THE EFFECT OF HEXAMETHYLENAMIN ON GUINEA-PIGS*

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Hexamethylenamin, or, as it is commonly called, urotropin, was first prepared in 1860 by Butlerow.¹ It is made by the action of ammonia on formaldehyd. It is readily broken up by heat, some acids, etc., into formaldehyd and ammonia. Nicolaier² first introduced this drug into medicine in 1894. He concluded that it stimulated the secretion of urine, prevented the growth of bacteria in the urine, and acted as a solvent of uric acid calculi. In some cases he gave as much as 150 grains in a day without producing symptoms. On the other hand, smaller doses after prolonged use would occasionally cause burning in the region of the bladder, and the appearance of epithelial cells in the urine. If the drug was still continued red blood corpuscles would appear in the urine. As a result of these observations many men began to employ this drug. Casper³ and Mendelsohn⁴ showed that it did not act as a solvent of uric acid calculi within the body. They and other authors, especially Richardson,⁵ confirmed Nicolaier's views in regard to the power of hexamethylenamin to act as a urinary disinfectant, especially in typhoid fever. Casper also thought that it was of value in curing phosphaturia. From that time on urotropin has been used in typhoid fever and in cases of cystitis and pyelitis due to other agents than the gonococcus or tubercle bacillus.

The fate of hexamethylenamin within the body has not been definitely decided. It has been reported as present in the blood and urine. Casper³ has found free hexamethylenamin in the blood of a rabbit after a subcutaneous injection of the drug.

Richardson⁵ found that in cases with an existing nephritis the administration of hexamethylenamin not only did not cause an increase in albu-

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1. Butlerow: *Ann. d. Chem. u. Pharm. (Liebig's)*, 1860, 115-332.
2. Nicolaier: *Deutsch. med. Wehnschr.*, 1895, 541.
3. Casper: *Deutsch. med. Wehnschr.*, 1897, *Therap. Beilage*, p. 75.
4. Mendelsohn: *Berl. klin. Wehnschr.*, 1898, 48.
5. Richardson: *Jour. Exper. Med.*, 1899, iv, 19.

min and casts, but in some cases apparently caused a diminution of these elements.

Since the introduction of hexamethylenamin in therapeutics reports have been gradually accumulating of evil effects caused by this drug. In the cases reported the amount of the dose and the length of time over which the drug were given vary greatly. Even in the same individual the same dose acts differently at different times. Coleman,⁶ from the literature and his own experience, reported in 1903 the following list of symptoms in human cases following the use of hexamethylenamin, which in some of the cases had been given in small doses: Irritation of the stomach, diarrhea and abdominal pain, irritation of the skin with a rash like that of measles, headache and ringing in the ears, irritation of the kidney and bladder, hematuria, and hemoglobinuria. In these cases Coleman thinks that the blood is probably derived from the bladder. In 1906 Karwowski⁷ and Rosen also summed up the ill effects which might arise from hexamethylenamin, but did not add anything essential to the report of Coleman.

As a result of all these communications hexamethylenamin, in small doses, is considered a safe drug for most people, and also in large doses up to the appearance of symptoms. When symptoms appear the discontinuance of the drug causes a subsidence of the symptoms. None of these writers, however, have inquired into the tissue changes back of these symptoms.

In 1908 Crowe⁸ showed that typhoid bacilli could be killed in the gall bladder of man as well as in the urine by the administration of hexamethylenamin provided that sufficiently large doses were used. He demonstrated the presence of hexamethylenamin in the bile, cerebrospinal fluid, synovial fluid, saliva, pleural effusions and blood of man. Also he asserted that it acted favorably on other forms of infection in cavities where the hexamethylenamin was found present. For killing typhoid bacilli in the gall bladder 75 grains a day for several days are necessary. As a result of these findings it seems possible that even larger doses might be of value in other infections.

In view of the fact that as a result of Crowe's work hexamethylenamin might be used in large doses the following experimental work was done to see if possibly any lesions could be found in any organs after the administration of large doses. As such work could not be tried out in human cases guinea-pigs were used.

