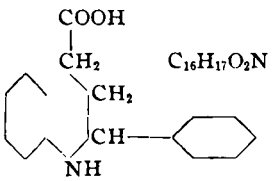
THE TOXICITY OF AN IMPURITY IN OFFICIAL CINCHOPHEN.*

BY ROBERT A. HATCHER.[1]

Part I—The Effects of Oral Doses on:
(*a*) Cats. (*b*) Dogs. (*c*) Rabbits.
Part II—The Effect of Intravenous Doses on:
(*a*) Cats. (*b*) Dogs.
Part III—The Effect on Vessels of Perfused Organs.
Part IV—The Effect on the Vomiting Center.

The Pharmacopœia states that when cinchophen is dried to constant weight over sulphuric acid, it contains not less than 99 per cent of $C_6H_5.C_9H_6N.COOH$, but Mr. John L. Smith, Secretary of Chas. Pfizer & Company, Inc., informed me that "Cinchophen may contain as much as 3 per cent of impurity and yet pass in a very satisfactory manner all the tests now prescribed by the U. S. P." It was in consequence of this fact that I was asked to determine the toxic effects of the impurity, which was supplied in pure form. Mr. Smith described it as follows:

Cinchophen Impurity (Substance 193).
This substance is γ-anilino-γ-phenyl-*n*-butyric acid.
Its structural formula is:



Molecular weight, 255.1. A white, crystalline body; melting point 193–194° C.

Like cinchophen, this substance forms stable salts with metallic bases. The sodium and ammonium salts have been obtained in crystalline form. Both are soluble in water and alcohol.

Two mols cinchophen and 1 mol of this substance form a crystalline double compound, which can be re-crystallized from alcohol without changing its composition. The melting point of this double compound is 183° C.

This substance when heated above its melting point splits up into CO_2 and a base, $C_{15}H_{17}N$. This base forms a characteristic hydrochloride and nitrate, both only slightly soluble in water.

Unlike cinchophen, this substance is readily attacked by oxidizing agents.

With sodium hypochlorite an oily decomposition product with a strong, unpleasant odor is produced.

By bromination in glacial acetic acid a crystalline bromination product, melting point 135° C., is obtained.

---

* The investigation of this problem was undertaken at the request of Chas. Pfizer & Company, Inc., and this paper embodies the essentials of the report made to that firm, but the details of the protocols and tracings are omitted. The urines were examined microscopically and tissues were examined by inspection by Dr. Harry Gold of the Department of Pharmacology; the specimens of the stomach, intestine and kidneys were examined microscopically by Professor L'Esperance of the Department of Pathology of Cornell University Medical College.

[1] Cornell University Medical College, New York City.

The cinchophen impurity forms a methyl ester. The ester is liquid at ordinary temperature. On the addition of alcoholic hydrochloric acid to the ester, a crystalline precipitate of the ester hydrochloride is produced.

The impurity was dissolved in four parts of normal sodium hydrate solution and diluted as required in the various experiments. Doses are expressed in milligrams per kilogram of body weight, but the words "per kilogram of body weight" are omitted.

PART I. THE EFFECT OF ORAL DOSES ON CATS, DOGS AND RABBITS.

(*a*) *Oral Doses to Cats.*

A dose of 250 mg. by the stomach tube caused nausea in five minutes, and vomiting five minutes later. The animal was apparently normal the next day. Each of two animals received doses of 500 mg. by stomach tube. In each case there was nausea followed by depression, and death from stoppage of the respiration in 18 hours in one case and in an hour and one-half in the other. Each of two cats received a dose of 1 Gm. per Kg. by the stomach tube. The first caused nausea in 29 minutes and vomiting at intervals during six hours. Reflex excitability occurred after about one-half hour and convulsions in about five hours. There was then diminished reaction to painful stimuli with general depression during observation. The animal was found dead in the morning, 72 hours after the last dose. The second animal had convulsions after two minutes and these were frequent until death occurred one hour and fifty minutes after the dose.

Each of three cats received a dose of 100 mg. of the impurity daily through a stomach tube from August 17th to September 12, 1927 (inclusive, except Sundays) and they were killed by bleeding on the 13th.

The gross examination of the liver, lungs, spleen, kidneys, heart and intestine revealed nothing abnormal in any case. The microscopic examination of specimens of the stomach, intestine and kidneys of these animals showed nothing abnormal or only unimportant changes, of which the pathologist reported, "I cannot tell you their significance."

Specimens of urine of all three cats were collected on September 9th and on the 13th. All showed traces of albumin but none contained casts or cells, and they were not perceptibly different from the urines of two apparently normal cats which served as controls.

