and four times respectively, and the results were pooled for either cow. Prior to each test the reducing substances normally encountered in

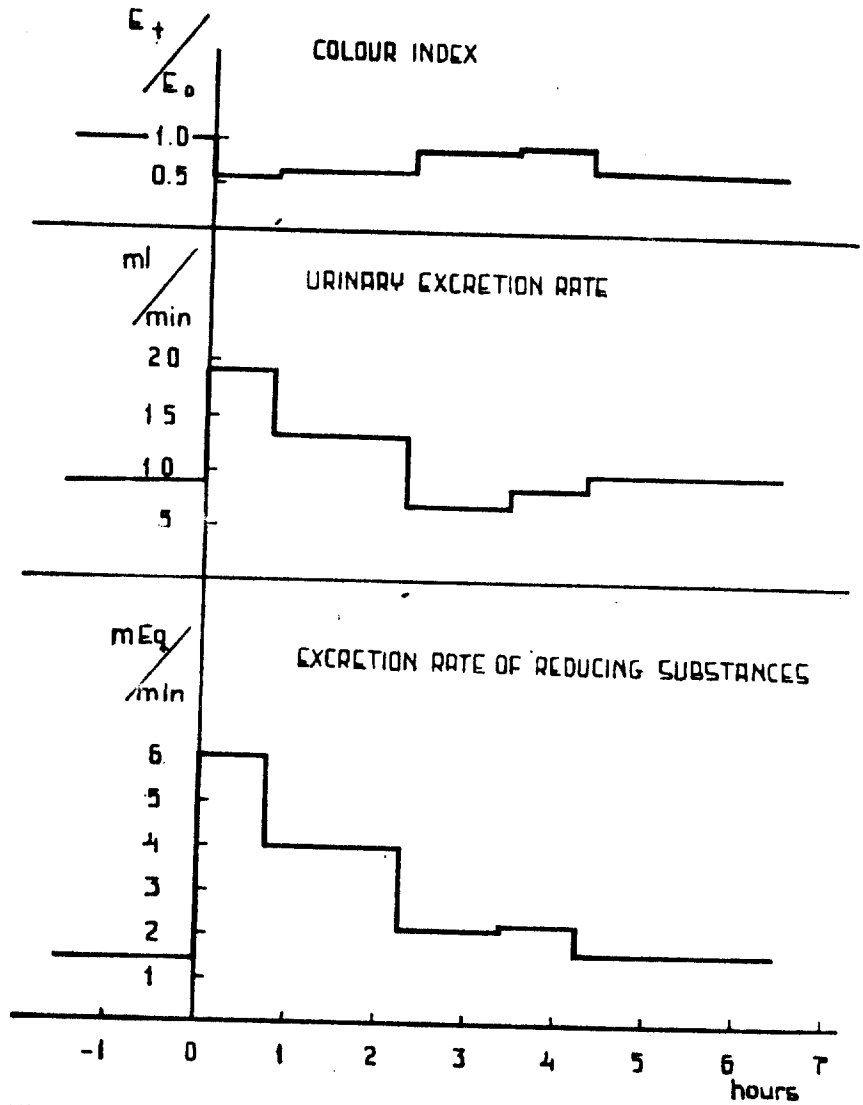


Fig. 1. The effect of an intravenous injection of 0.3 moles hypophosphite. Data from a test performed on a high-producing cow.

the urine were determined by titration, and the extinction at 420 mμ wave-length was determined with a photoelectric colorimeter for each urine sample as a check on diuresis.

