Title: The Toxicity of Caffein: An experimental study on different species of animals

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Release date: July 22, 2014 [EBook #46356]

Language: English

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Issued April 4, 1912.

U.S. DEPARTMENT OF AGRICULTURE,
BUREAU OF CHEMISTRY—BULLETIN No. 148.
H. W. WILEY, Chief of Bureau.

THE TOXICITY OF CAFFEIN:

AN EXPERIMENTAL STUDY

ON DIFFERENT SPECIES OF ANIMALS.

BY

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WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1912.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., November 14, 1911.

Sir: I have the honor to submit for your approval a manuscript on the toxicity of caffein, which is the first of a series of reports to be made by Dr. Salant on the pharmacology of this drug; the conclusions here reported are, therefore, in some particulars to be regarded as tentative. The data obtained are primarily of use in the execution of the food and drugs act, but are capable of much broader application.

Acknowledgment is made of the assistance rendered by Dr. John R. Mohler, Chief of the Pathological Division, Bureau of Animal Industry, and his assistants, in performing the autopsies recorded in this report. I recommend the publication of the manuscript as Bulletin No. 148 of the Bureau of Chemistry.

Respectfully,

H. W. Wiley, *Chief.*

Hon. James Wilson,
Secretary of Agriculture.

THIS PUBLICATION may be procured from the Superintendent of Documents, Government Printing Office Washington, D. C., at 15 cents per copy

CONTENTS.

THE TOXICITY OF CAFFEIN.

INTRODUCTION.

Comparative physiology has established the fundamental fact that some properties are common to all forms of living matter. But the same method of inquiry has also led to the recognition of marked differences in the physiological processes of various species of animals. Among the most important investigations which contributed to the knowledge of such variation of function are the studies in comparative metabolism. It is now recognized that metabolism is in some respects quite different in herbivora and in carnivora. Some forms of oxidation are much greater in the rabbit than in cats and dogs. Nuclein metabolism presents important differences in the rabbit and in man, while the mode of neutralizing acid in the body may be cited as another variation in the metabolism of these forms. Perhaps the most striking examples of differences in the metabolism of different organisms is furnished by the results of studies on the fate of certain poisons introduced into the body.

The classical experiments of Bunge and Schmiedeberg[15[A]] on the synthesis of hippuric acid are of interest in this regard. It will be recalled that in the dog the synthesis takes place in the kidney; the rabbit is able to form hippuric acid in the liver as well as in the kidney, while frogs can synthesize hippuric acid even when both of these organs have been removed or excluded from the circulation. Observations on the fate of some of the alcohols of the fatty acid series have likewise shown that these substances may be combined with glycuronic acid in some animals but not in others. According to Thierfelder and Von Mering,[84] tertiary alcohols are combined in this manner in the rabbit but not in the dog. According to Neubauer,[64] the primary and secondary alcohols are so combined in the dog as well as in the rabbit, but to a greater degree in the latter.

Pohl[73] found that amyl alcohol is largely eliminated by the lungs in the cat and in the dog. The protocols of his experiments show that 65 per cent of the alcohol given these animals was thus recovered, while he recovered only 22 per cent of this substance in the expired air of the rabbit. Examination of the urine showed the presence of glycuronic acid. Hofmeister's[37] work with tellurium in the dog is of interest in this connection. He made the important discovery that some animals possess the power of methylation as well as of demethylation. Abderhalden and Brahm's[1] experiments with pyridin show that the same is true of young dogs when on a meat diet. His experiments on rabbits with this substance were negative.

The metabolism of caffein and theobromin furnish another illustration of differences in the physiological mechanism of animals. Although the substances found in the urine of man, dog, and rabbit after the administration of caffein and theobromin were the same, the quantities varied considerably. According to Krüger and Schmidt,[47] over 14 per cent of the theobromin introduced into the rabbit is eliminated as 7-methyl xanthin in the urine. The dog eliminates only about 0.67 per cent. On the other hand, the amount of tri-methyl xanthin eliminated was only 3 per cent in the dog and not quite 1 per cent in the rabbit.

It appears, therefore, from studies in comparative metabolism, whether endogenous or exogenous, that well-marked physiologic and chemical differences exist in various species of animals. That pharmacological action may likewise vary in different species of animals is shown by the following investigations. According to Guinard,[31] who made an exhaustive study of morphin, the reaction to this alkaloid varies in different forms of life, both qualitatively and quantitatively. He established its narcotic effect in the dog, rabbit, guinea pig, white mice, and rats, while for the cat, horse, ox, sheep, hog, and goat it is, on the contrary, a stimulant. Moreover, there is no evidence of cerebral effect. The stimulating effect of morphin on the nervous system in some animals was also observed by Noe[65] in experiments with this substance on the hedgehog.

Guinard[29, 30] has also shown that morphin has no narcotic effect in the marmot, although this animal is very sensitive to the drug. Two milligrams per kilo were found to be a surely fatal dose for this animal. His experiments on the comparative toxicity of morphin[30, 31] show a considerable range of variation in different species. Thus the fatal dose for the dog was found to be 0.65 per kilo, while 7 mg per kilo is the fatal dose for the horse. About twice the amount is fatal for the ox and 0.2 mg per kilo kills the pig. Experiments with other drugs has shown that a considerable range of variation in resistance exists in animals of different species.

Noe's[65] studies on the comparative toxicity of chloral brought out the interesting fact that the rabbit is more resistant to it than the hedgehog and the latter more resistant than the guinea pig. Perhaps the most striking example of a difference in reaction of the same substance in widely different species is that furnished by apocodein, quinin, and yohimbin. According to Gunn[32] these substances have been found to cause vasodilation in warm-blooded animals, but they constrict the blood vessels of the frog.

Experiments with apomorphin likewise show that the reaction to this substance varies in different species of animals. The resistance of the cat to this drug is, according to Guinard[31], ten times greater than that of the dog, and the latter is more sensitive than the rabbit to the crystalline form of apomorphin when given intravenously. According to Kobert[45] amygdalin is without effect on dogs, but it is poisonous to rabbits. Lapicque[49] found that the toxicity of curara varies in different species of frogs, the dose required to produce paralysis in *Rana esculenta*

being three times greater than in *Bufo vulgaris*. Weir Mitchell[59] pointed out long ago that turtles stand enormous doses of curara. Schmiedeberg's experiments with caffein on *Rana temporaria* and *Rana esculenta* (and more recently those of Jacobi and Golowinski[42] with caffein, theobromin, and theophyllin) are also of interest in this connection. These experimenters observed well-marked differences of reaction to methyl-xanthins in these closely allied forms.

Experiments with quinin have shown that the action of this substance differs in some animals. It causes a fall of temperature immediately after its administration in the guinea pig, but frequently produces, at first, a rise of temperature, followed by an unimportant fall, in rabbits, dogs, and man.

The numerous investigations which have been carried out on the effect of atoxyl within recent years have contributed much to the comparative pharmacology of this substance. Although the symptoms and organic changes produced by this substance in a variety of animals present no great differences, the resistance of some has been found to vary; according to Köster[46] it is more toxic for dogs than for rabbits. A number of other substances have been found by various experimenters to vary in toxicity for different species of animals. Cantharadin, phenol, atropin, and strychnin may be mentioned as illustrations.

Pharmacological studies on lower forms of life have also revealed marked variations in the effect of some poisons. Observations made by Danilewski[18] with hydrochinone indicate that solutions of 1 to 100 or 200 are toxic to Celentrates, causing paralysis in these organisms. Echinoderms are killed within one or two hours in 1 to 1,000 or 2,000 solution, while in Vermes even weaker solutions cause tetanus and finally paralysis. The experiments of Drzewina[19] with potassium cyanid are also interesting in this connection. Teleosts placed in 100 cc of sea water containing twentieth-normal potassium cyanid showed signs of asphyxia and died in 10 to 20 minutes. Actinia placed in a solution of sea water containing five times as much potassium cyanid were active on the thirteenth day of the experiment. Similar results were obtained with other marine organisms.

From these data it is evident that the toxicity of a substance may vary considerably in different forms of life. It has been shown also by some investigations cited by Salant[78] that the action of drugs may be modified by different conditions in the environment as well as in the subject of the experiment. The recognition of the importance of these factors in determining pharmacological action has contributed much to the elucidation of the mechanism by which drugs and other substances produce physiological effects in the body. Moreover, such knowledge has often enhanced the therapeutic value of pharmaco-dynamic agents and has frequently served to avert effects of an undesirable character in man and domestic animals. The results obtained in one species of animals under a particular condition do not admit, therefore, of universal application. Furthermore, the nature of the action of a drug can only be partly learned from the manifestation of its acute effects. Equally important, therefore, especially in studies on toxicity, are the changes produced in chronic intoxication.

That the acute effects of a substance can hardly be considered a correct estimate of its toxicity is shown by the evidence obtained in experiments on tolerance and cumulative action of drugs; for the toxicity of a substance may diminish when the substance is given steadily for a long time if the body acquires tolerance for it. Arsenic, morphin, and cannabis indica may be cited as illustrations of drugs, the toxicity of which decreases with repeated doses, while digitalis and lead show a tendency to increased toxicity when similarly administered. Moreover the acute and chronic effects are sometimes qualitatively different. According to Igersheimer[41] the symptoms in acute atoxyl intoxication are nausea, vomiting, and diarrhea. These symptoms are absent in chronic intoxication, in which trophic disturbances of the skin and inflammation of the mucous membranes were the effects produced. That the acute action of atoxyl differs from the chronic effects was likewise shown by experiments on animals. The studies of von Anrep[5] on chronic atropin intoxication are of interest in this connection, as he found that after 10 to 15 injections of atropin there is no manifestation of symptoms such as is observed in acute intoxication, while the effects on the circulation are also less marked, the acceleration of the pulse being less than after the same dose in a normal subject not accustomed to its use. When the administration of atropin is continued for a longer time its usual effects on the pulse disappear altogether; there is, on the contrary a decreased frequency of the pulse. If atropin has been administered for from two to three weeks, respiration is likewise affected.

HISTORICAL REVIEW OF THE LITERATURE ON THE TOXICITY OF CAFFEIN.

Caffein was discovered in 1820 by Runge,[77] Pelletier,[60] and Robiquet[75] and was first analyzed by Dumas and Pelletier,[20] but its exact percentage composition was determined by Pfaff and Liebig,[71, 72] while to Herzog [13, 18] belongs the credit of having established that it is basic. Strecker[82] prepared caffein synthetically by heating theobromin silver and methyl iodid in a closed tube for 24 hours. Soon after its discovery in coffee Oudry[67] reported the presence of a substance in tea which he called "thein." Its identity with caffein was established 15 years later by Jobst[43] and also by Mulder.[62, 63] According to Brill,[13] Mulder (1838) was also the first to perform experiments with caffein on animals. After the administration of one-half grain to a pregnant rabbit he observed loss of appetite and kyphosis. The rabbit aborted but recovered from the effects of caffein. It has since been made the subject of numerous investigations which were carried out on a variety of animals. Observations with caffein were also made on the human subject. About four years after Mulder published his results, Lehmann[51] (1842) reported experiments on a number of people who were given caffein. The administration of from 2 to 10 grains of the alkaloid was followed by headache, palpitation of the heart, increased frequency and irregularity of the pulse, tinnitus aurium, photopsia, insomnia, and even delirium. Similar experiments reported by Frerichs[25] (1846) indicate that in doses of 25 grains it may induce severe symptoms about 15 minutes after its administration. He also observed circulatory as well as nervous symptoms and vomiting.

According to Albers[2] (1852), 4.5 grains of caffein citrate injected subcutaneously into the thigh of a rabbit was soon followed by diminished motion and tremors of the operated thigh. Other symptoms reported were spasms of the facial muscles, increased respiratory movements, and mental confusion. Of interest in this connection are the experiments of Cogswell[17] (1852) on frogs. He concluded that in point of destructive action on the tissues, caffein is far superior to morphin and may be compared to strychnin and coniin, its action on the nervous system he believed to be principally confined to the effect on the brain and spinal cord.

Lehmann[52] (1853) observed increased frequency of heart action after the administration of 4 grains, which were given with a normal diet to an adult man. When the dose was doubled the frequency of the pulse was still more increased, heart action became stronger, and tremors and confusion of thought with excitement of the imagination made their appearance. There was also an increased desire to micturate.

Stuhlmann and Falck[83] (1857) were the first to make a study of the toxicity of caffein on animals of different species. The administration of 0.5 gram of caffein subcutaneously or per rectum in rabbits induced tremors, tonic and clonic convulsions, paralysis, and increased frequency of respiration at first followed by violent dyspnœa. On autopsy he noticed congestion of the organs and in two of the three rabbits experimented upon punctiform hemorrhages of the brain with congestion of the meninges were found. In the other rabbit anemia of the brain was observed. Experiments on cats were carried out by subcutaneous, intravenous, and rectal injections. The symptoms observed after the administration of 0.5 to 0.7 gram of caffein were the same as in rabbits except that the cats developed diarrhea when caffein was given and no anatomic lesions were found on autopsy. The effect of caffein on dogs indicated that in subjects of medium weight a dose of 0.5 gram given by mouth might produce restlessness and increased frequency of respiration, while the injection of the same amount intravenously into such animals may cause death. Large, full-grown dogs, however, survived an intravenous injection of 2 grams of caffein, showing symptoms of incoordination, salivation, and frequent defecation. These investigators also made observations on caffein, using pigeons and other birds; 0.5 to 0.1 gram introduced into the stomach caused vomiting, diarrhea, tonic, but more frequently clonic, convulsions, incoordination, tremors, paresis, and paralysis.

