AN EXPERIMENTAL INVESTIGATION OF THE VALUE OF HEXAMETHYLENAMIN AND ALLIED COMPOUNDS *

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This study was undertaken to determine the capacity of infected kidneys to excrete hexamethylenamin. We had just had a small series of unilateral kidney infections in which large doses of hexamethylenamin had failed to be of any benefit. The frequent clinical failure of the drug it was felt might depend on its inability to pass through such impaired organs. At the outset, full credence was placed in the generally current valuation set on hexamethylenamin, i. e., when taken by mouth, it is excreted in the urine, bile, pancreatic, synovial and cerebrospinal fluids in sufficient quantities to be of marked bactericidal value. This confidence, however, was not strengthened by a rather thorough review of its extensive literature, and personal experimental results which quickly developed gave still greater uncertainty. Ever since Nicolaier introduced it into medicine, nearly twenty years ago, the efficiency of the drug has been ascribed to its decomposition into formaldehyd. The authors, however, are quite vague, using such adjectives as, a little, partial and almost complete, in describing the extent of this decomposition, and not a few hold that hexamethylenamin is antiseptic itself independent of the formation of formaldehyd.

Nowhere has there been serious endeavor to ascertain how much hexamethylenamin or formaldehyd are present in the fluids of the body after giving the drug by mouth. Its accredited efficiency in the body fluids where it has been described, rests, first, on a demonstration of the existence of either hexamethylenamin or formaldehyd in the fluid; second, on clinical improvement, and third, on the reduction in the number of bacteria as shown by the plate-culture methods after its use. In my personal studies, the seemingly necessary steps were, first, a quantitative determination of the amount of hexamethylenamin excreted in the urine after giving known quantities by mouth; second, a quantitative estimation of the amount of free formaldehyd present; third, determination of the bactericidal power of hexamethylenamin; fourth, determination of the bactericidal power of formaldehyd; fifth, determination of the strongest solutions of hexamethylenamin and of formaldehyd which can be tolerated by the kidneys and other urinary organs; sixth, a comparison of the chemical and clinical findings.

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The third, fourth and fifth steps proved simple and yielded positive results, the details of which follow. Great difficulty, however, was met at the very outset in connection with securing suitable chemical methods for estimating the amounts of the substances in the fluids. Not only were the quantitative methods uncertain, but the qualitative procedures were equally unsatisfactory, principally because they reacted identically with hexamethylenamin and formaldehyd.

The most delicate qualitative test in use and one which has been extensively employed is that of Hehner. It consists in adding 5 or 6 drops of milk to a few c.c. of the fluid to be tested. This mixture is then stratified over a reagent made by adding one drop of a 3 per cent. ferric chlorid solution to 100 c.c. of 99 per cent. sulphuric acid. The presence of formaldehyd or hexamethylenamin is indicated by the slow formation at the line of juncture of a deep amethyst ring. The color of urine and bile seriously interfere with the test and require a preliminary distillation, which is accomplished by adding a little sulphuric acid and water, when formaldehyd gas passes off in the distillate.

The objections to this test are two-fold. First, that it does not differentiate between formaldehyd and hexamethylenamin, and second, because of its extraordinary delicacy. Formaldehyd clearly shows in solutions of 1 to 1,000,000 and hexamethylenamin in solutions of 1 to 500,000 or less. This is in the proportion of 1 c.c. of formaldehyd to 1,000 liters of water. By the thickness of the ring or the rapidity of its appearance I was completely unable to distinguish between solutions of 1 to 100,000 and 1 to 10,000, nor could I determine from the ring formation whether formaldehyd or hexamethylenamin was the substance present.

