SCIENTIFIC SECTION, AMERICAN PHARMACEUTICAL ASSOCIATION

HEXAMETHYLENAMINA.*

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Hexamethylenamina U. S. P., known commercially as Urotropin, Formin, Cystogen, Aminoform, Oritone, and probably other trade names, occurs as colorless, lustrous, odorless crystals or in the form of white compressed tablets.

It is soluble in about 1.5 parts of water, 10 parts of alcohol, and 288 parts of ether at 25° C. (77° F.). When heated to 263° C. (505.4° F.) it sublimes without melting and with partial decomposition.

Hexamethylenetetramine, $(CH_2)_6N_4$, is a condensation product obtained by the action of ammonia on formaldehyde.

It is made by passing a current of dry ammonia gas over warm trioxymethylene (Paraformaldehyde) and purifying the product.

There are several compounds of hexamethylenetetramine:

Hexamethylenetetramine salicylate; hexamethylenetetramine bromethylate, known as Bromalin, Bromoform, Bromethylformin; hexamethylenetetramine tannin, known as Tannopin, Tannon; hexamethylenetetramine iodoform, known as Iodoformin; Ferrostyptin, a double salt of hexamethylenetetramine hydrochloride and ferric chloride.

The fact that Hexamethylenamine has been sold under various names led some to believe that ill effects of the drug came from inferior products. Such reports led Daniel Base[1] to analyze the products appearing under the trade names of Urotropin, Formin, Cystogen, Aminoform, and also various samples of the official Hexamethylenamine.

He used the tests described by Alfred Wohlk, as follows: Hexamethylenamine heated with Nessler's reagent should show no signs of coloration.

Base in his experiment gets a slight pale yellow precipitate; according to Romizn this is a double salt of hexamethylenetetramine and potassium mercuric iodide.[2] The precipitate dissolves on heating, producing a pale yellow solution. All of these samples showed negative tests with Nessler's reagent.

Base also used the method of estimation of nitrogen as ammonia for establishing the purity of these compounds as follows:

Hexamethylenamine heated with sulphuric acid gives off formaldehyde, and nitrogen is liberated as free ammonia which unites with the acid. On titration with normal potassium hydroxide solution he found that 1 Gm. of pure hexamethylenamine was found equal to 28.74 Cc. of NH_2SO_4.

All of these methods, he declares, while not exact, give the same results for all of the samples tested. He finds one form of hexamethylenamine as pure as another.

Puckner and Hilbert determined hexamethylenamine in galenical preparations

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by decomposition of hexamethylenamine with an acid and estimating the free ammonia by titration.[3]

A. Brown and B. Fotto suggest an analysis of hexamethylenamine alone or in a galenical mixture by hydrolyzing hexamethylene and estimating the amount of free ammonia, or by the amount of formaldehyde given off.[4]

DECOMPOSITION OF FORMALDEHYDE.

Perhaps the first work done on the decomposition products and various influences under which hexamethylenamine is decomposed was done by two Japanese, Ischidzu and Inouyu. They report that acids readily decompose hexamethylenamine into formaldehyde, carbon dioxide and methylamine. Boiling water will readily decompose it, liberating formaldehyde. It is more stable in alkaline solutions than in neutral.[5]

Wohlk suggests the use of Nessler's reagent for the detection of ammonia, amides, and para-formaldehyde. He finds that on boiling with sodium hydroxide solution there is not a trace of nitrogen liberated as ammonia.[6]

In some recent investigation of the decomposition products of hexamethylenamine by Paul J. Hanzlik, M.D., and R. J. Collins, we find hexamethylenamine is decomposed only in acid medium, and the decomposition is wholly dependent on the hydrogen ions present. Concentration of the acid, and temperature, have much to do with the decomposition. In the presence of 0.2 percent HCl if kept at a temperature of 37.5° for one to five hours or on boiling it will liberate formaldehyde. They conclude that no formaldehyde is liberated in alkaline solutions. It does not liberate formaldehyde in body fluids unless truly acid, namely, gastric juice and urine.[7]