6. Coleman: Medical News, New York, 1903, lxxxiii, 393.

7. Karwowski: Monatschr. f. prakt. Dermat., 1906, xlii, 8.

8. Crowe: Johns Hopkins Hosp. Bull., 1908, xix, 109.

There were three main problems to be solved in doing this work:

1. How much hexamethylenamin per body weight can be given to a guinea-pig without producing fatal symptoms?
2. What lesions, if any, are produced by one or two very large or fatal doses of this drug?
3. What lesions, if any, appear after repeated sublethal doses?

In these experiments the hexamethylenamin was dissolved in sterile water and injected subcutaneously.

Guinea-pigs vary somewhat in their ability to withstand the drug, but as a rule $4\frac{1}{2}$ to 5 grains per ounce of body weight will kill the pig or render it very quiet for twelve to fourteen hours. One pig, however, survived a dose of $6\frac{1}{2}$ grains to the ounce. As it takes a considerable amount of boiled water to put a fatal dose of hexamethylenamin into solution, one pig, as a control, was injected with this amount of water. The animal did not suffer any ill effects. If the dose proves fatal it usually does so within a few hours. If the animal survives twenty-four hours it recovers. After a fatal dose the pigs grow more and more quiet, and in the course of two or more hours usually topple over, have some slight twitching of the extremities, and gradually die. Others have been found in a sitting position, which suggested that they grew more and more quiet and then died. In none were any convulsions noted.

The pigs are able to withstand sublethal doses over a considerable period of time without any ill effects other than reaction at the point of injection and some quietness for a few hours after the injection. By the next day, however, they are lively again. One animal weighing 20 ounces, for instance, received 375 grains in eight doses during nine days. The doses were each 45 grains, except one of 60. Five days after the last injection the animal appeared lively and perfectly well, showed some reaction at the site of inoculation, which was rapidly improving and had lost only 2 ounces in weight. On that day it received a fatal dose. Another pig, weighing 18 ounces, took 15 doses in nineteen days, amounting in all to 319 grains, and gained 1 ounce in weight. Eight or nine days later it was given 75 grains, which it survived, and two weeks later had gained 2 ounces.

In the animals which received one or two large doses the findings were practically the same, whether the doses proved fatal or not. At the point of inoculation there was edema of the subcutaneous tissue and muscle, with congestion and some hemorrhage. Of the twelve guinea-pigs in this group, two showed free fluid in the peritoneal cavity. Both of these received fatal doses. It was felt at the time that one of these animals received some of the injection intraperitoneally; and this suspicion was practically confirmed at autopsy by a subperitoneal hemor-

rhage on the stomach wall near the site of suspected entrance of needle. As none of the other ten pigs in this group showed free fluid, it seems fair to assume that the other case of free fluid was due to a perforation into the peritoneum while injecting the animal.

In these twelve animals there were no macroscopic lesions of any organs other than those mentioned above, except in the stomach. In eleven of these twelve cases the stomach was examined and in two cases submucous hemorrhages were present. These areas will be referred to later under the microscopic findings.

Pieces of tissue from the heart, lungs, liver, spleen, kidneys, stomach, adrenals, bone marrow, brain, muscles and abdominal wall were preserved in Zenker's fluid and formalin. Sections mounted in paraffin and stained with eosin and methylene blue from two of these cases, one with one dose and one with two doses which proved fatal, were studied. Also three control guinea-pigs which had not received hexamethylenamin were studied. The hearts in these two cases showed an occasional area of round-cell infiltration, but otherwise were negative. The controls showed the same. The lungs showed an increase of leucocytes and lymphocytes around the vessels and bronchi, and occasionally a slight cellular infiltration into some of the alveoli. The controls showed the same. In the liver there was considerable vacuolization of the cells in both the injected animals and the controls. In one of the injected animals there were areas of necrosis of the parenchymal cells with invasion by phagocytic cells. They did not appear in any of the other injected animals of this or the other group and was attributed to chance focal necrosis in the liver, which is apt to occur in guinea-pigs, as Mallory⁹ has pointed out. The spleens are congested and show questionable hemorrhages around the edges of the lymph follicles, but the controls show the same. Pigment in the endothelial phagocytic cells in some cases was marked in both the injected animals and in the controls. In the kidneys of these two pigs were a few scattered areas of round-cell infiltration apparently replacing some of the kidney parenchyma. The controls presented the same appearance. In none was there any evidence of extensive destruction of the tubules or injury to the glomeruli. In one of the cases many of the nuclei of the convoluted tubules stained a solid purple and had lost their vesicular appearance. Under high power faint, round, pinkish bodies could be made out in the purple mass. As no other cases showed this condition it was considered as a freak of fixation, or an accidental lesion, rather than a lesion attributable to the hexamethylenamin. The adrenals,