The heart rate, respiration rate and rectal temperature were recorded just before the administration of the impurity and about two hours later on August 29th and 30th and September 1st and 2nd, and also on September 7th in case of cats 2 and 3. There was no change in heart rate that could be attributed to the substance with certainty. There was an increase in the heart rate in 9 of the 12 observations of an average of 31 beats, or an average of 15 per cent. The rate was unchanged in two, and in one it was slowed by 24 beats. Spontaneous changes in the heart rate occur in laboratory animals, and it is probable that such changes in rate as were observed were due to causes other than the drug, especially in the light of the obsèrvations on respiration and rectal temperature.

The respiration was slowed in 9 observations by an average of $7\frac{1}{2}$ per minute, or 13 per cent, but the average slowing in 7 observations was only 5. There was no change in rate in two observations, and in one there was a slight increase. The

changes in respiration were such as one observes without medication and are of no especial significance.

The rectal temperature fell in nine observations by an average of 0.15° C. It rose by an average of 0.2° in four and in one there was no change. In no case was there any significant change in temperature.

The weight of cat 1 fell constantly throughout the administration. The weight before starting the drug was 2.64 Kg. and 27 days later (after a total of 21 doses) it was 1.92 Kg.—a loss of 27.3 per cent. The weight of cat 2 was 2.56 Kg. before starting the administration. Nineteen days later it was 1.92 Kg. after which it increased to 2.0 Kg., and on September 13th it was 1.96 Kg., a loss of 23.4 per cent. Cat 3 lost 13 per cent in weight in the first five days, after which the weight fluctuated slightly, and on the 10th of September it weighed the same as before the experiment. The loss in weight was clearly due to interference with the appetite, which, in turn, is attributable to the local action, causing anorexia, but not distinct nausea.

(*b*) *Oral Doses to Dogs.*

Each of two dogs (A and B) received an oral dose of 500 mg. There was no constant effect on heart rate, respiration or rectal temperature. The dog is more tolerant than the cat toward this impurity as it is toward cinchophen. An oral dose of 500 mg. of the impurity caused vomiting in dog 1, after 50 minutes, on August 15th, hence the dose was reduced to 250 mg. and this was administered daily, except Sunday, to each of two dogs. Dog 1 was killed accidentally by injecting the solution into the lungs after a total of 18 doses. Dog 2 received 250 mg. daily, except Sunday, from August 15th until September 12th inclusive, and was killed by bleeding on the 13th after a total of 21 doses.

The gross examination of the liver, spleen, heart, lungs and kidneys of both animals and the stomach and intestine of dog 2 showed nothing abnormal. The stomach mucosa of dog 1 was congested and showed small ulcers. The entire small intestine and the upper part of the large showed swollen mucosa. This was mucinous, friable and easily rubbed off. It was suggestive of dipheritic enteritis. The lower part of the colon and the rectum were quite normal. It is improbable that the impurity caused the abnormality of the stomach and intestine in this dog, as no such effect was induced in any other animal. No such action can be expected from what we know of the nature of the substance. The microscopic examination of the stomach, intestine and kidneys revealed nothing that could be attributed to the effect of the drug.

Specimens of the urine of dog 1 of August 29th and September 7th, and one of dog 2 of August 30th were examined. None contained albumin or casts. The urine of dog 2 of September 13th contained a trace of albumin, but no blood, pus or casts, and no injurious action on the kidneys could be detected.

The impurity had no apparent effect on the heart and it was not responsible for the changes in rate that occurred, since similar changes occur spontaneously in untreated dogs. The heart rate of dog 1 was 156 before the administration on September 2nd, and 132 two hours later. The heart rate of dog 2 was 144 per minute before the administration of the impurity on September 2nd, and 168 two hours and 25 minutes after the administration. On September 9th the rate was 132 before the drug was given, and it was 144 two hours later.

The impurity may sometimes cause increased respiration but minor changes in rate appear to be quite independent of it. The respiration of dog 1 was 33 per minute before the administration, and two hours later it was 30. The respiration was 48 before administration on the 2nd, and the animal was panting two hours later; on the 9th the respiration before the administration was 24 and two hours later it was 30.

The impurity in the doses used has no effect on the body temperature. The rectal temperature of dog 1 was 39.1° before administration on September 2nd, and 38.9° two hours later. The temperature of dog 2 rose 0.25 of a degree after the administration on September 2nd and it fell to exactly the same extent after that on September 9th.