TESTS TO DISTINGUISH BETWEEN FORMALDEHYDE AND HEXAMETHYLENAMINE

The tests of the Pharmacopoeia are not suitable for this purpose on account of the fact the reagents used will liberate formaldehyde as H_2SO_4 and $C_6H_4OH.COOH$ tests. The silver ammonia iodide test is also unfit for testing for free formaldehyde in urine because of the precipitation of soluble salts of the urine by this reagent. Hanzlik and Collins recommend the following tests for distinguishing the presence of free formaldehyde in urine and body fluids:

Nitroprusside test: This test may be performed in cold or natural room temperature as follows:

To 5 to 10 Cc. of the solution to be tested contained in a test tube add three drops of phenylhydrazine, two drops of nitroprusside and three drops of alkali in the above mentioned order; if formaldehyde is present in an aqueous solution an emerald-green to a deep blue color is produced at the moment the alkali comes in contact with the solution. This color gradually diffuses through the liquid and almost at once begins to disappear. In highly dilute solutions of formaldehyde, there is finally formed an orange-yellow to a urine-red color. In urine containing formaldehyde the sequence of colors is as follows: as soon as the hydroxide is added a deep color is produced (generally, not always); this quickly changes to green then to yellow and finally to yellowish red. This test is directly applicable to all body fluids except bile and blood, owing to the color possessed by them.

Phloroglucin test for free formaldehyde: This can be performed in cold or natural room temperature and is as follows:

By the direct addition of about 0.5 Cc. of reagent to about 1 to 2 Cc. of the fluid to be tested; if the fluid contains formaldehyde, a deep, bright red color will appear instantly if the solution is concentrated; however, if of higher dilution it usually takes from one-half to one minute for the color to reach its intensity. The color persists for at least five minutes with dilute solutions. The test is applicable to all body fluids except bile and blood.[8]

"Nicolair in 1899 discovered the elimination of formaldehyde in the urine after an administration of hexamethylenamine. He observed it to have marked urolytic (uric acid solvent) properties. He found that after administration, uric acid and urate disappear. However, at this time, there was no clinical data.[9]

Since the discovery of formaldehyde in the urine, after the administration of hexamethylenamine, it has grown to be one of the largely used chemicals in medicine. For a long time the medical profession has sought some way of administering formaldehyde to obtain its antiseptic and disinfectant properties on the urinary tract. Owing to the irritating action of formaldehyde, which is said to be the equal of corrosive sublimate, this drug was but seldom given internally.

Dr. G. M. Bellch, of Chicago, in a paper before the American Medical Society, says "hexamethylenamine is unsurpassed by other drugs as an antiseptic to the urinary tract. Its value can be estimated clinically as well as bacteriologically. Decomposed by uric acid, in the bladder, into ammonia and formaldehyde, it destroys the bacteria found in the urine in from one to two days. It is valuable as a disinfectant because it will not undo the effects of any local medication."[10] H. Thursfield recommends hexamethylenamine in bacteriuria.[11] W. Coleman reports gastro-intestinal disturbance after administration of 1 Gm. of hexamethylenamine. He also reports other secondary effects.[12]

Frederick C. Shattuck reports having administered 7 to 10 grammes of hexamethylenamine in a case of typhoid twice daily two successive days of each week, until convalescence was complete, with no evil effects.[13]

THE LIBERATION OF FORMALDEHYDE FROM HEXAMETHYLENAMINE IN PATHOLOGICAL FLUIDS.

"These fluids were obtained from patients suffering with different clinical conditions. Usually about 60 grains of hexamethylenamine was given an hour before the fluid was obtained.

In urines of 12 persons suffering with chronic hemorrhagic nephritis, the average time of appearance of formaldehyde in the urine was about seventeen minutes. In all urine where the H^+ ion was concentrated, that is, the urine was truly acid, it gave tests for free formaldehyde and hexamethylenamine. In one case the urine was alkaline and only hexamethylenamine was present.

Other body fluids showed the presence of hexamethylenamine but no formaldehyde.