Through the valuable assistance of Dr. H. A. B. Dunning, I was fortunate in securing a very delicate test, and one which reacts to free formaldehyd, but not to hexamethylenamin. This test consists in adding to the suspected fluid, 3 drops of .5 per cent. aqueous solution of phenylhydrazin hydrochlorid and then 3 drops of a 5 per cent. aqueous solution of sodium nitroprussid; then an excess of saturated aqueous solution of sodium hydroxid. It is important that the solution to be tested as well as the sodium hydroxid be slightly warmed to a little more than body temperature. When formaldehyd is present in solutions of 1 to 20,000, or stronger, there follows an intense blue color which gradually changes to green and then after a few minutes to brown. In solutions of less than 1 to 20,000 the first color is the intense green which passes off into brown. The test is delicate down to 1 to 150,000 or less. When a solution is tested and found to be negative, as is the case when hexamethylenamin alone is present, it can be acidulated with sulphuric acid, heated to boiling, cooled off and tested, when the reaction will be positive, due to the breakdown of hexamethylenamin into formaldehyd. This has yielded striking results in the urine, the bile, the sputum, the saliva and the cere-

brospinal fluid, and so contradictory to the generally accepted beliefs that it seemed important to bring them to the attention of the profession. I have thus been diverted from my original purpose, the quantitative estimation of the hexamethylenamin and formaldehyd in the urine. I have at present a promising quantitative method for these determinations and will embody my findings with it in a later communication.

I wish to present here the results of my experiments in regard to the bactericidal powers of hexamethylenamin and formaldehyd, the toleration of the urinary organs to these substances, the question of the excretion of hexamethylenamin in the urine, the bile, the sputum, the saliva and the cerebrospinal fluids. In addition to hexamethylenamin, I have employed helmitol and several other formaldehyd compounds.

Before taking up the actual findings it seems desirable to give a short historical review of the subject.

Hexamethylenamin is formed by the direct action of four molecules of ammonia on six of formaldehyd gas. According to the formula $4NH_3+6HCO=(CH_2)_6N_4+6H_2O$. It occurs as colorless, odorless crystals, which are ready soluble in water, less so in alcohol. It was first prepared by Butlerow.[1] When acid is added to an aqueous solution there is partial decomposition into formaldehyd and ammonia, and on boiling, complete decomposition. Boiling alone partly causes decomposition. The drug was introduced into medicine as a urinary antiseptic by Nicolaier,[2] and has ever since enjoyed wide popularity and extensive use all over the world. It remained as a urinary antiseptic alone until S. J. Crowe[3] gave it reputation as a gall-bladder and pancreatic disinfectant, and one year later[4] brought it into its present use as a prophylactic and curative agent in cerebrospinal infections. Armstrong and Goodman,[5] following Crowe's method, introduced it into use as the disinfectant of the sputum in bronchitis, pneumonia, pulmonary tuberculosis and infections of the nose and throat. After giving it by the mouth, it has been found in the aqueous humor of the eye, in the synovial and pleural fluids. Many clinical contributions of a more or less confirmatory nature have been added and the use of the substance "as a bactericide" in these conditions has grown almost to equal its use as a urinary antiseptic.

TOXICITY OF HEXAMETHYLENAMIN

In the rabbit hexamethylenamin is practically non-toxic. I have given 100 grains at a dose, hyperdermically, without the slightest evidence of poisoning. This animal weighed 2 pounds, so that the equivalent dose in

1. Butlerow: Liebig's ann. d. Chem. u. Pharm., 1860, cxv, 322.
2. Nicolaier: Deutsch. med. Wchnschr., 1895, No. 34.
3. Crowe, S. J.: Bull. Johns Hopkins Hosp., 1908, xix, 109.
4. Crowe, S. J.: Bull. Johns Hopkins Hosp., 1909, xx, 102.
5. Armstrong and Goodman: Jour. Am. Med. Assn., May 27, 1911.

the human being of 150 pounds, would be about 18 ounces. However, there is one marked difference between the human being and the rabbit, i. e., in the rabbit, even on immense doses, there is no decomposition into formaldehyd. The drug is excreted as hexamethylenamin. The toxic effects noted in the human being have been hematuria and vesical irritation, both due to a liberation of formaldehyd gas in the urine at the level of the kidney.

BACTERICIDAL POWERS OF HEXAMETHYLENAMIN AND FORMALDEHYD

The technic employed was uniform, i. e., solutions of varying strength of each drug in sterile water were made. These solutions were then inoculated with the bacteria tested so that a slight clouding of the fluid was produced. The inoculated tubes were then incubated at body temperature in periods varying from a few minutes to a week. The bacteria employed were the colon bacillus, the typhoid bacillus, the bacillus pyocyaneus, the streptococcus and the staphylococcus aureus.