Dog 1 weighed 5.38 Kg. before starting the experiment. After the first dose of 500 mg., which caused vomiting, the weight fell to 4.88 Kg. on the following day. On September 7th it was 5.04 Kg., a loss of 0.34 Kg. or 6.3 per cent. Dog 2 weighed 6.94 Kg. before starting the experiment. The weight increased steadily and on September 12th it was 8.2 Kg., an increase of 1.26 Kg. or 18.2 per cent. The increase is not attributable to the effect of the impurity, but on the other hand, the results show that daily oral doses of 250 mg. do not interfere with the appetite in the dog, though a dose of 500 mg. may cause vomiting.

(*c*) *Oral Doses to Rabbits.*

Each of three rabbits received a dose of 100 mg. of the impurity daily through a stomach tube; one from October 19th to October 26th inclusive (it died the next day), two from October 19th to November 1st inclusive, except Sundays. There was nothing to indicate the cause of death of the rabbit which died on October 27th; each of the other two was killed on November 2nd by a blow above the medulla.

The gross and microscopic examination of the kidneys, stomach and intestine and the gross examination of the heart and lungs showed nothing abnormal.

In each case the urine in the bladder when the rabbits were killed on November 2nd contained traces of albumin, but there were no casts and no pus or blood in either. The urines of two normal rabbits were examined on November 3rd, 4th and 7th for controls. All showed traces of albumin, none showed blood, pus or casts.

The heart rate, respiration rate and temperature of normal rabbits show considerable normal daily variations, and these were not determined in the experiments. The weight of rabbit 1 showed slight fluctuations above and below that before the beginning of the experiment. Three days after beginning the experiment the weight had increased by 0.08 Kg., or 3 per cent. At the close of the experiment the weight had decreased by 0.14 Kg., or 6 per cent. The weight of rabbit 2 increased slightly after the first day and decreased slightly up to the time of death. The weight of rabbit 3 fluctuated slightly, and it was practically the same when killed as before starting the experiment. The weight before beginning the experiment was 1.98 Kg.; the weight on the last day of the experiment was 2.0 Kg.

PART II. THE EFFECTS OF INTRAVENOUS DOSES.

(*a*) *Intravenous Doses to Cats.*

The intravenous injection of a dose of 100 mg. caused nausea and depression lasting about 24 hours. A dose of 200 mg. caused nausea in 10 minutes in an-

other animal with marked hyperexcitability in about two hours, and lasting about an hour. The animal was apparently normal the next day. A third animal was anesthetized with chloretone, which caused low blood pressure. A dose of 75 mg. of the impurity injected rapidly caused no important effect on heart rate or blood pressure.

The heart rate, respiration rate and rectal temperature were recorded shortly before, and one hour after, intravenous doses of 25 mg. and 50 mg. (total 75 mg.) in each of two cats. Both showed a temporary increase in the heart rate after the first dose, but the second dose injected after an interval of one hour caused no change, or a slowing, in the first cat, and practically none (a slight increase) in the second.

There was no constant effect on respiration. It was rapid in the first cat before the experiment began; it became slower after the first dose and showed the same slower rate one hour after the second dose in the first cat. The respiration rate was normal in cat 2 before beginning the experiment; there was a slight decrease after the first dose, and a considerable increase after the second dose. It is probable that this increase in rate was due to manipulation, because the heart showed no corresponding change.

The rectal temperature of the first cat was 39.9° C. before beginning the experiment. It fell to 38.8° one hour after the first injection, and was 38.5° one hour after the second injection. The rectal temperature in the second cat was 39.4° before injection and it was 39.3° one hour after the second injection, showing that these doses had no important influence on body temperature, though it is possible that the fall in the first experiment was due to the drug.

The intravenous injection of 25 mg. of the impurity caused no change in heart rate during six minutes after starting the injection. The blood pressure rose markedly owing to increased pulse pressure. There was no evidence of vaso-construction. A similar intravenous injection (25 mg.) caused no change in heart rate, blood pressure or pulse pressure in another cat. The intravenous injection of 50 mg. in another cat caused a fall of blood pressure during the injection with a return to very nearly the original pressure after a second injection of 50 mg. The heart rate was slowed slightly. In this experiment, the injection of 100 mg. had very little influence on the circulation. In two other experiments, doses of 25 mg. were injected intravenously into cats. In one case the blood pressure, which had been very high, fell sharply, probably due to variation in the depth of ether anesthesia.