Ten specimens of bile from a patient who was ill with typhoid fever and had received hexamethylenamine for about four months was operated on for chololithiasis, showed the presence of hexamethylenamine and typhus bacilli at the beginning and the end. Hexamethylenamine has no bactericidal action by itself and only acts as a bactericide in the presence of acids."[14]

HEXAMETHYLENAMINE IN THE SALIVA, BILE AND PANCREATIC JUICE.

On several tests made on dogs and human beings it is concluded that hexamethylenamine is secreted in the saliva. It has no bactericidal effect as there is no trace of formaldehyde present.[15]

S. J. Crowe finds that hexamethylenamine when administered by mouth is rapidly absorbed and remains in the circulating blood for twenty-four hours. Apparently the maximum concentration in the blood is reached from five to eight hours after administration. It is excreted in the bile, pancreatic juice, and directly through the wall of the gall-bladder, in dogs.

It was found to be present in saliva and milk of dogs after intravenous injection of 1 Gm. of the drug.[16]

Crowe merely suggested that it liberated formaldehyde in the above cases.

Flexner and Clarke[17] and Hald[18] have shown that no free formaldehyde is secreted in these situations.

G. Markman finds it a most efficient food preservative for milk and chopped meat. A 0.1 percent solution will preserve milk for several days and as little as 0.01 percent will keep it fresh for twelve hours; 0.01 to 0.02 percent will keep meat fresh for days.

Markman suggests the following for use as a preservative for meat; hexamethylenamine 100 parts, common salt 850 parts, potassium nitrate 15 parts, and sugar 35 parts.[19]

On the addition of 1 percent hexamethylenamine to four ounces of milk I find it to act as a preservative. The preservative properties of hexamethylenamine depend entirely on its ability to liberate formaldehyde. Lactic acid found in milk probably liberates formaldehyde which destroys the ferments present and stops fermentation for a time.

I find that hexamethylenamine in aqueous solution on the addition of sulphuric acid, on distillation, gives off formaldehyde, also when gently heated, or even in cold concentrated solutions it will give off formaldehyde.

On boiling an aqueous solution of hexamethylenamine formaldehyde is given off which can be recognized by its odor and its response to the silver iodide test. If a few drops of silver ammonium iodide T. S. be added to the solution, it produces a gray precipitate of metallic silver which forms a mirror on the sides of the test tube.

On boiling, heating, or in cold solution concentrated hydrochloric acid decomposes hexamethylenamine into formaldehyde and other products, probably ammonia and carbon dioxide or amines.

A 0.2 percent solution of hydrochloric acid added to a solution of hexamethylenamine and kept at a temperature of 52° C. for one hour gave the odor of formaldehyde which was confirmed by other tests.

Uric acid added to a solution of hexamethylenamine and heated to a temperature of 52° C. was decomposed and responded to tests for formaldehyde.

In four tests using 250 mg. of uric acid and 1 Gm. of hexamethylenamine in aqueous solution, the solution being kept in a water bath regulated to 53° C. for one hour and being agitated every ten minutes, the amount of uric acid dissolved under these conditions was scarcely noticeable. I conclude that the action of hexamethylenamine as a uric acid solvent is very slight. I have had no opportunity to observe its action as a urolytic agent under pathological conditions.

HEXAMETHYLENAMINE ON THE HEART.

The action of 1, 2, 3, 4, 5 and 10 percent solutions of hexamethylenamine was used in this experiment.

A frog of 25 Gm. weight was used and the action recorded. The experiments were as follows:

The frog was prepared by pithing, consisting of probing to prevent sensibility to pain and causing possible movement during experiment.

The frog was placed on a frog board and pinned down with the abdomen uppermost; the skin over the abdomen was pinched up and slit to the mouth; abdominal wall was then divided slightly to one side to prevent cutting the anterior abdominal veins. By a transverse cut the sternum was divided, the anterior junction of the abdominal vein of the heart being preserved. The pectoral girdle was next divided and pulled far apart. The heart was removed from the pericardium. A small hook was attached to the anterior end of the heart; this hook, by means of a thread, was connected to a heart lever. The heart lever was counterpoised so as to record the movements of the heart on a smoked drum. The results of this experiment, using solutions from 1 to 10 percent, are as follows:

Total amount of solution, 270 minims; total amount of hexamethylenamine, 10.6375 grains; total length of time during administration, $2^1/_2$ hours. At the end of this time the rate of heart-beat was 54; there was no perceptible change in the beat of the heart except every other diastole was slightly shortened.