Hexamethylenamin solutions free of formaldehyd were obtained by adding a drop of ammonia to each tube. The hexamethylenamin proved to have no bactericidal power; the organisms tested all lived in from 5 per cent. to 10 per cent. solutions without any deterioration.

The formaldehyd solutions, on the contrary, proved very bactericidal. A solution of 1 to 100 destroyed all the organisms studied in twenty minutes. A 1 to 1,000 solution destroyed all of them within twenty-four hours. Marked differences in the toleration toward formaldehyd was noted between the different bacteria. The most resistant organism was the *Staphylococcus aureus,* and the least resistant, the typhoid bacillus. A solution of 1 to 5,000 formaldehyd destroyed the typhoid bacillus and the streptococcus within twenty-four hours. It destroyed the *Bacillus pyocyaneus* within forty-eight hours, and the colon bacillus in four days. The *Staphylococcus aureus* was still alive at the end of a week. The *Staphylococcus aureus* was completely destroyed in forty-eight hours by a solution of 1 to 2,000 formaldehyd. Solutions of 1 to 20,000 formaldehyd had little or no effect on any of the organisms except the typhoid bacillus and the streptococcus; these were not destroyed at the end of four days, but as shown by reinoculations, were somewhat diminished in vitality. A 1 to 50,000 formaldehyd solution had apparently no effect either in destroying the organism or in inhibiting their growth.[6]

TOXIC EFFECTS OF FORMALDEHYD

In dilutions of 1 per cent. and less, formaldehyd solution is an irritant to the skin; in weaker solutions it is not irritant. Every year there are a few reports of serious poisonings resulting from accidental or

6. All were formaldehyd solutions made from a carefully standardized 10 per cent. aqueous solution of the gas.

suicidal drinking of formaldehyd solutions. In a majority of the cases there are violent gastro-intestinal symptoms, and in the more serious ones, coma, which may last several days. In the fatal cases, death has resulted from gradual paralysis of the cardio-respiratory system. So far as is known to me, there has never been a case in the human being of poisoning from hexamethylenamin due to liberation of formaldehyd in the tissues. Jacobson[7] states that dogs can take daily 3.2 gm. without serious results. On the other hand, 1 c.c. per kilo is said to be lethal in a single dose. In association with Dr. H. A. Kelly, I have been using, locally, solutions of formaldehyd varying in strength from 1-250 to 1-7,500, in treating infections in various parts of the body, and have never noted any general toxic symptoms.

TOLERANCE OF THE URINARY ORGANS TO FORMALDEHYD

Our method of investigation here was, first, to try various strengths of formaldehyd solution in the bladder, beginning with a very small percentage and gradually increasing it. Having found a solution which was well tolerated in the bladder, it was then injected into the kidney pelvis through a renal catheter. There are marked individual variations in the tolerance of the vesical mucous membrane to formaldehyd solution, and this is independent of the state of inflammation present. Of course, an acutely inflamed bladder is much more intolerant than a healthy bladder. In chronic cystitis and in healthy bladders we have found it practical to use solutions varying in strength from 1 to 3,750, to 1 to 7,500. Occasionally a bladder is met with which does not tolerate even this weak solution. We have had no cases which would not tolerate a 1 to 12,500 solution of formaldehyd. The kidney pelvis will tolerate solutions as strong as does the bladder, and we have never noted any irritation in the kidney itself after an irrigation.

The formaldehyd irrigations for bladder and kidney infections have proved very effectual. They are especially valuable in cystitis, associated with ammoniacal decomposition of the urine, such as occurs with enlarged prostate or tumor of the bladder.

The gall-bladder tolerates formaldehyd readily in solutions of 1 to 3,750. In infected incisions and sinuses, a 2½ per cent. solution can be used without giving undue pain.

A few tests were made with solutions of hexamethylenamin. It is not at all irritating and can be used in the bladder and kidney in 50 per cent. strength without any ill result, but apparently with no effect on the infection.