In a few, but not in all of the birds, there was at first increased frequency of respiration followed by dyspnœa and circulatory disturbances. These amounts of caffein proved fatal in all of the experiments on birds. Inflammation of the intestinal mucosa and congestion of the meninges were the only changes found on autopsy. Stuhlmann and Falck also studied the effects of caffein on fishes and toads. Mitscherlich[60] (1858) fed 0.4 gram of caffein with bread to a rabbit and noticed lowered temperature, fatigue, convulsions, first increased then decreased frequency of respiration, and on autopsy congestion of all the viscera. He also reported observations on two frogs, one of which was given one-sixteenth of a grain of caffein in a pill with bread. It was administered to the other frog in aqueous solution, but the mode of administration was not published. The symptoms observed were in the main the same as in rabbits. In pigeons 0.125 gram introduced into the stomach caused severe vomiting, muscular incoordination, tonic rigidity of the limbs, and retraction of the head. Respiration was increased in frequency. Death followed within 3 hours and 15 minutes.

From a series of experiments on frogs which Hoppe[38] carried out (1858) by applying one-fourth of a grain of caffein to the muscles of the back, he concluded that caffein causes paralysis of the nerves, spinal cord, and brain, sensation being paralyzed before movement. The injurious action of caffein proceeds, according to Hoppe, from the spinal cord. This was based on experiments on

two frogs, *Rana esculenta*, in which the right leg was amputated, the nerve being left intact, while the nerve of the other leg was ligated. At the end of 30 minutes paralysis was more marked on the right than on the left side. In another frog of the same species he resected the femoral nerve on the right side; about 1½ hours after the administration of caffein convulsions were observed. The left leg was rigid, but the right was relaxed.

Voit[85] (1860) ligated the vessels of the right lower extremity, cut the nerves of the left leg, and introduced a few drops of caffein solution into the stomach. Shortly afterwards tetanus of the right leg occurred on touching the back of the animal; the left leg was motionless. Later the entire body exhibited tetanic convulsions. From this and similar experiments Voit concluded that caffein acts first and principally on the central nervous system, and that caffein is also poisonous to nerve and muscle fibers, as they die when a solution of caffein is applied to them. The action of caffein, according to Voit, is similar in great part to that of strychnin. The effect on the blood vessels is particularly interesting, as Voit observed dilatation of the vessels, due as he thought to muscular paralysis, and also transudation and congestion of the capillaries.

Kurzak[48] (1860) made a study of the comparative toxicity of caffein in frogs and rabbits and came to the conclusion that the lethal dose for frogs is about one-seventh of that for rabbits. Caffein citrate in the form of crystals was administered in both cases by mouth. The doses given to frogs were 1 to 1.5 grains. He observed convulsions and increased respiratory activity at first; after one hour respiration diminished and voluntary muscular activity disappeared. Even on the second day convulsions were sometimes noticed. Death occurred at the end of the first or second day. Experiments on only two rabbits were reported, 0.8 gram of caffein citrate causing the death of one at the end of 13 hours. The symptoms noticed were the same practically as in frogs, but it is interesting to observe that ecchymosis of the mucous membranes of the stomach near the cardia was the only lesion found on autopsy. Several experiments made on different days on the other rabbit indicated that the toxic dose exceeded 0.5 gram, while smaller doses caused but very mild symptoms.

According to Gentilhomme[27] (1867), after caffein the reflexes are at first diminished and then disappear altogether. Death is produced by stiffness and immobility of all the muscles, particularly of the muscles of respiration, thus causing asphyxia. He furthermore held that caffein has no effect on cardiac or smooth muscle fiber, its action being specific on voluntary muscle fiber, contractions of which he observed under the microscope, thus differing completely from strychnin, which is a nerve poison.

These observations seemed to be confirmed by Pratt[74] (1868), who reported that the isolated posterior extremities and muscle fibers of the toad placed in a solution of caffein (1 grain to a wineglassful of water) for three minutes were contracted, while controls placed in distilled water were relaxed. This experiment is, of course, defective, as normal salt solution should have been used in both cases. When the muscular fibers previously immersed in caffein solution were placed under the microscope violent contractions were observed. The same author administered from 2 to 18 grains at a dose to five healthy young men. After the administration of 12 grains he noticed mental anguish, tremors of the hands and arms, and insomnia. Doses under 5 grains had no marked effect except a diminution in the frequency of the pulse and wakefulness.

About the same time Amory[4] (1868) published the results of his studies on the toxicity of caffein in cats, dogs, rabbits, and pigeons. In all cases very large doses were introduced directly into the stomach by means of a temporary gastric fistula. Ten grains given in meat to a dog caused restlessness, but no other symptoms. Doses of 30 grains and above were invariably fatal. Seventy-three grains given to a cat caused death within 20 minutes.

From observations on frogs, guinea pigs, rabbits, and on one dog, Leven[53] (1868) concluded that caffein which he gave in the form of the citrate in doses of 10 mg to frogs, from 150 to 200 mg to guinea pigs, and three to four times the latter amount to rabbits, stimulates the central nervous system and the voluntary, cardiac, and smooth muscles. He found that 0.9 gram caffein was fatal for a rabbit when injected subcutaneously, while 1 gram of the citrate was not toxic for a dog of medium size. Caffein applied directly to muscle fiber causes tetanus and destroys muscular contractility, while a nerve fiber similarly treated loses its irritability.

According to Johansen[44] (1869), caffein acts directly on the muscular fiber. After the subcutaneous injection of 0.02 gram of caffein into frogs, he observed contraction of the muscles at the site of injection, then contraction of the anterior extremities, and finally the posterior extremities become rigid and extended. Johansen observed muscular rigidity after caffein, even after curara was injected, or after ligating the vessels, or cutting the nerves which supply the muscles. He also observed that large doses of caffein diminish muscular irritability. When cardiac muscle was poisoned with caffein, microscopical examination showed that the striations disappeared. Johansen also states that reflexes disappear after caffein poisoning. He never observed tetanus in frogs, but reported tonic and clonic convulsions as a result of caffein poisoning in mammals. Somewhat different effects of caffein in frogs were observed by Buchheim and Eisenmenger[14] (1870). After the injection of 2 per cent of the citrate the frogs soon become inactive. He also observed muscular twitching of the extremities, which gradually increased, with rigidity of the muscles and opisthotonos, while respiration became slow and superficial, finally stopping altogether.

Aubert[6] (1872) studied the toxicity of caffein in man and other animals. After the ingestion of

0.36 gram, he observed dizziness, but doses of 0.12 and 0.24 gram were without any apparent effect. On the other hand, a dose of 0.5 gram of caffein was followed by increased frequency of the pulse, which soon disappeared. After one hour he noticed dizziness and trembling of the hands, which likewise passed away soon. The injection of 0.16 gram of a 2 per cent solution of caffein into the jugular vein of a rabbit weighing 1,090 grams caused tetanus and death in two and one-half minutes, and 0.12 gram injected into a rabbit weighing 980 grams caused death in one minute. Much larger doses could be borne, however, when artificial respiration was resorted to. A dog which was given 3 grams of caffein survived when artificial respiration was performed. Aubert reports, on the other hand, a similar experiment with 0.25 gram of caffein which terminated fatally.

That caffein may give rise to different effects in various species of animals was observed for the first time by Bennett.[9] He studied its action on frogs, mice, rabbits, and cats, and attempted to determine the minimum fatal dose in rabbits and cats. He also reported experiments with thein. In his first communication on the subject he states that the administration of thein to rabbits first increased and then diminished the frequency of respiration, while the pulse was decreased in frequency. Caffein, which he apparently thought was different from thein, caused increased frequency of respiration, while the pulse was markedly retarded after a preliminary acceleration. He also noticed congestion of the ears, muscular incoordination, tetanus, paralysis, diminished reflexes, and contraction of the pupils. Bennett reported the minimum fatal dose of caffein for a rabbit weighing 3.25 pounds as being 5.25-5.5 grains. The symptoms in cats after the administration of toxic doses of thein or of caffein were great excitement, paralysis alternating with convulsions, and profuse salivation. The minimum fatal dose for a cat weighing 5 pounds was, according to Bennett, 6 grains of caffein and 5.5 grains of thein. Only one experiment on a mouse is reported; the administration of 0.1 grain proved fatal. The symptoms were the same as those observed in cats and rabbits after the administration of caffein. The experiments on frogs indicate that the symptoms were about the same as those previously described in the case of warm-blooded animals except that the reflexes are almost completely lost after the subcutaneous injection of doses of one-sixteenth to one-twelfth of a grain. The latter dose was fatal for frogs. It would be of interest to know the comparative toxicity of caffein to frogs and mammals, but unfortunately the weights were not reported.

Schmiedeberg[79] (1874) noticed that the administration of 20 mg of caffein to frogs weighing about 45 grams was followed, in *Rana esculenta*, in about 25 minutes, by increased reflexes, 7 minutes later by tetanus. Several attacks occurred, but tonic spasms were never observed. On the contrary, when the same amount of caffein was given to *Rana temporaria* weighing 45 grams he noticed a marked diminution of the reflexes and tonic rigidity of the muscles after 23 minutes; the reflexes were greatly increased, however, about 24 hours later. The frogs were under observation for three days, and although symptoms were still present at the end of this time in the subjects of both species tetanus was never observed in *Rana temporaria*.

Peretti's[70] (1875) studies on the effects of caffein were confined chiefly to observations on dogs. He also made observations on a few rabbits and reported an experiment on one cat to which he administered, by subcutaneous injection, 0.18 gram of caffein per kilo and noticed increased frequency in lachrymation and crying. The cat was found dead the next day. The subcutaneous injection of a rabbit in which artificial respiration was instituted with 0.36 gram of caffein per kilo proved fatal soon after the injection without any manifestation of symptoms. Small doses of caffein, 0.1 gram, given to a rabbit weighing 3,670 grams, failed to produce any visible effects. Doses under 0.1 gram per kilo likewise failed to induce any symptoms in dogs. When 0.1 gram of caffein per kilo was given by mouth or subcutaneously it was followed by restlessness, salivation, rigidity of hind legs, and vomiting. In both instances the dogs recovered. The symptoms were more severe when the dose was increased to 0.185 gram per kilo, but even in this case the dog recovered. A dose of 0.2 gram per kilo, however, proved fatal.

Henneguy[36] (1875) experimented on three frogs to which he gave 0.01 gram of caffein citrate subcutaneously. He observed mild stimulation of the nervous system and of the muscles, as well as increased cardiac activity. Later, voluntary movement and respiration disappeared and sensations diminished, but convulsions of the extremities appeared. Cardiac activity was then diminished, the heart being finally arrested in systole. Since the motor nerves retained their irritability even after the reflexes disappeared, he concluded that the loss of motion was due to the action of caffein on the nerve centers.

Binz[11] (1878) reported experiments on dogs and also made some observations on man with caffein. The subcutaneous injections of 0.2 gram caffein may prove fatal to dogs, although some survive such a dose. The toxic dose in man varies from 0.5 to 1.5 grams. Disturbance of the circulation, such as palpitation of the heart and fullness of pulse, restlessness, and diarrhea were the symptoms he observed.

Extensive investigations on the action of caffein were carried out by Leblond[50] (1883), who studied its effect on the circulation in man and lower animals, and its toxicity in the lower animals alone. Five to twenty centigrams of caffein and 0.06 to 0.25 gram of salicylate of soda were dissolved and injected into the muscles of the thigh of young guinea pigs weighing a little over 300 grams. In the three experiments reported the death of the animals occurred after 23 minutes, 40 minutes, and 1 hour and 20 minutes. Symptoms appeared in from 10 to 15 minutes after the injection of caffein. Incoordination of movements, convulsions, both tonic and clonic, opisthotonos, tremors, increased frequency of respiration, ataxia, paralysis were the symptoms

observed. It is worthy of note that the appearance of paresis preceded the convulsions. Diminished sensation was reported in one pig, but no sensory disturbances nor reflexes had been observed in the other. Two rabbits, one of which received 0.5 and the other about 0.3 gram of caffein per kilo with equal parts of salicylate of soda, were injected subcutaneously into the thigh. Diminished sensation, paresis of the posterior extremities, hyperexcitability, convulsions, opisthotonos, dilation of the veins of the ear were observed. Death followed in 1 hour and 23 minutes in one rabbit and in 3 hours and 7 minutes in the other.