I conclude that hexamethylenamine itself has no action on the heart. Its decomposition products may have marked action.

THE USE OF HEXAMETHYLENAMINE IN AQUEOUS SOLUTIONS ADMINISTERED HYPODERMICALLY AS AN ANAESTHETIC.

The experiment was made on a frog and the results were as follows: Five grains were injected in the abdomen of a frog; after 3 minutes 3 grains more were given; after 18 minutes there were no signs of anaesthesia. Eight grains were injected and at the end of 10 minutes there was no sign of anaesthesia, frog being sensitive to an electric stimulus. Coördination of the muscles was not as complete as in a normal frog.

One hour and nine minutes after the first injection ten grains more were given. Twelve minutes after the last injection there was internal hemorrhage in the lower portions of the body, frog had lost all coördination of the muscles but was still sensitive to touch.

After one hour and thirty minutes after last injection ten grains more were given. There was continued loss of coördination of muscles but no anaesthetic effect, frog remaining sensitive to electric stimulus until time of death.

HEXAMETHYLENAMINE INCREASES THE AMOUNT OF FREE AMMONIA IN THE URINE.

The method used in these experiments is as follows: Free ammonia (ammonia combined with free acids) (Folin's method). Place 25 Cc. of urine in a tall cylinder provided. To the urine add 10 Gm. NaCl to prevent oxidation, add a few Cc. of kerosene oil to prevent frothing and 1 Gm. Na_2CO_3. Connect this cylinder with another cylinder containing 30 Cc. of $\frac{N}{10}$ H_2SO_4 and an equal amount of water. Connect the second cylinder to a suction pump and suck air through the two cylinders as rapidly as possible; continue the suction from four to five hours, the time necessary depending on the strength of the suction. At the close of the time disconnect the cylinder and titrate the acid solution with $\frac{N}{10}$ NaOH solution, using alizarine red as an indicator. Calculate the amount of ammonia N as follows: Subtract the amount of base used from the amount of acid solution. This is equal to the amount of acid neutralized by the

ammonia. The amount of acid neutralized multiplied by 0.0014 equals the amount of N calculated as free ammonia in 25 Cc. of urine. The total output in a day multiplied by the equivalent of 1 Cc. equals the amount of free ammonia in a day's output.

Four experiments by the above methods were made: Seven and one-half grains of hexamethylenamine were taken every two hours during the day, the urine for the day was collected and the amount of ammonia estimated. A total amount of hexamethylenamine taken during the day was 45 grains. The average of N calculated as free ammonia from the four experiments was 0.805 Gm.

The total output of urine varied from 1150 to 1500 Cc. per day. The urine remained acid to litmus during the whole time.

The urine voided during four days under normal conditions was tested for free ammonia stated in terms of nitrogen. The amount was 0.638 Gm. The total amount of normal urine during the day's output varied from 1100 to 1350 Cc. The increase in free ammonia in urine after taking hexamethylenamine was 0.167 Gm.

The variation of the ammonia in urine makes it difficult to get exact results of the increase in ammonia caused by the administration of hexamethylenamine, but from the above results it seems that the administration of hexamethylenamine does increase the amount of ammonia. There is a field for investigation along this line. If the ammonia is liberated only in the bladder it will cause a great increase of ammonia but if it is liberated before getting to the bladder, the ammonia will probably be eliminated as amines or urea.

It is true that hexamethylenamine has diuretic properties and also liberates formaldehyde in the urine, but the question arises whether formaldehyde is liberated before or after it reaches the bladder. From previous experiments we find that hexamethylenamine is readily decomposed in an acid medium and experiments have shown that as low as 0.2 percent of HCl will decompose hexamethylenamine into formaldehyde and other products.