7. Jacobson: Berl. klin. Wchnschr., 1904, p. 114.

EXCRETION OF HEXAMETHYLENAMIN IN THE URINE

When given by mouth, hexamethylenamin begins to appear in the urine in fifteen minutes, reaches its maximum excretion in two hours, continues to be excreted abundantly for about eight hours, and has, if the dose given was not greater than 30 grains, disappeared in twenty-four hours. After the twelfth hour, the amounts excreted are very small. In a communication to follow this, I will report more in detail the quantitative eliminative amounts of hexamethylenamin.

The question of interest here is, how much formaldehyd is present in the urine after giving hexamethylenamin by mouth? As yet, I have not sufficiently worked out this question, but can say roughly that in some patients it is possible to secure solutions of from 1 to 5,000, and stronger. My important finding was here that on the customary doses, of from 5 to 10 grains, given three times a day, not more than two patients out of ten showed any decomposition of the drug into formaldehyd at all. This was tested out, not only on patients suffering with infections of the urinary tract, but on a series of normal individuals, and on a number of patients convalescent from operations. When a patient was found to show abundant formaldehyd after a 10-grain dose, it also was found that a 2- or 3-grain dose often sufficed to show the breakdown. While not more than 10 per cent. of those examined showed the formaldehyd free after the smaller doses, at least 60 per cent. showed it when the dosage was made from 20 to 30 grains, repeated every four to six hours. In a few instances in which the formaldehyd was not present after dosage of 30 grains, the quantity was raised to as high as 100 grains at a single dose without causing a decomposition of the hexamethylenamin. There are some individuals who do not break down hexamethylenamin into formaldehyd. This, however, is the exception, not the rule.

PLACE OF DECOMPOSITION

At what point in the urinary tract is hexamethylenamin transformed into formaldehyd? In every case in which free formaldehyd was found in the bladder urine and catheterization of the kidney carried out the formaldehyd was found at the level of the kidney. This fact was established in five successive patients. In one patient of this group the blood-serum was examined and found to contain hexamethylenamin, but no formaldehyd. It is my impression that the formaldehyd liberation is due to some specific activity of the renal epithelium. It is, of course, impossible to conceive of free formaldehyd in a highly ammoniacal urine. The urine's alkalinity, however, is normally not due to free ammonia. The greatest decompositions have been observed in highly acid urines. This, however, is not invariably the case, and it was frequently noted that in an acid urine there was no free formaldehyd after giving the hexamethylen-

amin. The same thing holds for alkaline urines. With the reaction definitely alkaline, large amounts of formaldehyd are occasionally met with.

CLINICAL OBSERVATIONS

The clinical results obtained have conformed in every way with the assumption that it is the free formaldehyd which is the effective agent, and that it must be present in fairly strong solution. In not a single case has there been observed the slightest improvement from giving hexamethylenamin when the urine showed hexamethylenamin, but no free formaldehyd. On the contrary, every patient showing free formaldehyd has shown prompt improvement and a number of the very chronic and obstinate colon infections have entirely cleared up. To obtain this result the treatment in some cases had to be carried over several months.

A pertinent example of this kind was afforded by the case of Mrs. E. C. S., aged 29, first seen Oct. 26, 1911. This patient had always been healthy, and had had three normal labors. In March, 1911, when four months pregnant, she was seized with an attack of renal colic in the left kidney region and two days later had a similar one in the right side. She was allowed to continue the pregnancy and gave birth to a healthy child in July, 1911. From the very first attack, the patient began having pus and blood in the urine. This continued after the labor and was associated with irregular fever, marked deterioration of general health and great reduction in hemoglobin. In November, 1911, she had the kidneys catheterized, functional tests made, and (*x*-ray pictures taken) collargol injection of the pelves. The results of these examinations were to show that there was bilateral colon bacillus infection of the kidneys, greatly dilated pelves, the left having a capacity of 120 c.c. and the right of 320. The functions of the kidneys, however, were fairly well maintained, as shown by the phenolsulphonephthalein test. Ten grains of hexamethylenamin by mouth sufficed to give free formaldehyd from both kidneys. At the time of the examination there was an enormous amount of pus on both sides. The patient was started on 120 grains of hexamethylenamin per diem. This occasionally caused vesical distress, when the dose was reduced. By February 3, the urine had practically cleared of infection. On that date Dr. Kelly suspended and plicated the pelvis of the right kidney. On the twentieth of the same month a similar operation was carried out on the left side. The hexamethylenamin was continued during her stay in the hospital. A recent examination shows a perfectly sterile urine, free from pus and blood.