Filehne[22] (1886) experimented with caffein on *Rana esculenta* and *Rana temporaria*. The subcutaneous injection of 7 mg of caffein into *Rana esculenta* caused tetanus, while 50 mg given by mouth caused tonic spasms. He further stated that the difference between *Rana esculenta* and *Rana temporaria* as regards the reaction to caffein was one of degree only.

Amat[3] (1889) reported experiments on three guinea pigs, in which 0.4 to 0.5 gram per kilo injected subcutaneously proved fatal within 38 and 44 minutes. One guinea pig which received 0.1 gram of caffein per kilo survived. The symptoms observed in the two fatal cases were general muscular rigidity and convulsions.

Parisot[68] (1890) made a study of the toxicity of caffein on different species of animals. Unlike most of his predecessors, however, he reported, at least in some cases, the weight of the animals on which he worked. After the subcutaneous and intramuscular injections of from 5 to 20 mg of caffein into *Rana temporaria* weighing from 14 to 16 grams, he noticed increased irritability at first; later, a loss of reflexes, inability to use the muscles, complete muscular rigidity resembling rigor mortis, and also cessation of heart action. The effect of caffein produced in the green frog was analogous to that observed in strychnin poisoning. Parisot found, however, that muscular rigidity developed, although very gradually, also in the green frog, but it set in much later than in frogs of the other species and without superseding the clonic convulsions. According to Parisot, the muscular rigidity after caffein persists after the destruction of the brain and spinal cord, thus showing that it is not of nervous origin. He further emphasized the difference in the behavior of these two species of frogs toward caffein by stating that he never observed tetanic convulsions in the red frog. His experiments also indicate that the green frog is more resistant to caffein than *Rana temporaria*, as the same doses which are fatal for the latter were only toxic for *Rana esculenta*. The number of experiments, however, is too few to justify a positive conclusion on this point. Parisot also made some experiments on turtles. The results he obtained show that caffein is at least as toxic for these animals as for the frogs he experimented upon, 0.33 gram per kilo (carapace not included in weight) having proved fatal within 24 hours. Two experiments on one pigeon were also reported by the same observer; two doses of 0.06 gram per kilo given at an interval of four hours caused mental depression and muscular rigidity, but the pigeon survived.

Experiments with caffein on the human subject made by Parisot showed that man is far more susceptible to this substance than the other animals he investigated. After the ingestion of 0.3 gram of caffein symptoms of intoxication pointing to cerebral disturbance appeared, which became more marked when the size of the doses was increased.

It will be noticed that the nature of the action of caffein, whether it is a nerve or a muscle poison, formed the subject of several investigations. Binz[11] (1890) brought forward additional evidence in support of the view that caffein acts primarily on the ganglion cells, and not on the muscle directly. This he has shown by injecting 0.5 gram into each of two rabbits after cutting the sciatic nerve on one side; in one case he also resected the obdurator and crural nerves on the same side. Clonic spasms developed in both subjects soon after caffein was given, but in each rabbit the side operated upon remained paralyzed. Baldi[8] (1891) studied the action of caffein on *Rana esculenta*. After injecting from 4 to 20 mg tetanus, such as observed in strychnin poisoning, was noticed. Fröhner[26] (1892) made observations on the comparative toxicity of caffein in domesticated animals. After the administration of 5 grams of caffein sodium salicylate by mouth to a dog weighing 10 kilos, he noticed salivation, restlessness, vomiting, and convulsions as in strychnin poisoning. Death occurred three hours after the drug was given. On autopsy he noticed mild inflammation of the mucous membranes of the stomach and intestines and edema of the lungs; the heart was in diastole. A dose of 2 grams of caffein sodium salicylate given to the same animal subcutaneously two days previously provoked only very slight symptoms. The subcutaneous injection of 10 grams of the same preparation into a pig weighing 30 kilos caused death in two and a half hours, with the production of symptoms of disturbance of the nervous system and of gastrointestinal irritation. The same dose per kilo of body weight given to a goat likewise caused death in two and a half hours after its administration. Examination on autopsy revealed inflammation of the gastrointestinal tract. Similar lesions were found in a horse killed by 100 grams of caffein, in which he also noticed hemorrhage of the mucosa in the fundus of the stomach.

Gourewitch[28] (1907) conducted experiments with caffein on rabbits, pigeons, and white rats. It appears from his protocol that single doses of about 0.2 to 0.25 gram caffein per kilo given subcutaneously proved to be fatal. He states, however, that the resistance to caffein was markedly diminished, when its administration was repeated daily, for much smaller amounts sufficed to cause death in these animals. A dose of 120 mg of caffein per kilo proved fatal after the third injection. When the dose was increased to 170 mg per kilo, the animal succumbed to the effects of caffein after the second injection. His experiments on the other animals do not indicate the degree of resistance to caffein, since the weights for some were not given while for the others

no attempt was made to determine the minimum toxic or fatal dose.

Maurel[55] (1907) studied the influence of different methods of administration on the toxicity of caffein on frogs and rabbits. He determined the minimum toxic and lethal doses of caffein hydrobromid which he employed in 1 to 2 per cent solutions. He concluded from his experiments that the toxicity of caffein when given by mouth is twice as great for the frog as for the rabbit.

More recently Hale[33] carried out a number of experiments on guinea pigs in which he determined the toxicity of caffein given in the form of the citrate and made into a pill with mucilage of acacia and arrow-root starch. After the pill was dried it was fed to the animal, due precaution being taken that none of it was lost during feeding. From experiments on guinea pigs which received doses of 0.3 to 0.6 gram caffein citrate, the following data have been reported: Three decigrams per kilo given to one pig was not fatal. Of three pigs which received 0.4 per kilo, one died and two survived. Exactly the same results were obtained in three others which received 0.5 per kilo. Two guinea pigs, which received 0.55 and 0.6 per kilo each, died after 15 and 7 hours, respectively, while another animal survived a dose of 0.45 per kilo.

This review of the literature on the toxicity of caffein, although bearing evidence of considerable investigation and extending over three-quarters of a century, is largely qualitative in character. It appears from the experiments that the main object of the investigations was to ascertain the nature of the action of caffein, whether it is a muscle or a nerve poison. The comparative toxicity in different species of animals by the accurate determination of the toxic and fatal doses received but little attention. To fill the gap in our knowledge of the toxic effects of caffein, the present investigation was undertaken. This, it will be seen, proved to be a most laborious task, because in the large number of experiments careful observations showed that individuals of the same species varied considerably in their reaction to the drug. Numerous other factors, as will be shown, were also found to play an important part in the determination of the toxicity of caffein.

ACUTE CAFFEIN INTOXICATION.

The object of these experiments was to determine the resistance to caffein in various species of animals and by various methods of administration. Caffein was therefore given by mouth and injected subcutaneously into the peritoneal cavity, into the muscles, and intravenously. As far as could be judged by appearance, healthy animals were selected for the subjects of the experiments, but as it is impossible to diagnose with any degree of accuracy the condition of the animal while it is alive, post mortem examinations were resorted to in many cases in which the issue of the experiment was fatal. Since the age of the animal may modify toxicity full grown, as well as young, animals were employed for these experiments; diet, race, and season also play an important part in determining the toxicity of a drug and these factors were also taken into account in the present investigation.

EXPERIMENTS ON RABBITS.

Animals of different varieties were used and were given caffein by all of the methods indicated in the preceding paragraph. Some of the rabbits employed in these experiments received oats, others received a diet exclusively of carrots for several days or weeks previous to the administration of caffein. The experiments were conducted at all seasons of the year.

SUBCUTANEOUS INJECTION.

From a study of the literature on the toxicity of caffein it seemed that about 150 mg per kilo is probably the lethal dose for the rabbit when the drug is injected subcutaneously. Preliminary observations were therefore carried out with such a dose, but it was found, on the contrary, that this amount per kilo was hardly sufficient to induce symptoms in the great majority of cases.

SERIES A.

[Doses of 147 to 167 mg of caffein per kilo were employed in these experiments.]

Rabbit 332. Belgian hare, female. Weight, 1,070 grams. Diet, oats.

March 25: 8.5 cc 2 per cent caffein (158 mg per kilo) injected subcutaneously at 2.15 p. m.; 4 p. m., reflexes increased; 5.45 p. m., increases of reflexes still more marked.

March 26: Rabbit looked normal; no symptoms observed.

Rabbit 331. Belgian hare, female. Weight, 1,170 grams. Diet, oats.

March 25: 2.15 p. m., 9 cc 2 per cent caffein (153 mg per kilo) injected subcutaneously; 4 p. m., reflexes increased; 5.45 p. m., condition the same.

March 26: Rabbit looks normal; no symptoms observed.

Rabbit 328. Belgian hare, female. Weight, 1,200 grams. Diet, oats.

March 25: 9 cc 2 per cent caffein injected subcutaneously (150 mg per kilo); 4 p. m., reflexes increased; 5.45 p. m., reflexes increased but not markedly.

March 26: No symptoms; rabbit looks normal.

Rabbit 322. White female. Weight, 1,065 grams. Diet, oats.

March 17: 8 cc 2 per cent caffein (150 mg per kilo) injected subcutaneously at 11.55 a. m.; 12.55 p. m., reflexes increased, but no tetanus nor any other symptoms.

March 18: Rabbit running around in cage; condition apparently normal.

March 25: Condition of rabbit good.

Rabbit 217. White. Weight, 1,355 grams. Diet, oats.

October 29: 10 cc 2 per cent caffein (147 mg per kilo) injected subcutaneously at 1.51 p. m. 5.15 p. m., rabbit alive; survived.

Rabbit 219. Maltese. Weight, 1,820 grams. Diet, oats.

October 29: 14 cc 2 per cent caffein injected subcutaneously at 1.40 p. m. (153 mg per kilo); 5.15, rabbit alive; survived.

Rabbit 194. White female. Weight, 1,490 grams. Diet, oats.

October 14: 13 cc 2 per cent caffein (174 mg per kilo) injected subcutaneously; increased reflexes and tremors were observed.

October 15: Condition of rabbit good; no symptoms.

Rabbit 191. Brown male. Weight, 1,915 grams. Diet, oats.

October 14: 16 cc 2 per cent caffein (167 mg per kilo) injected subcutaneously; reflexes increased and tremors present.

October 15: Condition of rabbit good.

A study of this series shows that about 150 mg of caffein per kilo caused increased reflexes within one to two hours after injection. When the dose was increased, as in rabbits 194 and 191, the symptoms were more pronounced; 150 mg per kilo may be regarded as the minimum dose which produces symptoms of nervous irritability when caffein is injected subcutaneously. Experiments with larger doses were therefore carried out in order to determine the minimum fatal dose.

SERIES B.

Approximately 0.2 gram of caffein per kilo was employed in these experiments. Diet and race as possible factors which may influence the toxicity of caffein were made the subject of study in these experiments which were divided into two groups as shown in the table, page 25.

Rabbit 95. Gray and white male. Weight, 1,478 grams. Diet, oats.

February 27: 11.30 a. m., 15 cc 2 per cent caffein (210 mg per kilo) injected subcutaneously; 2.20 p. m., no symptoms, tremors observed when handled, but not marked, reflexes slightly increased, no muscular rigidity nor any other symptoms; 2.45 p. m., rabbit suddenly became very restless, jumped off the table, and had convulsions; 3.45 p. m., rabbit died, rigor mortis set in almost immediately after death.

Rabbit 96. Gray and white male. Weight, 1,585 grams. Diet, oats.

February 27: 16 cc 2 per cent caffein (200 mg per kilo) injected subcutaneously at 3.40 p. m.; increased reflexes observed about one hour after caffein was injected, but no other symptoms.

February 28: Rabbit found dead.

Rabbit 112. Black female. Weight, 875 grams. Diet, oats.

March 18: 9 cc 2 per cent caffein (205 mg per kilo) injected subcutaneously at 3 p. m.; 3.30 p. m., rabbit became restless, reflexes were increased, tremors were observed, but no other symptoms; 4.15 p. m., rabbit had tremors, was handled but this failed to induce tetanus, 10 minutes later tetanus of short duration with recovery occurred.

March 19: 9 a. m., found dead.

Rabbit 119. Yellow white female. Weight, 1,060 grams. Diet, oats.

April 17: 10 cc 2 per cent caffein (188 mg per kilo) injected subcutaneously at 2.10 p. m.

April 18: Rabbit found dead.

Rabbit 195. White female. Weight, 1,300 grams. Diet, carrots, since October 7.

October 14: 13 cc 2 per cent caffein (0.2 gram per kilo) injected subcutaneously at 11.15 a. m.; 2.25 p. m., rabbit had convulsions and died. *Note*: Ulceration of rectum was noticed.

Rabbit 208. Gray. Weight, 1,068 grams. Diet, carrots, October 7-15, inclusive.

October 15: 10 cc 2 per cent caffein injected subcutaneously at 11 a. m.; 1 p. m., increased reflexes and tremors observed; 3.45 p. m., tremors were marked when rabbit was handled.

October 16: Rabbit found dead. *Note*: Looked poorly nourished.

Rabbit 247. Belgian hare, female. Weight, 1,295 grams. Diet, oats last 10 days before experiment.