The intravenous injection of 400 mg. of the impurity in 30 seconds caused little change in the circulation during the injection, but the heart stopped suddenly about 30 seconds later. The respiration continued for a short time. In another experiment the intravenous injection of 428 mg. in a period of 18 minutes caused great slowing of the heart during the injection and death by stoppage of the heart. Convulsions occurred when about 300 mg. had been injected, with a rise of blood pressure during the convulsions (probably stimulation of the vasomotor center), followed by a fall of blood pressure. Blood pressure tracings were taken with a Huerthle manometer but their publication here is not necessary.

(*b*) *Intravenous Doses to Dogs.*

The heart rate and the rectal temperature were virtually unchanged after

the intravenous injection of 50 mg., and also after the intravenous injection of 100 mg. of the impurity in the same dog one hour and fifteen minutes later. The respiration rate was temporarily slightly increased after each dose but one hour after the last dose it was the same as before the first dose. One can say that such doses of the impurity have no important effect on the heart rate, respiration rate or temperature in the dog.

The intravenous injection of 100 mg. of the impurity within three minutes in an anesthetized dog caused a slight fall in blood pressure but this dose had no lasting effect on the heart rate or blood pressure. A dose of about 400 mg. of the impurity was injected intravenously in an anesthetized dog in a period of about twenty-eight minutes. There was no important effect when 200 mg. had been injected. Marked toxic effects occurred when 300 mg. had been injected and the total dose caused stoppage of the heart before failure of respiration.

PART III. EFFECTS ON VESSELS OF PERFUSED ORGANS.

A solution of the impurity in 1000 parts of Locke's solution was perfused from a Marriott's bottle at a height of 100 cm. through a cannula in the renal artery, with the kidney in an oncometer. The outflow from the renal vein showed practically no change within fifteen minutes in any case. In one case there was no change in the kidney volume and in the other three the changes were negligible. A solution of epinephrine 1–100,000 was used as a control in the fourth experiment after the perfusion with the impurity; it caused a sharp drop in kidney volume and the outflow from the vein, showing that the vessels were capable of reacting to any constricting substance perfused through them. These results suggest that any changes in blood pressure caused by the impurity were due to the action on the heart or the vasomotor center and not to any effect on the vessels.

PART IV. EFFECTS ON VOMITING CENTER.

Each of four cats received an intravenous injection of 100 mg. of the impurity in ten parts of normal salt solution with NaOH. All showed slight symptoms of nausea within ten to fifteen minutes but none vomited. A dose of 200 mg. also caused nausea without actual vomiting. These results indicate that the impurity has a slight action on the vomiting center in the cat.

Each of two dogs received doses of 100 mg. intravenously. The first showed slight nausea within two minutes; the second showed probable nausea within one minute. Another dog showed nausea two minutes after the intravenous injection of 150 mg. and this dose was followed by muscular twitching in fifteen minutes and increased reflex excitability twenty-five minutes after the injection. One of the dogs which had received a dose of 100 mg. received a dose of 200 mg. dissolved in normal salt solution with NaOH twenty-four hours later. It showed no perceptible effect within about two hours.

These results indicate that the impurity has only a slight action on the vomiting center, but the increased reflex excitability points to action on the cord. The fact that moderately large doses have little action on the respiratory center is in harmony with the observation that it has little action on the vomiting center, since nearly all drugs which stimulate one of these centers stimulate or depress

the other. It is probable that changes in blood pressure are caused mainly by the cardiac actions, though there is probably some action on the vasomotor centers.

SUMMARY.

Intravenous doses of 25 to 75 mg. of the impurity have no important effect on the heart rate, respiration rate or rectal temperature in the cat or dog.

A solution of the impurity in 1000 parts of Locke's solution has practically no effect on perfused blood vessels.

The impurity has only a slight action on the vomiting center after its intravenous injection in cats and dogs. Large doses increase the reflex excitability of the cord and induce convulsions similar to those induced by strychnine. Such doses probably stimulate the vasomotor center briefly.

The cat is much more susceptible to the toxic action of the impurity than is the dog. It is absorbed fairly well from the gastro-intestinal tract of the cat and dog.

The drug usually kills the cat or dog by stoppage of the heart after the intravenous injection of a fatal dose.

Repeated oral doses of the impurity do not cause perceptible gross or microscopic changes in the organs of cats, dogs or rabbits, but fairly large doses sometimes interfere with the appetite in the cat and cause loss in weight.

The latter conclusions are based on the administration of moderate amounts of the impurity to apparently healthy animals for limited periods of time, and one would not be justified in assuming that the impurity would have no effect on diseased organs in man with continued use. It is in the interest of therapeutics that drugs should be as pure as circumstances permit, even though an impurity is of slight toxicity.

The Blaine House—the Governor's Residence, Portland, Maine.