THE DOSAGE OF HEXAMETHYLENAMIN

The average dose advised for hexamethylenamin is 7.5 grains, repeated two or three times per diem. In an occasional case of irritable bladder, even this amount causes a sufficient liberation of formaldehyd to produce irritation. Such, however, is the exception. If it does cause irritation, free formaldehyd will always be found and the indication is to reduce the dose. From what has been said, it is evident that there is no fixed dose. This is to be obtained by testing the urine and observing the toleration of the patient. Where 10 grains causes no free formaldehyd liberation, the dose should be increased to 20 grains, and if there is still none, to 30 or 40 grains, repeated every four hours. The only toxic effect due to

hexamethylenamin is occasioned by the liberation of formaldehyd in the urine, and when this does not occur, it is safe to push the dose until it does appear. The first disagreeable symptom in our experience is vesical irritability. It has always led us to discontinue or diminish the dose. In such a case, pushing the dose might easily lead to hematuria. We have never observed a macroscopic hematuria from the use of hexamethylenamin.

The proper treatment is to give a dose just large enough to be under that necessary to cause bladder irritation. Using this dose, improvement will follow so rapidly in most cases that long continuation in the use of the drug is unnecessary. On the other hand, it is certainly possible to keep up the dosage for months without any serious impairment of either the general health or of the kidneys.

SOME OF THE PROPRIETARY HEXAMETHYLENAMIN COMPOUNDS

The first trade name and one which has been so universally used as to have become fixed in the popular mind is urotropin. It is, however, sold under various other names, such as formin, cystamin, hexamin, etc. These products are identical with hexamethylenamin and show no variations from it in chemical or pharmacologic reaction.

Helmitol, the methylene citrate of hexamethylenamin, also responds like the pure drug. There is this difference, however: When hexamethylenamin is dissolved in water no free formaldehyd is formed; when helmitol is dissolved in water a considerable amount of free formaldehyd is liberated. Formaldehyd, however, when taken by the mouth, is not excreted through the kidneys. When helmitol is taken, its excretion is identical with that of hexamethylenamin; i. e., in some cases, there is free formaldehyd liberated, and in others, none.

COMBINATION OF DRUGS WITH HEXAMETHYLENAMIN

In the hope that the presence of some other substance in the urine would cause decomposition of the hexamethylenamin, a great variety of substances have been given with it. Among these are potassium citrate, potassium iodid, sodium benzoate, salol, oil of wintergreen, etc. The results have not been encouraging; in no instance has the combination been more effective than the pure drug.

OTHER FORMALDEHYD-CONTAINING DRUGS

Dr. H. A. B. Dunning has furnished me with a number of formaldehyd compounds, some of them new ones; some of them have been excreted through the kidneys as in the case of a compound between formaldehyd and phenolsulphonephthalein. None, however, has shown a tendency to break down, liberating free formaldehyd. This phase of the subject is still under investigation.

EXPERIMENTAL STUDIES WITH RABBITS

The use of hexamethylenamin in rabbits was carried out in order to determine its toxicity and its method of excretion. Only three animals were employed. The results obtained were identical. In the rabbit, hexamethylenamin is excreted primarily and principally through the kidneys. It is excreted unchanged, and is not broken down into formaldehyd. In small doses of from 2 to 5 grains, hypodermically, in a 2-pound rabbit, the excretion is practically entirely through the kidneys. When from 30 to 100 grains are given at a dose, the principal excretion is through the kidneys, but there is a large excretion through the bile. The drug is eliminated as hexamethylenamin, there being no formaldehyd liberated. The hexamethylenamin does not seem in the least toxic to the rabbit.