November 10: 11 a. m., urine obtained from the bladder was acid to litmus and did not contain sugar or albumen, 13 cc 2 per cent caffein was injected subcutaneously; 1.30 p. m., 15 cc urine obtained was markedly alkaline to litmus and reduced Fehling's solution; 2.30 p. m., reduction of urine considerable, marked tremors observed but no tetanus.

November 11: 10.30 a. m., 95 cc urine collected gave moderate reduction of Fehling's solution, no symptoms, condition of rabbit seemed to be good.

Rabbit 248. Belgian hare, female. Weight, 1,305 grams. Diet, oats the last 10 days before the experiment.

November 10: 11 a. m., urine markedly acid to litmus, no albumen, no sugar; 13 cc 2 per cent caffein injected subcutaneously; 1.30 p. m., urine was slightly alkaline to litmus, no reduction of Fehling's solution; 2 p. m., reflexes increased; 2.30 p. m., 2 cc urine obtained from bladder, sugar abundant; 4.45 p. m., reflexes increased as before, but no tetanus.

November 11: 10.30 a. m., urine collected showed slight reduction of Fehling's solution; otherwise condition of rabbit was good; rabbit did not show any effects of caffein.

Rabbit 337. Belgian hare. Weight, 1,040 grams. Diet, carrots, March 31 to April 6, inclusive.

April 6: 3 p. m., 11 cc 2 per cent caffein injected subcutaneously in the back (0.211 per kilo); 4.30 p. m., reflexes much exaggerated.

April 7: 8.15 a. m.; condition good, no symptoms.

Rabbit 336. Belgian hare. Weight, 1,040 grams. Diet, carrots, March 31 to April 6, inclusive.

April 6: 3 p. m., 11 cc 2 per cent caffein injected subcutaneously into tissues of the back.

April 7: 8.15 a. m., no symptoms, condition good.

Although symptoms appeared in rabbits of Group I (see table, page 25) about the same time after the administration of caffein as in the rabbits of the preceding series all of them terminated fatally 2¼ hours to 24 hours after its administration. Two of these rabbits (Nos. 195 and 208) were fed carrots for several days before the injection of caffein, the others were fed oats. Since symptoms and death appeared in these two rabbits about the same time as in the rest of this group it may be concluded that caffein is not less toxic when carrots are fed than when oats form the exclusive diet. But since rabbit No. 208 was poorly nourished and ulceration of the rectum was observed in No. 195 it is quite possible that caffein might be less toxic in normal rabbits on this diet. This was tested in rabbits Nos. 336 and 337, both of which seemed to be free from abnormality and were well nourished. Since these rabbits survived and manifested mild symptoms only of intoxication it would seem that a carrot diet decreases the toxicity of caffein.

It was suggested, however, that another factor might be the cause of the greater resistance to caffein in these two rabbits, namely, race. This was tested in rabbits 247 and 248, both Belgian hares. Since the toxicity of caffein in these two rabbits was the same as in Nos. 336 and 337, diet as a factor in acute caffein intoxication may be disregarded. The greater resistance to caffein of these four rabbits is in all probability due, therefore, to a difference of race. This suggestion gained additional support from the experiments of the next series.

Series C.

The object of these experiments was to determine the minimum fatal dose for the gray rabbit and to obtain additional evidence as to the toxicity of caffein in the several varieties of rabbits. Eight experiments were performed, in which from 236 to 252 mg per kilo were given. The white rabbits, three in number, received 250, 242, and 238 mg per kilo. All the others (which were Belgian hares) received from 236 to 252 mg per kilo. Two of the white rabbits were fed carrots for one week preceding the injection of caffein. The other was fed oats. Three of the Belgian hares were on a diet of oats, two were fed carrots the week before the experiment with caffein.

Rabbit 122. White, female. Weight, 2,060 grams. Diet, oats.

April 14: 25 cc of 2 per cent caffein (250 mg per kilo) in aqueous solution injected subcutaneously in the back at 1.35 p. m.; 4.30 p. m., tremors, reflexes increased, condition otherwise good.

April 16: 9 a. m., found dead in cage. *Autopsy*: Liver deeply congested; kidneys congested in cortex and medulla; stomach showed small hemorrhagic areas, perforating ulcers in pyloric portion; small intestine petechiated on mucosa; lungs and spleen normal.

Rabbit 234. White, female. Weight, 1,650 grams. Diet, November 2-9, carrots.

November 9: 10.45 a. m., 20 cc 2 per cent caffein (242 mg per kilo) administered subcutaneously.

November 10: 9 p. m., found dead.

Rabbit 335. Gray hare, female. Weight, 1,170 grams. Diet, March 31 to April 7, carrots.

April 7: 9.30 a. m., 14 cc 2 per cent caffein solution (240 mg per kilo) injected subcutaneously in the back; 10.30, reflexes much increased, rabbit is extremely sensitive.

April 8: 9 a. m., found dead. *Autopsy*: Liver was congested and contained several coccidiosis nodules; stomach distended with rather dry food mass; mucosa exhibited mild catarrhal inflammation; mucosa of intestines also slightly inflamed.

Rabbit 249. Belgian hare, female. Weight, 1,185 grams. Diet, oats.

November 11: Urine, 5 cc, from bladder acid to litmus, no sugar, no albumin; 11.50 a. m., 14 cc 2 per cent caffein (236 mg per kilo) administered subcutaneously; 3.45 p. m., reflexes increased, hyperæsthesia marked, but no tetanus, even when handled; 30 cc urine collected at 4 p. m., reduction of Fehling's solution considerable.

November 12: 10 a. m., 8 cc urine collected, reduction heavy, only a few cubic centimeters obtained from bladder, did not contain any sugar, general condition of rabbit good, no symptom of caffein intoxication.

Rabbit 321. Yellow, female. Weight, 1,135 grams. Diet, oats.

March 16, 1910: 11.50 a. m., 14 cc 2 per cent caffein (246 mg per kilo) injected subcutaneously in the back; 2 p. m., reflexes increased, is very sensitive, started to run when put on floor, no handling except what was required for removal and return to cage, feces soft.

March 17: 9.30 a. m., condition good, rabbit put on floor, gait normal, but does not care to walk.

March 18: 9 a. m., walks around when put on floor, appetite good, condition seems to be normal.

March 25: 11 a. m., rabbit still alive, condition good.

Rabbit 250. Belgian hare, female. Weight, 1,435 grams. Diet, oats at least two days before the

experiment.

November 11: 11 a. m., urine obtained from bladder acid to litmus, no albumin, no sugar; 11.10 a. m., 18 cc, 2 per cent caffein (252 mg per kilo); 3.45 p. m., reflexes and hyperæsthesia, no tetanus; 4 p. m., 60 cc urine, marked reduction of Fehling's solution.

November 12: 10 a. m., condition of rabbit good, no symptoms of caffein intoxication, 80 cc urine collected, sugar considerable, only a few cubic centimeters of urine obtained from bladder, no reduction of Fehling's solution.

Rabbit 834. Belgian hare, female. Weight, 1,270 grams. Diet, carrots, March 31 to April 7.

April 7: 9.30 a. m., 15 cc 2 per cent caffein (240 mg per kilo) injected subcutaneously in the back; 10.30 a. m., reflexes much increased, rabbit extremely sensitive.

April 8: 9 a. m., condition good, no symptoms.

Rabbit 233. White, male. Weight, 1,675 grams. Diet, carrots, November 2 to 9.

November 9: 10.50 a. m., 20 cc 2 per cent caffein (238 mg per kilo) injected subcutaneously, no symptoms observed until 5 p. m., when increased reflexes and hyperæsthesia were noticed, but no tetanus.

November 10: 9 a. m., paralysis of posterior extremities; died at 1 p. m.

Analysis of the results obtained in the experiments of this series and inspection of Table I, page 25, show that all four of the rabbits which survived doses of 236 to 252 mg of caffein per kilo were Belgian hares. Of the four which died one only was a Belgian hare. The other three were white rabbits. Two of these were fed oats; the other two received carrots during seven days preceding the administration of caffein. This diet does not seem to be a factor, therefore, in the toxicity of caffein. Moreover, it may be observed that rabbit No. 122, which was fed oats, died after receiving 250 mg per kilo, while rabbit No. 250 received the same diet and survived the same dose of caffein per kilo.

Experiments 234 and 334 offer another illustration that the toxicity of caffein is not dependent upon diet, since both rabbits were fed carrots, but the same dose of caffein caused only symptoms in one while it proved fatal to the other. It is evident, therefore, that the difference in resistance to caffein shown in these experiments is in all probability due to race, the Belgian hare being more resistant to caffein than rabbits of other varieties. Rabbit No. 335 seems to be an exception, but the post-mortem examination showed the presence of coccidiosis of the liver. As will be shown later, wherever this condition prevailed even smaller doses of caffein proved fatal.

Series D.

To obtain additional evidence regarding the resistance of the various races of rabbits to caffein and to ascertain the smallest dose which is surely fatal to the gray rabbit or Belgian hare was the object of this series of experiments. The diet in all cases consisted of oats, which was given ad libitum excepting to rabbit No. 235, which received carrots for one week previous to the injection of caffein. The doses administered ranged from 267 to 300 mg per kilo and were administered to different varieties of adult rabbits.

Rabbit 253. Brown and black, male. Weight, 1,600 grams. Diet, oats, November 9 to 12.

November 12: 11.30 a. m., urine from bladder acid, no albumen, no sugar; 11.35 a. m., 22 cc 2 per cent caffein (275 mg per kilo) injected subcutaneously; 11.45 a. m., rabbit jumped, off the table, had convulsions, retraction of head and opisthotonos, general tremors, anterior extremities stretched out, posterior extremities almost normal, frequent twitchings; died at 12.15 p. m.

Rabbit 252. Black, female. Weight, 1,335 grams. Diet, oats, November 9 to 12.

November 12: 11.30 a. m., 18 cc 2 per cent caffein (270 mg per kilo) injected subcutaneously. Urine obtained from bladder before injection, acid, no albumen, no sugar, color normal, tremors and great excitement noticed about 12 noon; 4.30 p. m., when handled, showed unusual restlessness and excitement followed by convulsions with opisthotonos; occasional twitching, condition bad. Died 4.35 p. m.

Rabbit 327. White, female. Weight, 820 grams. Diet, oats, March 8 to 16.

March 16: 11.45 a. m., 12 cc 2 per cent caffein (292 mg per kilo) injected subcutaneously in the back; 2 p. m., found dead, but was still warm. *Autopsy*: Hemorrhagic area at point of injection into spinal muscles; subcutaneous abdominal region exhibited a large area of cheesy purulent material; liver and spleen were engorged; bladder filled; intestines normal.

Rabbit 340. White and brown male. Weight, 1,465 grams. Diet, oats.

March 30: 3.20 p. m., 20 cc of 2 per cent caffein (273 mg per kilo) injected subcutaneously in back.

March 31: 9 a. m., found dead.

Rabbit 341. White and brown. Weight, 1,450 grams. Diet, oats.

March 30: 3.20 p. m., 20 cc 2 per cent caffein (270 mg per kilo) injected subcutaneously in back;

4.40 p. m., found in dying condition, had convulsions; 4.45 p. m., dead.

Rabbit 326. White, male. Weight, 1,645 grams. Diet, oats, March 8 to 16.

March 16, 1910: 12 noon, 20 cc 2 per cent caffein (243 mg per kilo) injected subcutaneously in the back; 2 p. m., tremors marked, hypersensitive, started to run when put on floor; rabbit was not handled any more than was required for his removal from and return to cage.

March 17: 9.30 a. m., tremors still present and marked, otherwise general condition good; no other symptoms.

March 18: 9.30 a. m., no appetite, tremors still present, general condition poor; died about 2 p. m.

Rabbit 235. Belgian hare, male. Weight, 1,870 grams. Diet, carrots, November 2 to 9.

November 10: 11.05 a. m., 25 cc 2 per cent caffein (267 mg per kilo) injected subcutaneously; reflexes increased and tremors, but no tetanus observed; found dead next morning.

Rabbit 316. Belgian hare, female. Weight, 860 grams. Diet, oats, March 8 to 16.

March 16, 1910: 11.40 a. m., 12 cc 2 per cent caffein (267 mg per kilo) injected subcutaneously in the back; 2.15 p. m., reflexes somewhat increased, but not markedly so; walked when put on floor; gait clumsy and slow; tremors of head observed; 2.35 p. m., rabbit lying in his cage, posterior extremities extended and rigid, anterior extremities flexed, head retracted; is still breathing; occasional spasms observed. Rabbit died at 3 p. m. *Autopsy*: No lesion at point of injection in dorsal spinal muscles; liver and spleen engorged; intestines injected; other organs apparently normal.

Rabbit 395. Belgian hare, male. Weight, 1,410 grams.

August 18: 1 p. m., 20 cc 2 per cent caffein (283 mg per kilo) injected subcutaneously in the back; 4 p. m., reflexes markedly increased; 5 p. m., reflexes about the same, but no tetanus.

August 19, 9.15 a. m.: Reflexes increased markedly.