Working on this theory we naturally suppose that hexamethylenamine is broken up in the stomach and formaldehyde is liberated. This being the case we know that the formaldehyde acting as a bactericidal agent will kill the ferments present in the stomach and impair if not totally destroy the action of pepsin on proteids and also the action of milk curdling ferment rennin.

The action of pepsin on egg albumen in the presence and absence of hexamethylenamine has been carried out by the following method: Pepsin, 0.1 Gm.; egg albumen, 10 Gm.; diluted hydrochloric acid and distilled water, of each a sufficient quantity. Mix 9 Cc. of dilute hydrochloric acid with 291 Cc. of distilled water and dissolve the pepsin in 150 Cc. of the acid solution. Immerse a hen's egg, which should be fresh, during fifteen minutes in boiling water; remove the pellicle and all of the yolk; rub the white coagulated albumen through a No. 40 sieve. Reject the first portion that passes through the sieve and place 10 Gm. of the succeeding portion in a wide mouth bottle of 100 Cc. capacity. Add 20 Cc. of the acid liquid and by means of a glass rod tipped with cork, completely disintegrate the albumen; then rinse the rod with 15 Cc. of the acid solution and add 5 Cc. of the pepsin solution. Cork the bottle securely and invert it three times; place in a water bath that has been previously regulated to 52° C. (125.6° F.). Keep it at this temperature for two and a half hours, agitating every ten minutes by inverting the bottle one time. Then remove from the water bath, add 50 Cc. distilled water, transfer the mixture to a narrow cylinder and allow it to stand for half an hour. The deposit of egg albumen should not then measure over 1 Cc. By this method the pepsin was first standardized and the amount of egg albumen deposited was less than 1 Cc.

Two assays were made under the same conditions, one containing $7^1/_2$ grains of hexamethylenamine, the other 15 grains. The amount of egg albumen deposited in each case was

approximately 10 Cc. There was no appreciable action of pepsin on egg albumen in the presence of hexamethylenamine.

The above experiment is calculated to give a mechanical representation of the conditions which exist in the normal stomach. This being true, we readily believe that hexamethylenamine is decomposed in the stomach and the action of the ferments present are absolutely retarded in its presence.

These conditions being true in physiological and pathological conditions of the stomach, long use of hexamethylenamine will result in gastro-intestinal troubles. Indigestion will naturally ensue and cause harmful action which cannot be overcome by its therapeutic value as an antiseptic to the urinary tract. Further investigation along this line should be conducted.

HEAT FROM HEXAMETHYLENAMINE.

Tablets of hexamethylenamine affords a very efficient way of obtaining heat for physicians at the bedside, for example, in dissolving hypodermic tablets. They are inexpensive and easy to carry. From the following experiment it can easily be seen that these tablets on burning afford sufficient heat for this purpose.

Hexamethylenamine tablets ignite on the application of a lighted match and burn with a practically smokeless flame. The following experiments were made: Hexamethylenamine tablets, $7^1/_2$ grains, were ignited in the open under a test tube containing 10 Cc. H_2SO_4 of sp. gr. 1.84.

RAISE OF TEMPERATURE.

No. 1.		No. 2.	
1st minute	64.5° C.	1st minute	74.0° C.
2nd minute	68.5° C.	2nd minute	66.0° C.
3rd minute	24.0° C.	3rd minute	22.0° C.
25 seconds	1.5° C.	50 seconds	1.0° C.
Time of burning: 3 min. 25 sec.	..	Time of burning: 3 min. 50 sec.	..
Total	158.5° C.	Total	163.0° C.
No. 3.		**No. 4.**	
1st minute	67.0° C.	1st minute	70.0° C.
2nd minute	72.0° C.	2nd minute	67.0° C.
3rd minute	29.0° C.	3rd minute	27.0° C.
58 seconds	2.5° C.	50 seconds	1.0° C.
Time of burning: 3 min. 58 sec.	..	Time of burning: 3 min. 50 sec.	..
Total	170.5° C.	Total	165.0° C.

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