9. Mallory: *Jour. Med. Research*, 1901, vi, 264.

muscle, bone marrow, duodenum, cerebrum and cerebellum were practically the same in appearance as the controls and all apparently normal. The abdominal wall at the site of inoculation showed edema of the tissues and necrosis of some of the muscle fibers, with invasion of phagocytic endothelial cells and polynuclear leucocytes. In addition, the surrounding edematous tissues often contained large numbers of polynuclear leucocytes.

The stomach was examined microscopically in eight of these cases, including the two which showed submucous hemorrhages in the gross. The six which showed no lesion in the gross did not show any lesion other than a congestion of the vessels throughout all the layers. Both of the stomachs which showed submucous hemorrhages presented microscopic lesions. In one there was marked congestion of all the vessels and hemorrhage into the mucosa. Also the mucosa was necrotic in places; and surrounding these necrotic areas were leucocytes wandering toward the necrosed area. This reaction on the part of the leucocytes was present throughout all layers of the stomach wall in the vicinity of the necrotic areas. In the other case the congestion and hemorrhage were not so marked, but the necrotic mucosa had been eroded in one place down to the submucosa. This was the only necrotic area in the sections from this animal. Surrounding this area, as in the other case, there was evidence of activity on the part of the leucocytes. The stomachs from seven pigs, as a control, were examined, and they showed nothing abnormal in the gross or under the microscope. The vessels in the controls were not so much congested as those in the animals injected.

Rosenau and Anderson¹⁰ in 1906 showed that hydrocyanic acid and chloral-cyanhydrin caused hemorrhagic areas in the stomach wall of guinea-pigs when given in fatal doses. They did not give in their paper the microscopic description of these areas. It seems probable, however, that some evidence of necrosis would have been present in these areas. This congestion and hemorrhage with necrosis that may occur after hexamethylenamin must be looked on as a lesion which may occur in guinea-pigs after lethal or very large doses of several drugs, and not as specific to hexamethylenamin.

Five guinea-pigs received sublethal doses for a period of a week or more. The one observed the longest was watched forty-four days, on sixteen of which he received doses of hexamethylenamin varying from $7\frac{1}{2}$ to 75 grains. Of these five animals three were chloroformed apparently in good health and two received fatal doses after many sublethal pre-

10. Rosenau and Anderson: Bull. 32, Hyg. Lab. U. S. P. H. and M.-H. S., 1906.

ceding ones. The animals showed the same subcutaneous lesions at the point of inoculation as described above. One animal had free fluid in the abdominal cavity. This animal received a fatal dose at the end of eight days, but here again it was suspected that some of the drug was given intraperitoneally. One of these animals that received a fatal dose after six former injections showed a small submucous hemorrhage of the stomach. Only one other stomach was examined in this group, but it did not show anything abnormal. All five of these cases were examined microscopically, and they showed nothing different from the other case described under the other group and the controls. The stomach in the animal that had the hemorrhage in its stomach wall simply appeared congested. Presumably the hemorrhagic area was missed in the sections examined.

In conclusion it may be said that hexamethylenamin, when given subcutaneously in guinea-pigs, either in single doses or in repeated doses, produces necrosis of the muscle and cellular reaction at the point of inoculation. It also causes congestion of the stomach vessels, and in some cases hemorrhage into the mucosa, with ulcer formation.

This work offers no proof as to what lesions in man may be the cause of the symptoms occasionally produced by hexamethylenamin, but it suggests that these lesions are not organic. The most interesting points brought out are the enormous amount of hexamethylenamin a guinea-pig can receive subcutaneously without apparent injury and the fact that hexamethylenamin is another drug to add to the list of those that may cause hemorrhage in the stomach walls of guinea-pigs.

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