August 21, weight, 1,215 grams. Given 275 mg per kilo of caffein; no symptoms observed.

August 23, found dead. *Autopsy*: Liver greatly engorged; stomach fairly well distended and mucous membrane in a slightly inflammatory condition; contents of small intestine liquid in nature, but walls of same appeared normal; other organs normal in appearance.

Rabbit 396. Belgian hare, female. Weight, 1,475 grams. Diet, oats.

August 18: 1 p. m., 20 cc 2 per cent caffein (272 mg per kilo) injected subcutaneously in the back; 4 p. m., reflexes increased markedly; 5 p. m., reflexes increased markedly but no tetanus.

August 19: 10.30 a. m., reflexes still increased very markedly; rabbit jumps when touched.

August 21: Weight, 1,245 grams. Injected subcutaneously 275 mg of caffein per kilo; reflexes increased, posterior extremities stiff over hour later.

August 22: 9 a. m., found dead. *Autopsy*: Thoracic organs normal in appearance; stomach distended and mucous membrane affected with a catarrhal inflammation; contents of stomach were covered with a shiny mucus; contents of small intestine liquid in nature and bile stained; liver showed a coccidial infestation; kidneys and spleen normal in appearance.

Rabbit 397. Belgian hare, male. Weight, 1,375 grams. Diet, oats.

August 19: 10.30 a. m., 20 cc 2 per cent caffein (290 mg per kilo) injected subcutaneously in the back.

August 22: 9 a. m., found dead. *Autopsy*: Stomach distended with ingesta; mucous membrane exhibited a catarrhal inflammation with excessive secretions; major portion of intestines showed a condition similar to that of stomach, contents consisting mainly of a shiny mucus; liver enlarged; other organs apparently normal.

Rabbit 398, Belgian hare, female. Weight, 1,570 grams. Diet, oats.

August 19: 10.30 a. m., 23 cc 2 per cent caffein (293 mg per kilo) injected subcutaneously in the back; 4 p. m., found dead. *Autopsy*: Thoracic organs seemingly normal; mucous membrane of stomach exhibited a catarrhal inflammation generally; large intestines somewhat impacted but walls appeared normal; other organs normal.

Rabbit 399, Belgian hare, male. Weight, 1,725 grams. Diet, oats.

August 19: 10.30 a. m., 26 cc 2 per cent caffein (300 mg per kilo) injected subcutaneously in the back; found dead at 4.30 p. m. *Autopsy*: Lungs slightly congested; liver engorged and friable; gall cyst well filled; stomach exhibited catarrhal gastritis; injection of mesenteries and intestines; kidney showed marked cortical congestion.

The results of the experiments of this series likewise indicate that the Belgian hare is more resistant to caffein than the rabbits of other varieties. Thus, of the four gray rabbits (Nos. 235, 316, 395, and 396), which received 267 to 283 mg of caffein per kilo, two died and two lived,[B]

one of which, 396, showed the presence of coccidiosis of the liver. On the other hand it will be observed that the black and white rabbits which received from 270 to 275 mg of caffein per kilo all died from the effects of the drug; one within 1 hour and 25 minutes and another within 50 hours after the administration of the caffein, while No. 340 died in the night. Furthermore it will be noted that of the last three rabbits of this series, which were Belgian hares and received 290, 293, and 300 mg of caffein, two died six hours after the injection, while the other, No. 397, lived three days. The minimum fatal dose of caffein for Belgian hares is, therefore, about 290 to 300 mg per kilo when injected subcutaneously, which is about 50 per cent greater than for rabbits of other varieties.

SERIES E.

It was shown in series A that 0.15 caffein per kilo caused symptoms of intoxication. Before concluding, however, that this is the smallest dose which causes symptoms of poisoning, a number of experiments were performed with smaller doses. It was found that in the great majority of cases 0.1 caffein per kilo may cause diuresis, but no nervous or muscular symptoms. In some rabbits, however, even such a dose proved fatal. Post-mortem examinations in these cases showed the presence of coccidiosis of the liver, and it will be recalled that similar observations were made before. It is quite possible, therefore, that coccidiosis of the liver is an important factor in decreasing the resistance to caffein. Experiment 551 (p. 25) shows that other conditions may likewise increase the toxicity of caffein.

Rabbit 325. White, female. Weight, 1,065 grams. Diet, oats.

March 17: 11 a. m., 6 cc 2 per cent (112 mg per kilo) caffein injected subcutaneously in the back. About 5 cc of urine squeezed out from bladder before injecting caffein.

March 17: 1 p. m., hind legs crossed and stretched out, front legs also extended; rabbit lying stretched out on her belly.

March 17: 5.40 p. m., rabbit still alive, condition somewhat improved.

March 18: 9 a. m., found dead, stiff and cold. *Autopsy*: Hemorrhagic area at point of inoculation; subcutaneous region of both thighs presented a hemorrhagic infiltration of the tissues; liver contained lesions of coccidiosis; other organs apparently normal.

Rabbit 330. Belgian hare, female. Weight, 935 grams; poorly nourished.

March 18: 3.35 p. m., 5 cc 2 per cent caffein (107 mg per kilo) injected into subcutaneous tissues in the back; 5.30 p. m., no symptoms.

March 19: 9 a. m., no symptoms.

March 25: Weight, 825 grams.

Rabbit 329. Belgian hare, male. Weight, 775 grams; poorly nourished. Received March 18.

March 18: 3.30 p. m., 4 cc 2 per cent caffein (103 mg per kilo) injected into subcutaneous tissues in the back; 5.30 p. m., no symptoms.

March 19: 9 a. m., no symptoms.

March 25: Rabbit alive in good condition; weight, 825 grams.

Rabbit 320. Black, male. Weight, 1,040 grams. Diet, oats.

March 17: 11 a. m., 6 cc 2 per cent caffein (115 mg per kilo) injected subcutaneously in the back; only a few drops of urine obtained from bladder before injecting caffein; 1 p. m., rabbit very restless; ran away when placed on floor; cried when touched with a piece of paper; no tremors observed, but rabbit became exhausted and was unable to walk; legs extended out; after running for about a minute dyspnœa was very marked, but rabbit soon raised himself on his legs; 5.40 p. m., rabbit up on his legs.

March 18: 9 a. m., found dead, but still warm. *Autopsy*: Lungs studded with small grayish white nodules, adhesions to costal pleura; probably lesions of coccidiosis; liver studded with coccidiosis nodules. Hemorrhages at point of inoculation.

Rabbit, 551. Gray, female. Weight, January 26, 1,650 grams. Diet, oats; fed 20 cc of 25 per cent alcohol daily from January 26-31.

January 31: Weight, 1,450 grams; 10.20 a. m., temperature 101.6°; 10.45, a. m., temperature 101.6°; received 7 cc 2 per cent caffein subcutaneously into back; 11.15 a. m., convulsions of short duration; raised himself on posterior legs, anterior legs wide apart; 4.10 p. m., looked normal, not hypersensitive; 4.30 p. m., condition seemed to be good.

February 1: 9 a. m., found dead, was alive at 5.30 p. m. of previous day. *Autopsy*: Lesions found involved thoracic cavity mainly; lungs were hepatized and a fibro plastic exudate caused them to adhere to costal pleura; liver engorged and appeared fatty; no marked lesions affecting digestive tract, a slight catarrh of stomach being the only noticeable feature; kidneys and spleen normal.

TABLE 1.—*Subcutaneous injections of caffein—rabbits.*

SERIES A.

No.	Weight.	Caffein per kilo	Appearance of symptoms in	Duration of life.	Diet.	Remarks.
	Grams.	*Mg.*				
332	1,070	158	1 hour 45 minutes	Survived	Oats	Gray.
331	1,170	153	do.	do.	do.	Do.
328	1,200	150	do.	do.	do.	Do.
322	1,065	150	1 hour	do.	do.	White.
217	1,355	147		do.	do.	Do.
219	1,820	153		do.	do.	Maltese.
194	1,490	174		do.	do.	White.
191	1,915	167		do.	do.	Light brown.
			SERIES B, GROUP I.			
95	1,478	210	2 hours 50 minutes	3 hours 10 minutes	Oats	White.
96	1,585	200	1 hour	About 18 hours	do.	Gray white.
112	875	205	30 minutes	do.	do.	Black.
119	1,060	188		do.	do.	Yellow white.
195	1,300	200		3 hours 10 minutes	Carrots	White.
208	1,068	188	2 hours	About 24 hours	do.	Gray.
			SERIES B, GROUP II.			
247	1,295	200	2.5 hours	Survived	Oats	Gray.
248	1,305	200	3 hours	do.	do.	Do.
337	1,040	211	1.5 hours	do.	Carrots	Do.
336	1,045	211	do.	do.	do.	Do.
			SERIES C.			
122	2,060	250	2 hours 55 minutes	1.5 days	Oats	White.
234	1,650	242		About 24 hours	Carrots	Do.
335	1,170	240	1 hour	do.	do.	Gray coccidiosis.
249	1,185	236	4 hours	Survived	Oats	Gray.
321	1,135	246	2 hours 10 minutes	do.	do.	Yellow.
250	1,435	252	4 hours 35 minutes	do.	do.	Gray.
334	1,270	240	1 hour	do.	Carrots	Do.
233	1,675	238	6 hours 10 minutes	26 hours	do.	White.
			SERIES D.			
253	1,600	275	10 minutes	35 minutes	Oats	Brown and black.
252	1,335	270	30 minutes	4 hours 55 minutes	do.	Black.
327	820	292		2 hours 15 minutes	do.	White.
340	1,465	273		About 18 hours	do.	White and brown.
341	1,450	270		1 hour 25 minutes	do.	Do.
326	1,645	243	2 hours	50 hours	do.	White.
235	1,875	267		20 hours	Carrots	Gray.
316	860	267	2 hours 45 minutes	3 hours 20 minutes	Oats	Do.
395	1,410	283	3 hours	Survived		Do.
395	1,215	275	do.	About 2 days	Oats	Do.
396	1,475	272	do.	Survived	do.	Do.
396	1,245	275	1 hour	About 18 hours	do.	Do.
397	1,375	290		3 days	do.	Do.
398	1,570	293		5.5 hours	do.	Do.
399	1,725	300		6 hours	do.	Do.
			SERIES E.			
325	1,065	112	2 hours	Less than 22 hours	Oats	White female.
330	935	107	None	Survived		Gray.
329	775	103	do.	do.		Gray male.
320	1,040	115	2 hours	46 hours	Oats	Black male.
551	1,450	100	30 minutes	Less than 24 hours	do.	Gray female.

ADMINISTRATION BY MOUTH.

These experiments were carried out on two varieties of rabbits, the white and the gray. The diet consisted chiefly of oats, but in a few cases carrots formed the exclusive diet. Food and water were given ad libitum. A 2 per cent solution of caffein was administered through a stomach tube. Since the resistance to most drugs is commonly supposed to be greater when given by mouth than when administered by any other path, doses of 175 to 200 mg per kilo were fed in a series of preliminary experiments, all of which were performed on gray rabbits weighing from 865 to 1,135 grams, and which were fed carrots for several days previous to the experiment. Three of the rabbits survived, two without showing any symptoms; in the other case paralysis of the posterior extremities was observed five hours after he received caffein and he was found dead the next morning. Unfortunately no autopsy was performed. The low resistance to caffein of this animal was probably due to some abnormal condition which developed about the time of the experiment, since this rabbit received 325 mg of caffein per kilo two weeks previously and increased reflexes only were observed as a result of this treatment. Hence 200 mg of caffein per kilo can not be considered the toxic dose when fed by mouth. In the following experiments larger doses were therefore given.

Series A.

Rabbit 248. Belgian hare. Weight, 1,170 grams. Diet, oats.

November 17: 1.20 p. m., 19.5 cc 2 per cent caffein (330 mg per kilo) administered by the mouth; 4.30 p. m., somewhat hypersensitive.

November 19: No symptoms; at 9 a. m., urine collected, no reduction of Fehling's solution; rabbit survived.

Rabbit 241. White male. Weight, 1,380 grams. Diet, oats.

November 17: 1.15 p. m., 20 cc 2 per cent caffein (290 mg per kilo) administered by the mouth; 4.30 p. m., some hypersensitiveness, but no other symptoms.

November 18: 9 a. m., urine collected, no reduction of Fehling's solution; no symptoms; rabbit survived.

Rabbit 249. Belgian hare. Weight, 890 grams. Diet, oats.

November 17: 1.30 p. m., 14.5 cc 2 per cent caffein (325 mg per kilo) administered; 4.30 p. m., hypersensitiveness; no other symptoms.

November 18: 10 a. m., no symptoms; urine collected, no reduction; rabbit survived.

Series B.

The object of these experiments was to determine the minimum fatal dose of caffein in the two varieties of rabbits, the white and the gray. All of the animals selected were approximately of the same weight.

Rabbit 239. Belgian hare, male. Weight, 935 grams. Diet, oats.

November 19: 4 p. m., 17 cc 2 per cent caffein (363 mg per kilo) administered by mouth, followed by 10 cc of 0.9 per cent salt solution.