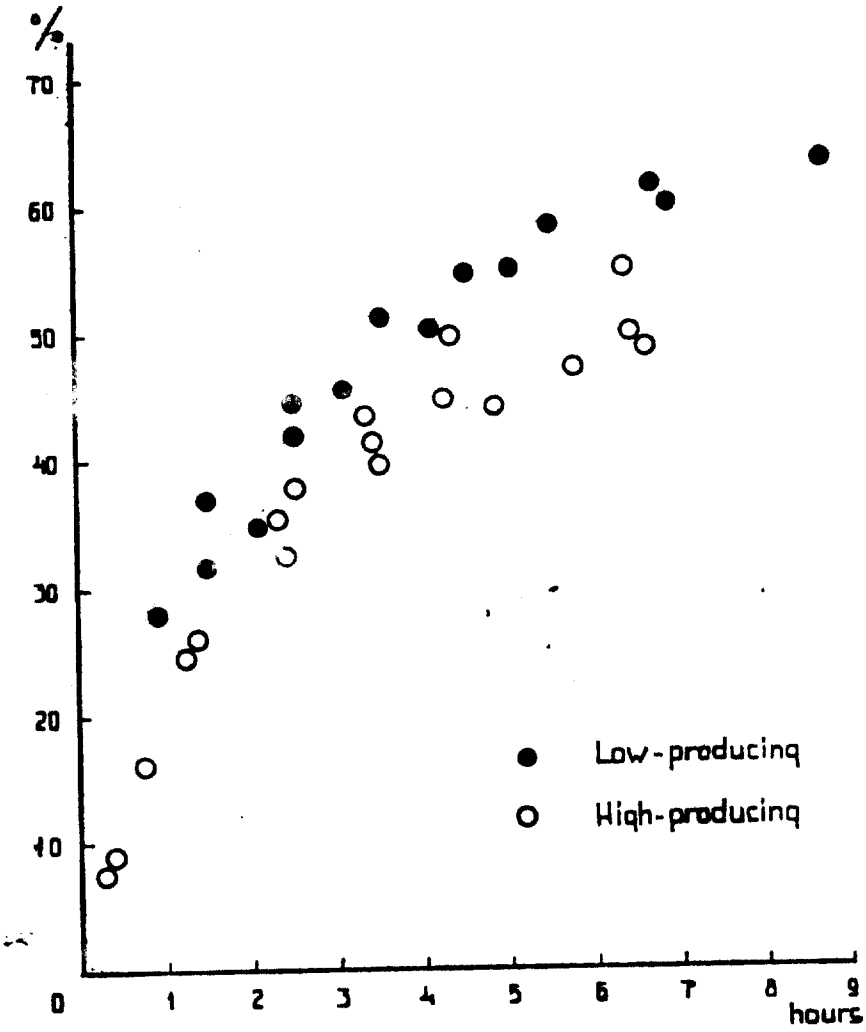


Fig. 2. Total hypophosphite output in the urine of two cows, one low-producing and the other high-producing, measured at various intervals and expressed as per cent of the initial 0.3 moles injected.

Results

In Fig. 1 the excretion of reducing substances, diuresis, and colour index in one of the three experiments performed in the cow with good productive capacity are exemplified. It was found that simultaneously with the increase in excretion of reducing

substances after the injection there was also a considerable increase of diuresis, and as a result of same a decrease in colour intensity of the urine, which caused considerably lower extinction values to be recorded. It is obvious from this that the manner in which the "normal" iodine-consuming substances are taken into account as corrections has an appreciable effect on the final results. In Fig. 2 the increase in excretion of reducing substances, calculated as hypophosphite, has been shown on the assumption that the content of the urine of normally occurring reducing substances is proportional to the colour intensity. If, again, the correction is based on the hypothesis that the normal excretion continues as before, unaffected by the injection, the increase in the excretion of reducing substances, calculated as hypophosphite, would be higher. The results in Fig. 2 would thus rather represent a minimum level of hypophosphite excretion, but on the strength of our experiments we think that they are closest to the actual truth.

After this correction the results indicate that

(1) the total quantity excreted in the urine was about two thirds of the injected quantity in the tests with the cow with low productive capacity, and slightly less in those with the cow with good productive capacity, and

(2) about one half of the hypophosphite excreted through the kidneys left the body within 1½ to 2 hours.

Discussion

At a critical assessment of the present results most attention will naturally be attracted to the question of where the hypophosphite quantity which has not immediately been excreted in the urine may have gone. If it has been converted into phosphate, the implication is that hypophosphite may have a certain significance as a source of phosphorus. However, such conversion would necessarily be through a phosphite stage, and there is nothing in our results supporting such an assumption. As other possible paths of excretion one might think of the udder, the saliva, or the intestinal wall. Some corroboration of the first alternative could be seen in the fact that the hypophosphite ex-

cretion in the urine was less in the case of the cow with high productive capacity. Against this one should consider that a cow with good productive capacity has a higher metabolic rate and, consequently, conversion to phosphate could take place more easily. A high metabolic rate, of course, also necessitates an increase in food consumption and thus increased salivary secretion. The digestive tract is an important path of excretion for phosphates under normal conditions (*Scheunert & Trautmann* 1957). It is not known to which extent hypophosphite may be excreted by this path, and the iodometric technique cannot be used in a study of this question any more than for investigation of the potential excretion in the milk.

In our opinions the present experiments serve to show that the significance of hypophosphite as a source of phosphorus is questionable. It seems, however, that the problem can hardly be definitively clarified without the aid of investigations involving radioactive tracer techniques.

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Summary

The urinary excretion of hypophosphite administered to cows has been investigated by an iodometric technique. After an intravenous injection about two thirds of the administered quantity was excreted unchanged in the urine, the excreted quantity being slightly less in the case of a cow with good productive capacity. About half of the entire excreted quantity left the body within 1½ to 2 hours. On the strength of the experiments the value of hypophosphite as a source of phosphorus for the organism is considered questionable.

Zusammenfassung

Die Ausscheidung von intravenös appliziertem Hypophosphit im Harn

Die Ausscheidung von an Kühe verabreichtem Hypophosphit im Harn wurde mittels eines jodometrischen Verfahrens untersucht. Nach erfolgter intravenöser Injektion wurden etwa zwei Drittel der verabfolgten Menge unverändert im Harn ausgeschieden; bei einer Kuh mit hoher Leistungskapazität war der ausgeschiedene Anteil etwas geringer. Von der gesamten ausgeschiedenen Menge verliess etwa die Hälfte den Körper innerhalb 1½ bis 2 Stunden. An Hand der Versuche wird der Wert von Hypophosphit als Phosphorquelle für den Organismus als fragwürdig erachtet.

Resumé

Utsöndring i urin av intravenöst applicerad hypofosfit

En jodometrisk teknik gav till resultat, att av en intravenöst injicerad terapeutisk dos hypofosfit ungefär två tredjedelar utsöndrades oförändrad i urinen. Hos en högmjölkande ko var den utsöndrade mängden något mindre. Ungefär hälften av denna mängd elimminerades inom 1½—2 timmar. Då även andra utsöndringsvägar kunna komma i fråga, ifrågasättes hypofosfitens värde som fosforkälla.

Modtaget af redaktionen den 16. marts 1964.