When a rabbit is given 30 grains of the drug hypodermically and then killed within an hour the findings in the different tissues are as follows: Urine, large amount; bile, considerable amount; cerebrospinal system, traces; blood, considerable amount; spleen, liver and kidneys, considerable amount; muscles, trace; skin, trace; in no tissue was any free formaldehyd found. These findings in the tissues and body fluids of the rabbit suggested the examination of some of the fluids of the human being where the drug has been thought to act efficiently.

EXAMINATION OF THE BILE

I examined in all ten patients with biliary fistula. None of them were getting less than 60 grains a day, and in one case I gave 100 grains at a dose and collected the bile for twelve hours. The bile in every case was treated identically, i. e., it was diluted slightly with distilled water, acidulated with sulphuric acid, and distilled. In every case the distillate gave a clear formaldehyd reaction with Hehner's test. In not one could a trace be found by the test which I have employed.

As a control, I put a solution of 1 to 50,000 hexamethylenamin into bile, allowed it to stand an hour and then distilled. The presence of formaldehyd was easily determined and there seemed to be an actual concentration in the distillate.

EXAMINATIONS OF THE SPUTUM AND SALIVA

In five healthy people I gave hexamethylenamin in 30-grain doses, at the rate of 120 grains a day for twenty-four hours, and then examined the saliva. By the Hehner test, either hexamethylenamin or formaldehyd was found to be present. By the phenolhydrazin-sodium-nitroprussid test, neither could be detected.

In three cases of bronchitis with mucopurulent expectoration, exactly similar technic was followed with identical results. By my test there was no free formaldehyd, and heating and acidulating proved that there was no free hexamethylenamin in amount sufficient to give the reaction.

EXAMINATION OF THE CEREBROSPINAL FLUID

The cerebrospinal fluid was examined in one case through the courtesy of Dr. Morse, of the Johns Hopkins Hospital staff. The patient, James B. D., aged 45, had normal temperature and no cerebral or spinal symptoms. For purposes of diagnosis, some of his cerebrospinal fluid was desired. Before the puncture he had been given, for twenty-four hours, 15 grains of hexamethylenamin every three hours. I obtained about 4 c.c. of clear fluid which showed a positive reaction with Hehner's test, but none whatever with the other test, even when boiled and acidulated.

CONCLUSIONS

These examinations of the bile, sputum, saliva and cerebrospinal fluid show that even after rather large doses of hexamethylenamin, there appears in them but traces of the drug, certainly in percentages less than 1 to 150,000. Whether this trace is of hexamethylenamin, as seems most likely, or of formaldehyd, it is impossible to state, because the only test which would show it is Hehner's which does not differentiate these two substances. So far as any therapeutic value is concerned, it does not make any difference because, as already shown, solutions of formaldehyd of the weakness indicated, do not possess any antiseptic value. I believe, therefore, that the use of hexamethylenamin for the curing or bettering of, or as a prophylactic against, infections of the bile passages, respiratory passages and cerebrospinal system is illusory, and cannot possibly yield results. I have no explanation to offer for the reported clinical and bacteriologic improvements, for with the exception of the urine, I have not tested this side of the question. In the urine the clinical and bacteriologic findings have conformed in every way with the chemical findings, viz., only those patients who show free formaldehyd have been improved by the drug.

The phenolhydrazin-nitroprussid test is very simple, and when applied gives the physician an easy method of determining the dose of hexamethylenamin which he should use, and also shows those cases in which no results from this drug can be expected.

The test is of value in determining the efficiency of compounds whose value rests on the liberation of free formaldehyd, and it is to be hoped that an endeavor will be successful in securing a substance which, when taken by the mouth, will be excreted through the kidneys and will liberate formaldehyd in the urine in every case.

Although it has its limitations, these experiences show that hexamethylenamin, when properly given, in more than a half the cases of urinary infection is of immense value, and at the present time superior to any other drug in common use.

Finally, I want to express my gratitude for the enthusiastic support and many valuable suggestions given me by Dr. Howard A. Kelly during the progress of this work.

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