November 20: Urine examined, no sugar found, no symptom noticed at any time after injection.

Rabbit 254. Belgian hare, female. Weight, 975 grams. Diet, oats.

November 19: 4.05 p. m., 18 cc 2 per cent caffein (369 mg per kilo) administered by mouth, followed by 10 cc of 0.9 per cent salt solution.

November 20: 9 a. m., rabbit found dead.

Rabbit 267. White. Weight, 1,050 grams. Diet, oats.

November 23: 12.10 p. m., 18 cc 2 per cent caffein (342 mg per kilo) given by mouth, followed by 18 cc salt solution; 1 p. m., increased reflexes, tremors marked but no tetanus; 1.05 p. m., rabbit stretched on abdomen, posterior extremities in extended position and paralyzed, soon after clonic spasms set in, which recurred about every minute; 1.14 p. m., tetanus and death. *Autopsy*: Liver showed fatty degeneration; slight inflammation of stomach and intestines; other organs normal.

Rabbit 268. White. Weight 1,100 grams. Diet, oats.

November 23: 20 cc 2 per cent caffein (363 mg per kilo) administered by mouth, followed by 20 cc salt solution; 1.15 p. m., somewhat hypersensitive; 4.30 p. m., tremors fairly marked, no urine passed, about 2 cc of bloody looking urine obtained from bladder, which contained albumen and a considerable amount of glycogen; rabbit died.

Rabbit 419, Belgian hare, male. Weight, 1,600 grams. Diet, oats.

September 26: 10 a. m., 28 cc 2 per cent caffein (350 mg per kilo) given by mouth; reflexes increased at 4 p. m.; 6 p. m., reflexes still increased, no other symptoms.

September 27: 9 a. m., found dead. *Autopsy*: Lungs, liver, and kidneys congested; other organs

normal.

Rabbit 420. Belgian hare, male. Weight, 1,250 grams. Diet, oats.

September 26: 10 a. m., 22 cc 2 per cent caffein (352 mg per kilo) given by mouth; 11.35 a. m., convulsions; 12 noon, found dead. *Autopsy*: Liver showed very extensive coccidiosis; no other lesions.

Rabbit 421. Belgian hare, male. Weight, 1,485 grams. Diet, oats.

September 26: 10 a. m., 26 cc 2 per cent caffein (351 mg per kilo) administered by mouth; 4 p. m., reflexes increased; 6 p. m., reflexes as before, no tetanus observed.

September 27: 9 a. m., rabbit found dead. *Autopsy*: Congestion of lungs and kidneys; liver congested and slightly fatty.

Rabbit 424. White, male. Weight, 1,295 grams. Diet, oats.

September 26: 2 p. m., 19 cc 2 per cent caffein (293 mg per kilo) administered by mouth; 4 p. m., reflexes increased, no other symptoms; 6 p. m., no change since 4 p. m.

September 27: 12 noon, convulsions and death. *Autopsy*: Congestion of the lungs; no other lesions.

Rabbit 423. White, male. Weight, 1,205 grams. Diet, oats.

September 26: 2 p. m., 18 cc 2 per cent caffein administered by mouth; 4 p. m., reflexes increased, no tetanus; 6 p. m., condition unchanged since 4 p. m.

September 27: 9 a. m., found dead. *Autopsy*: Lungs, liver, and kidneys congested; other organs normal.

Rabbit 422. White, male. Weight, 1,440 grams. Diet, oats.

September 26: 2 p. m., 21 cc 2 per cent caffein (291 mg per kilo) given by mouth; reflexes increased at 4 p. m.

September 27: 3 p. m., alive, no symptoms; 4 p. m., convulsions with recovery, this was soon followed by a violent attack of tetanus, which lasted about one minute and was succeeded by paralysis; rabbit died at 4.30. *Autopsy*: Liver slightly congested; a small portion of the intestine showed congestion and edema; other organs normal.

A study of these experiments shows also considerable variation in the toxicity of caffein when given by mouth. In some cases a dose of 300 mg per kilo, and even less, caused death, as in rabbits 423 and 424. In other rabbits, however, approximately the same doses of caffein produced increased reflexes only. The same symptoms were produced in Nos. 248 and 249 after the administration of 325-330 mg of caffein per kilo, while another rabbit (No. 239) survived a dose of 363 mg per kilo. That this is exceptional, however, appears from the result of the following experiments on rabbits Nos. 419, 420, and 421, all of which died after receiving 350 mg of caffein per kilo, and rabbits 267 and 268, to which doses of 363 and 342 mg, respectively, per kilo proved fatal. It will be observed further that the gray rabbits are more resistant to caffein than the white animals, as 350 mg per kilo was the smallest fatal dose for rabbits 419, 420, and 421, all of which were gray rabbits, while a dose of 290 mg per kilo was fatal for some of the white rabbits. Again, it will be noticed that of the two gray rabbits, Nos. 254 and 239, which received the largest doses in these experiments, namely, 369 and 363 mg, respectively, one survived. The largest doses given to the white rabbits were 363 and 342 mg caffein per kilo. Both of these died from the effects of the drug. It may be concluded, therefore, that the minimum toxic dose for the gray rabbit is about 325 mg of caffein per kilo, and the minimum fatal dose is at least 350 mg per kilo. It is to be remarked in this connection that post-mortem examination showed extensive coccidiosis in rabbit 420 and fatty liver in No. 421, while the macroscopical examination of the organs of Nos. 424 and 423 failed to show the presence of such abnormalities. Since, as was observed in the section on subcutaneous injection and elsewhere in this investigation, pathological changes are apt to decrease the resistance to caffein, it is quite possible that 350 mg per kilo is not the minimum fatal dose for the normal rabbit. Indeed, the experiment on rabbit 239 lends support to this view, thus furnishing additional evidence of difference in the resistance to caffein in the two varieties of rabbits.

TABLE 2.—*Administration of caffein by mouth.*

Rabbit No.	Weight.	Caffein per kilo	Symptoms.	Duration of life.	Diet.	Remarks.
	Grams.	*Mg.*				
SERIES A.						
248	1,170	330	3 hours	Survived	Oats	Gray.
241	1,380	290	2 hours	do.	do.	White male.
249	890	325	3 hours	do.	do.	Gray male.
SERIES B.						
239	935	363		Survived	Oats	Gray male.

254	975	369		About 2 days	do.	Gray female.
267	1,050	342	50 minutes	1 hour	do.	White.
268	1,100	363		About 3 hours	do.	Do.
419	1,600	350	6 hours	Less than	do.	Gray male.
420	1,250	352	1 hour	2 hours	do.	Do.
421	1,485	351	6 hours	Less than	do.	Do.
424	1,295	293	2 hours	22 hours	do.	White male.
423	1,205	300	do.	Less than	do.	Do.
422	1,440	291	do.	2½ hours	do.	Do.

INJECTION INTO THE PERITONEAL CAVITY.

In a number of experiments caffein was introduced into the peritoneal cavity. Rabbits of different varieties receiving a diet of oats or carrots were employed for this purpose; food and water were given ad libitum. The minimum doses required to induce symptoms or cause death in these animals were determined; tests with caffein were also made on young rabbits in order to study the influence of age on the resistance to this substance. The results are shown in the following experiments:

Series A.

In this series large doses were administered, approximating 0.3 gram per kilo.

Rabbit 71. Gray female. Weight, 1,659 grams.

January 20: 2.20 p. m., 25 cc aqueous solution 2 per cent caffein (300 mg per kilo) were injected into the peritoneal cavity; 3.45 p. m., when doors of cage were opened rabbit had spasm of short duration.

January 21: Rabbit found dead.

Rabbit 72. Gray and white. Weight, 1,402 grams.

January 21: 11.17 a. m., 20.2 cc (aqueous solution) of 2 per cent caffein (300 mg per kilo) injected into peritoneal cavity from burette; 11.25 a. m., paralysis; 11.30 a. m., rabbit had convulsion when picked up from the floor, followed by several spasms later; 11.35 a. m., typical tetanus; 12.30 noon, found dead.

Rabbit 61. Black female. Weight, 2,143 grams.

January 19: 40 cc 2 per cent caffein, aqueous solution (300 mg per kilo), injected into peritoneal cavity from burette; tetanus when about 30 cc were injected; when removed from holder, repeated and violent convulsions, terminating in death.

Series B.

The object of these experiments was to determine the minimum lethal dose; 0.2 to 0.15 gram of caffein per kilo was injected into the rabbits of this series.

Rabbit 69. White female. Weight, 1,714 grams.

January 20: 10.15 a. m., 6 cc 2 per cent caffein, aqueous solution, injected into peritoneal cavity. No symptoms, under observation for 45 minutes, rabbit defecated rather copiously; feces were soft; 11 a. m., 6 cc 2 per cent caffein, aqueous solution, injected into peritoneal cavity, no symptoms, under observation for 40 minutes; 11.40, 6 cc 2 per cent caffein injected into peritoneal cavity; 11.45, rabbit is restless, reflexes increased.

Rabbit 70. Gray and white female. Weight, 1,487 grams.

January 20: 1.30 p. m., 15 cc 2 per cent aqueous solution of caffein (0.2 gram per kilo) injected into the peritoneal cavity; 2.20 p. m., no symptoms.

January 30: About 2 p. m. rabbit died.

February 1: *Autopsy*: Cirrhosis of the liver; enteritis of small intestines; stomach and kidneys normal.

Rabbit 93. Maltese, male. Weight, 1,197 grams.

March 2: 11.30 a. m., 12 cc of 2 per cent caffein (200 mg per kilo) injected into peritoneal cavity; 11.35 a. m., while being released from holder, tetanus occurred, repeated attacks later, clonic convulsions with tonic rigidity of posterior extremities during the attacks as well as during intervals, anterior extremities were relaxed during the intervals between the attacks, opisthotonos of cervical region but kyphosis in lumbar region were observed, no salivation nor dilatation of the pupils; 2 p. m., rabbit died.

Rabbit 92. Yellow female. Weight, 1,388 grams.

February 25, 4.15 p. m., 14 cc 2 per cent caffein (0.2 gram per kilo) injected into peritoneal cavity; 4.20 p. m., restlessness and increased reflexes, rabbit found stretched out in cage, but raised himself on his legs again; 4.45, general tremor when touched.

February 26: 9 a. m., rabbit found dead. *Autopsy*: Hemorrhage into abdominal muscles at site of injection; hemorrhage also in walls of stomach opposite similar spot in abdominal wall.

Rabbit 309. Belgian hare, female. Weight, 1,500 grams. Diet, oats.

March 2: 2.05 p. m., 2 per cent caffein solution (0.2 gram per kilo) injected into peritoneal cavity; 2.25 p. m., found dead, no urine found in bladder.

Rabbit 307. Belgian hare, female. Weight, 1,320 grams. Diet, oats.

March 2: 12 noon, urine obtained from bladder, clear amber colored, no albumin, no reduction; 12.06 p. m., 10 cc of 2 per cent caffein (0.151 gram per kilo) injected into peritoneal cavity; 1.30 p. m., rabbit placed on floor, runs around but anterior and posterior extremities soon extended, in tonic condition; 2.25 p. m., reflexes increased, paralysis of extremities, dyspnœa; 4.50 p. m., about 100 cc urine collected, no albumin, reduction of Fehling's solution moderate.

March 3: 9.30 a. m., posterior extremities extended and rigid, anterior extremities paralyzed, respiration less frequent and deeper than normal. Rabbit died at 11.50 a. m.; urine collected since 4.50 p. m. previous day gave very heavy reduction of Fehling's solution. *Autopsy*: Animal in good condition; in the left axillary region was observed a hemorrhage into the subcutaneous and muscular tissue of that region. The ventral portion of the large colon, in contact with the ventral abdominal wall, showed a hemorrhagic area about one-half inch in length, such as might be produced by a puncture or bruise of the colon through the abdominal wall. A small portion of the small intestine adjacent to the colon was affected in a similar manner. All internal organs were apparently normal.

Rabbit 308. Belgian hare, female. Weight, 1,350 grams. Diet, oats.

March 2: 11.45 a. m., urine obtained from bladder, no albumen, no reduction; 11.50 a. m., 10 cc 2 per cent caffein (0.15 gram per kilo) injected into peritoneal cavity; 1.30 p. m., not very active, no abnormal symptoms otherwise; 3.30 p. m., rabbit looked depressed, made very little attempt to move about, remained in one position most of the time when placed on floor; 4.30 p. m., 180 cc urine collected, no albumen, reduction of Fehling's solution moderate.

March 3: 9.30 a. m., rabbit looks normal, is able to walk but is easily fatigued when made to walk about or when placed on his side, followed by paralysis of anterior extremities, posterior extremities apparently normal, about 90 cc of urine collected at noon was free from albumen, did not reduce Fehling's solution.

March 4: 11 a. m., lying on his side in cage, anterior extremities limp, posterior extremities extended and rigid, is in dying condition.

March 5: 9 a. m., found dead. *Autopsy*: Liver engorged; spleen congested, but not enlarged; kidneys, some congestion in cortex; stomach filled, mucosa thickened and easily pulled off; petechial hemorrhages on serosa of colon.

Series C.

The experiments of this series were made to determine the minimum toxic dose.

Rabbit 295. Belgian hare, female. Weight, 1,205 grams. Diet, carrots.

March 1: 10.40 a. m., 6 cc 2 per cent caffein (0.1 gram per kilo) injected into peritoneal cavity; about 2 cc of urine obtained before injecting caffein; 2 p. m, 100 cc urine, bloody in appearance, collected, a moderate quantity of albumen present, no reduction; 3.40 p. m., no symptoms.

March 4: 2 p. m., rabbit looks well.

Rabbit 293. Belgian hare, female. Weight, 1,605 grams. Diet, carrots.

March 1: Urine from bladder clear, alkaline; 11.55 a. m., 8 cc 2 per cent caffein (0.1 gram per kilo) injected into peritoneal cavity; 3 p. m., 90 cc urine normal in color collected, no albumen, no reduction; 3.40 p. m., no symptoms.

March 4: 1.15 p. m., rabbit looks normal.

Rabbit 292. Belgian hare, male. Weight, 1,595 grams. Diet, carrots.

March 1: 10.10 a. m., 8 cc 2 per cent caffein (0.1 gram per kilo) solution injected into peritoneal cavity; 10.40 a. m., rabbit urinated, reflexes increased, but no other symptoms; 10.50 a. m., no urine obtained from bladder; 2 p. m., 105 cc of clear pale urine collected; no albumen, no reduction; 3.40 p. m., no symptoms.

March 4: 2 p. m., rabbit looks well, urine collected, did not contain sugar.

Rabbit 298. Belgian hare, female. Weight, 1,205 grams. Diet, carrots.

March 1: 4.06 p. m., 7.5 cc 2 per cent caffein solution (0.125 gram per kilo) injected into peritoneal cavity, urine obtained from bladder immediately after injection, no albumen, no

reduction; 5.30 p. m., reflexes increased, rabbit was able to run around, but became paralyzed soon; 5.40 p. m., rabbit is again able to run around.

March 3: 10 a. m., anterior extremities paralyzed, is able to use posterior extremities.

March 4: 1 p. m., rabbit looks normal.

Rabbit 223. Belgian hare, male. Weight, 1,165 grams. Diet, carrots.

March 1: 3.50 p. m., urine obtained from bladder clear, amber colored, no albumen, no sugar; 3.52 p. m., 7.5 cc 2 per cent caffein (125 mg per kilo) injected into peritoneal cavity; 5.40 p. m., rabbit makes little attempt to run when put on the floor, weakness of extremities marked.

March 4: 1.15 p. m., rabbit normal.

SERIES D.

The object of the experiments of this series was to study the effect of age on the resistance to caffein. Half-grown rabbits were, therefore, used in the following experiments.

Rabbit 310. Belgian hare, female. Weight, 880 grams. Diet, oats.

March 2: 3.25 p. m., 9 cc 2 per cent caffein (0.2 gram per kilo) injected into peritoneal cavity.

March 3: 9.30 a. m., no symptoms, rabbit looks normal.

March 4: 11 a. m., posterior extremities abducted, walked when placed on the floor, made no attempt to change attitude when placed on its side, remained some time in this position.

March 5: 9 a. m., found dead. *Autopsy*: Liver showed areas of degeneration; kidneys congestion and petechial hemorrhage on cortex; small and large intestines, inflammation marked; bladder distended.

Rabbit 75. Gray and white, female. Weight, 842 grams.

January 25: 3 p. m., 8.5 cc 2 per cent caffein solution (0.2 gram per kilo) injected into peritoneal cavity; 3.15 p. m., anterior extremities weak and reflexes increased.

January 27: Rabbit paralyzed but is able to turn over when placed on back.

Rabbit 74. Gray and white, female. Weight, 692 grams.

January 25: 3 p. m., 7 cc 2 per cent caffein (0.2 gram per kilo) solution injected into peritoneal cavity; 3.15 p. m., reflexes increased and anterior extremities paralyzed.

January 27: Rabbit recovered and is able to walk about in the room.

Rabbit 312, maltese, female. Weight, 740 grams. Diet, oats.

March 3: 11.47 a. m., urine obtained from bladder, appearance normal, no albumen, no reduction of Fehling's solution; 11.50 a. m., 7.5 cc 2 per cent caffein (0.2 gram per kilo) injected into peritoneal cavity; 2.30 p. m. anterior extremities paralyzed, posterior extremities rigid and extended; 5 p. m. (about), rabbit died.

Rabbit 311. Belgian hare, female. Weight, 650 grams. Diet, oats.

March 3: 11.26 a. m., urine obtained from bladder normal in appearance, albumen considerable, reduction of Fehling's solution none; 11.27 a. m., 6 cc 2 per cent caffein solution (0.2 gram per kilo) injected into peritoneal cavity; 2.30 p. m., rabbit seemed to be normal, no symptoms had developed; urine collected contained a large amount of sugar, reduction was very heavy, but no albumen was found.

March 4: 11 a. m., condition good, moves about when put on floor; gait, normal.

Rabbit 78. Yellow and white. Weight 659 grams.

January 26: 1.30 p. m., 8.5 cc 2 per cent caffein (250 mg per kilo) injected into peritoneal cavity, under observation the rest of the afternoon, no symptoms.

January 27: 4 p. m., no symptoms developed.

Rabbit 317. Belgian hare, female. Weight 635 grams. Diet oats.

March 15: 10.35 a. m., 8 cc 2 per cent caffein (0.252 gram per kilo) injected into peritoneal cavity; 12 noon, marked abduction of hind legs, was unable to walk after a little exertion, rabbit died between 12.30 and 12.50 p. m. *Autopsy*. Right lung hepatized and showed adhesions to costal and mediastinal pleura; liver studded with nodules of coccidiosis; spleen congested; stomach filled, mucosa normal; intestines injected; colon hemorrhagic on serosa in ventral region, near point of injection; kidneys normal.

Rabbit 323. White, female. Weight 820 grams. Diet oats.

March 15: 10.45 a. m., 10 cc 2 per cent caffein (250 mg per kilo) injected into peritoneal cavity; 12 noon, reflexes increased, hind legs abducted but is able to walk, symptoms are mild; 1.40 p. m., tremors, weakness, and abduction of head and legs much more marked than at 12 noon.

March 16: Condition good.

March 17: Condition good, recovery apparently complete.

Since the experiments of Series A, which were intended as preliminary tests, have shown that 0.3 gram of caffein per kilo when introduced into the peritoneal cavity is rapidly absorbed and is fatal, much smaller doses were employed in subsequent trials with the drug. This is shown in series B, which may be divided into two groups. Group I, consisting of rabbits 69, 70, 92, 93, and 309, which received 0.2 gram of caffein per kilo, and Group II, Nos. 307 and 308, into which 0.15 gram of caffein per kilo was injected. Three rabbits of Group I (Nos. 92, 93, 309) died from the effects of caffein; rabbit 309 twenty minutes after injection, and rabbits Nos. 92 and 93, twenty hours and two and one-half hours, respectively, after the administration of caffein. In both of these rabbits symptoms appeared within five minutes after the injections were made. Rabbits 69 and 70, it will be noticed, survived the same amount of caffein in proportion to body weight as was given to the other members of this group. Increased peristalsis and the distribution of the dose may account for the greater resistance of rabbit No. 69. The case of rabbit No. 70 is evidently one of exceptional resistance to caffein, since both the rabbits of Group II died from the effects of a much smaller dose, namely, 0.15 gram of caffein per kilo. Moreover, macroscopical examination at the autopsy of Nos. 307 and 308 failed to show any lesions which might tend to lessen the resistance to caffein.

That a dose of 0.15 gram per kilo is therefore in all probability the minimum fatal dose for the rabbit when injected into the peritoneal cavity appears from the results of the experiments in series C, in which smaller doses, 0.125 gram of caffein per kilo caused mild symptoms only, while 0.1 gram per kilo rarely induced any symptoms. It may be remarked that the rabbits of series C were fed carrots while rabbits Nos. 307 and 308 received oats. Their resistance to caffein may be different, but, as was pointed out in the earlier part of this investigation, diet does not seem to influence the toxicity of the single dose of caffein. Doses of 150 and of 100 to 125 mg per kilo, when injected into the peritoneal cavity, may be considered, respectively, as the minimum fatal and minimum toxic doses for the gray rabbit. Analysis of the experiments in series D shows much greater resistance to caffein than in the other rabbits which received it intraperitoneally. Thus, after the administration of 0.2 gram per kilo to each of five rabbits, no effect was observed in two cases (Nos. 310, 311), while in two others (Nos. 74, 75) symptoms developed, but they survived. Only one rabbit, No. 312, died from the effects of this dose; the autopsy showed the presence of degeneration of the liver and petechial hemorrhages on the cortex of the kidneys in the case of No. 310, which was probably the cause of death rather than the caffein.

Two decigrams of caffein can not be considered, therefore, the fatal dose for rabbits. This is further corroborated by the results obtained in experiments with larger doses. Rabbit 78, which received 257 mg per kilo, failed to show any symptoms. The same amount in proportion to body weight in No. 323 caused mild symptoms only, while the rapid death of rabbit No. 317 after the same dose of caffein may be explained by the lesion found at autopsy, thus affording additional evidence that disease may decrease the resistance to caffein. It will be observed that all the members of this series were young rabbits and, as will be shown later, young animals of other species are likewise more resistant to caffein than adult animals. Similar results were obtained by von Anrep, who observed that atropin is less toxic in young than in full-grown animals.

Observations were also made on the diuretic effect of caffein when injected into the peritoneal cavity. The results shown in the following table indicates the stimulating effect on renal secretion whether the diet consisted of oats or of carrots. The urine of some rabbits contained moderate amounts of sugar after from 0.2 to 0.15 gram of caffein per kilo was given; albumen was observed in one case, but in none of the others. In rabbit No. 311 albumin was found before the injection of caffein, but none in the urine which was collected three hours after caffein was injected.

Effect of caffein on renal secretion.

No.	Weight.	Caffein per kilo.	Urine.	Time.	Diet.
	Grams.	*Gram.*	cc.		
307	1,320	0.150	100	4.5 hours	Oats.
308	1,305	.150	180	do.	Do.
295	1,205	.100	100	2 hours 20 minutes	Carrots.
293	1,605	.100	90	3 hours	Do.
292	1,595	.100	105	4 hours	Do.

NOTE.—The amount of urine secreted in three hours by control rabbits, on a carrot diet, varied between 35 and 50 cc, the average weight of the animals being a little above 1,600 grams. The secretion of urine on an oat diet was much less for an equal period of time.

TABLE 3.—*Intraperitoneal injections.*

SERIES A.

No.	Weight.	Caffein per kilo	Time of appearance of symptoms in	Duration of life.	Remarks.
	Grams.	*Gram.*			
71	1,659	0.3	85 minutes	24 hours	Gray.
61	2,143	.3		At the end of	Black.

				injection	
72	1,402	.3	8 minutes	1.25 hour	Gray and white.
			SERIES B, GROUP I.		
70	1,487	0.2		10 days	Gray and white.
93	1,492	.2	5 minutes	2.5 hours	Maltese; given second dose after 3 days, died 2.5 hours later.
69	1,492	.2	About 5 minutes	Survived	White.
92	1,388	.2	5 minutes	24 hours	Yellow.
309	1,500	.2		20 minutes	Belgian; oats.
			SERIES B, GROUP II.		
308	1,350	0.15	3 hours and 40 minutes	About 2.5 days	Belgian; oats.
307	1,320	.15	1 hour 24 minutes	24 hours	Do.
			SERIES C.		
223	1,165	0.125	2 hours	Survived	Belgian; carrots.
293	1,605	.1		do.	Do.
295	1,205	.1		do.	Do.
292	1,595	.1		do.	Do.
298	1,205	.125	1.5 hours	do.	Do.
			SERIES D.		
310	880	0.2	2 days[1]	About 2.5 days[1]	Belgian; oats.
311	650	.2		Survived	Do.
312	740	.2	40 minutes	4.5 hours	Maltese; oats.
78	659	.257	15 minutes	Survived	Yellow and white; oats.
75	842	.2	do.	do.	Gray and white.
74	692	.2	do.	do.	
317	635	.252	1 hour 25 minutes	About 2 hours	Belgian; oats.
323	820	.25	1 hour 15 minutes	Survived	White; oats.

[1] Not due to caffein.

INTRAMUSCULAR INJECTION.

Well-fed rabbits, which received a diet exclusively of oats, were used for these experiments. The injections were made into the lumbar or into the gluteal muscles.

Series A.

In this series the caffein was injected into the gluteal muscles.

Rabbit 284. Brown and white, female. Weight, 1,100 grams.

December 14: 2 p. m., 11 cc 2 per cent caffein injected into the gluteal muscles (0.2 gram per kilo), under observation until 5 p. m., had frequent convulsions; at 5 p. m. in a comatose condition. Rabbit was found dead the next morning.

Rabbit 286, white and black, female. Weight, 1,315 grams.

December 15: 2.30 p. m., 13 cc 2 per cent caffein injected into the gluteal muscles (0.1977 gram per kilo), tremors and increased reflexes observed during the next two hours, but no other symptoms.

December 17: Rabbit alive.

Rabbit 285, yellow and white, female. Weight, 1,385 grams.

December 14: 10.15 a. m., 14 cc 2 per cent caffein injected into the gluteal muscles (0.2 gram per kilo), general tremors, but no convulsions observed. Rabbit survived.

December 17: Rabbit still alive.

Rabbit 287. Belgian hare, female. Weight, 1,140 grams.

December 15: 2.15 p. m., 11 cc of 2 per cent caffein injected into the gluteal muscles; 2.30 p. m., tonic contractions of posterior limbs. Paralysis and death at 2.40 p. m.

Series B.

In series B the caffein was injected into the lumbar muscles.

Rabbit 307. Belgian hare, female. Weight, 1,175 grams.

February 16: 11.05 a. m., 8 cc 2 per cent caffein injected (0.136 gram per kilo) into the lumbar muscles; under observation until 4 p. m., no symptoms; 4 p. m., allowed to walk on the floor; after walking a short distance loss of coordination and paralysis of posterior extremities; 5.20 p. m.,

found dead.

Rabbit 306. Belgian hare, female. Weight, 1,860 grams.

February 16: 11 a. m., 12.5 cc 2 per cent caffein injected into the lumbar muscles; 12 noon, no symptoms; 2 p. m., walked about 10 feet, exhaustion and paralysis; 3 p. m. found dead.

Rabbit 181. Belgian hare. Weight, 1,230 grams. (Was experimented on some time previously.)

February 16: 10.55 a. m., 8 cc 2 per cent caffein injected into the lumbar muscles; (0.130 gram per kilo); 12 noon, no symptoms; 2 p. m., no symptoms; 3 p. m., put on the floor, walked about 10 feet and was exhausted, posterior extremities paralyzed; 4 p. m., found dead.

SERIES C.

In the fall of the same year additional experiments were carried out with doses ranging from 100 to 200 milligrams of caffein per kilo, which were injected into the lumbar muscles. The results are given in the following abbreviated protocols:

Rabbit 425. Belgian hare. Weight 1,520 grams.

September 27: 10.30 a. m., 7.5 cc 2 per cent caffein injected into the lumbar muscles; 2 p. m., reflexes increased.

September 28: Rabbit normal.

October 5: Weight, 1,620 grams; 2.50 p. m., 10 cc 2 per cent caffein injected into lumbar muscles; 3.05 p. m., reflexes increased.

October 13: Weight, 1,520 grams; 10.30 a. m., 10 cc 2 per cent caffein (131 mg per kilo) injected; 11 a. m., no symptoms; 11.30 a. m., reflexes much increased.

October 14: Alive, no symptoms.

Rabbit 426. Belgian hare, female. Weight, 1,425 grams.

September 27: 7 cc 2 per cent caffein injected into the lumbar muscles at 10.30 a. m.; 2 p. m., reflexes increased.

September 28: Rabbit normal.

October 5: Weight, 1,425 grams; 2.55 p. m., 9 cc 2 per cent caffein injected into lumbar muscles; 3.05 p. m., reflexes increased.

October 13: Weight, 1,405 grams; 10.30 a. m., 10 cc 2 per cent caffein (142 mg per kilo) injected; 11 a. m., no symptoms; 11.30 a. m., reflexes increased.

October 14: Rabbit alive, no symptoms.

Rabbit 427. Belgian hare, female. Weight, 1,780 grams.

September 27: 9 cc 2 per cent caffein injected into the lumbar muscles; 2 p. m., reflexes increased.

September 28: Rabbit normal.

October 5: Weight, 1,850 grams; 3 p. m., 11.5 cc 2 per cent caffein injected into lumbar muscles; 3.10 p. m., reflexes increased.

October 13: Weight, 1,830 grams; 10.40 a. m., 14 cc 2 per cent caffein (153 mg per kilo) injected into lumbar muscles; 11 a. m., no symptoms; 11.30 a. m., reflexes increased.

October 14: Rabbit alive, no symptoms.

Rabbit 453. Belgian hare, male. Weight, 1,160 grams.

October 12: 3.45 p. m., 11.5 cc 2 per cent caffein in aqueous solution injected into lumbar muscles; 4.15 p. m., reflexes increased; 4.30 p. m., paralyzed.

October 13: 9 a. m., found dead. *Autopsy*: Gastric mucosa hemorrhagic; liver darkened; other organs normal.

Rabbit 455. Belgian hare, gray, female. Weight, 1,185 grams.

October 12: 3.30 p. m., 11.5 cc 2 per cent caffein injected into the lumbar muscles; 4 p. m., reflexes increased.

October 13: Rabbit weighed 1,070 grams, no symptom of caffein poisoning, reflexes normal; 10.30 a. m., 10 cc 2 per cent caffein injected into the lumbar muscles; 11.30 a. m., jumped off the table, had attack of convulsions and died. *Autopsy*: Findings same as in No. 453.

Rabbit 428. Belgian hare, gray, male. Weight, 1,650 grams.

October 5: 4 p. m., 14.8 cc 2 per cent caffein (0.18 gram per kilo) injected into the lumbar muscles.

October 6: Found dead.

Rabbit 429. Belgian hare, male. Weight, 1,340 grams.

October 5: 4 p. m., 13.5 cc 2 per cent caffein (0.2 gram per kilo) injected into lumbar muscles.

October 8: Rabbit found dead.

SERIES D.

Further experiments making injections into both the lumbar and the gluteal muscles, were made in this series.

Rabbit 577. Gray male. Weight, 1,380 grams.

February 14: 3 p. m. 14 cc 2 per cent caffein injected into the gluteal muscles of the right side; 3.10 p. m., restless, jumped off the table and walked about, reflexes increased; 3.45 p. m., passed 30 cc clear, straw-colored urine; 4.45 p. m., allowed to walk about, ran across the room, about 20 feet, looked tired, stretched himself out on the floor, then raised himself and walked about showing no disturbance of gait.

February 15: 9 a. m., found dead.

Rabbit 578. Gray, female. Weight, 1,670 grams.

February 14: 3.05 p. m., 18 cc 2 per cent caffein solution injected into the gluteal muscles of the right side; 3.15 reflexes increased, but not restless; 5 p. m., allowed to walk about, no symptoms observed.

February 15: Found dead.

Rabbit 579. White and gray, male. Weight, 1,490 grams.

February 14: 3.15 p. m., 15 cc 2 per cent caffein solution injected into the gluteal muscles of the right side; put in cage; 3.30 p. m., reflexes increased; 5 p. m., taken out of cage and allowed to walk across the room, no special symptoms noticed.

February 23: Still alive.

Rabbit 580. Gray male. Weight, 1,510 grams.

February 14: 3.35 p. m., 15 cc 2 per cent caffein solution injected into lumbar muscles.

February 23: Still alive, in good condition.

Rabbit 581. Gray female. Weight, 1,680 grams.

February 14: 3.45 p. m., 17 cc 2 per cent caffein solution injected into the lumbar muscles of the right side; 4 p. m., reflexes increased; 4.15 p. m., jumped off the table and had wild convulsions, became very restless, walked about the laboratory; 4.25 p. m., had convulsions occasionally; 4.30 p. m., extremities extended and quite rigid; 4.35 p. m., convulsions and death.

Rabbit 582. Gray male. Weight, 1,870 grams.

February 14: 4.15 p. m., 18 cc 2 per cent caffein solution injected into the lumbar muscles of right side; 5 p. m., reflexes increased; walked about in the room, then rested; 5.15 p. m., had short spasm when handled.

February 23: Alive; good condition.

The data presented in these experiments show that the toxicity of caffein when injected into the muscles of the lumbar regions is the same as when injected into the gluteal muscles. The rabbits of series A received approximately 0.2 gram caffein per kilo and two died as a result of this treatment. The other two survived but symptoms of caffein intoxication were observed.

In series B smaller doses proved fatal, from which it would appear that caffein is more toxic when injected into the lumbar muscles. Further observations, however, failed to corroborate the results obtained in this series. Thus, in series C, 130 to 150 mg of caffein per kilo injected into the lumbar muscles produced mild symptoms only. Experiments with larger doses showed that 0.180 gram caffein per kilo may cause death. It will be noticed, on the other hand, that rabbit No. 455 survived a dose of 0.2 gram per kilo. New experiments were therefore carried out in which the same amounts of caffein in proportion to the weight of the animals were injected into the lumbar muscles as into the gluteal muscles. As shown in the experiments of series D, one rabbit (No. 581) died shortly after caffein was injected into the lumbar muscles; two recovered. Two of the three which received injections into the gluteal muscles were found dead the next day; one recovered. Post-mortem examination failed to indicate the presence of any abnormalities. The rate of absorption of caffein from the gluteal and from the lumbar muscles seems to be, therefore, the same, or not to differ very much. The observations of Auer and Meltzer[7] are of interest in this connection. According to their investigations adrenalin is more rapidly absorbed from the lumbar than from the gluteal muscles. This is in all probability due to the greater delicacy of the test they employed (since they judged the rate of absorption by the effect of adrenalin on blood pressure) as well as to the much greater activity of the substance.

TABLE 4.—*Intramuscular injections.*

SERIES A.

No.	Weight.	Caffein per kilo	Symptoms after—	Duration of life.	Site of injection.	Remarks.
	Grams.	*Gram.*				
284	1,100	0.200	3 hours	Less than 20 hours	Gluteal	White and brown female.
286	1,315	.1977	2 hours	Survived	do.	White and black female.
285	1,385	.200	Present	do.	do.	Yellow and white female.
287	1,140	.210	15 minutes	25 minutes	do.	Gray female.
			SERIES B.			
307	1,175	0.136	5 hours	6 hours, 20 minutes	Lumbar	Gray female.
306	1,860	.134	3 hours	4 hours	do.	Do.
181	1,230	.130	4 hours	5 hours	do.	Gray.
			SERIES C.			
425	1,520	0.131	1 hour	Survived	Lumbar	Gray.
426	1,405	.142	30 minutes	do.	do.	Gray female.
427	1,830	.153	50 minutes	do.	do.	Do.
453	1,160	.200	30 minutes	Less than 20 hours	do.	Gray male.
455	1,185	.200	do.	Survived	do.	Gray female.
428	1,650	.180		Less than 20 hours	do.	Gray male.
429	1,340	.200		do.	do.	Do.
			SERIES D.			
577	1,380	0.200	10 minutes	Less than 18 hours	Gluteal	Gray male.
578	1,670	.210	do.	do.	do.	Do.
579	1,490	.200	15 minutes	Survived	do.	White and gray male.
580	1,510	.200		do.	Lumbar	Gray male.
581	1,680	.200	15 minutes	50 minutes	do.	Do.
582	1,870	.192	45 minutes	Survived	do.	Do.

Examination of Table 4 shows that 14 rabbits received from 180 to 210 mg caffein per kilo. The appearance of symptoms in these rabbits varied considerably. In some increased reflexes could be noticed in 10 to 15 minutes after the injection of caffein; in others it was delayed 2 or 3 hours. It might be added that the onset of symptoms occurred in many cases very soon after the administration of the drug—on an average about 10 to 30 minutes after the drug was injected. After smaller doses were administered by injection into the lumbar muscles the appearance of symptoms was delayed several hours in some cases. The duration of life in these 14 rabbits varied considerably. Eight of them died within 1 to 20 hours; six survived. About 0.2 gram caffein per kilo may be regarded as the minimum fatal dose, while the minimum toxic dose is somewhere between 130 and 150 mg per kilo.

INTRAVENOUS INJECTION.

These experiments were carried out on well-fed, full-grown gray rabbits. The diet for several days preceding the experiments consisted of oats or carrots, which were given ad libitum. The injections were made into the ear veins from a burette or by means of a syringe, the temperature of the caffein solution being about 40° C. Attention was also directed to the effect of the rate of injection and of the concentration on the toxicity. The minimum toxic as well as lethal doses were determined as shown in the following experiments.

SERIES A.

In these experiments the rate of injection was about 1 cc of 2 per cent caffein solution per minute.

Rabbit 194. White, female. Weight, 1,310 grams.

October 19: Injected 7.5 cc 2 per cent solution caffein (115 mg per kilo) into the ear vein. Rabbit showed stiffness; paralysis of extremities appeared soon after.[C] Rabbit survived.

Rabbit 556. Gray, female. Weight, 1,635 grams.

January 31: 2 p. m., 11 cc 2 per cent caffein (134 mg per kilo) injected into ear vein, in about 11 minutes; 2.10 p. m., convulsions, rabbit remained lying on its side; during the rest of the hour it had convulsions occasionally; 3.20 p. m., convulsions and died. Rabbit did not urinate after the injection of caffein.

Rabbit 557. Gray, female. Weight, 1,580 grams.

January 31: 2.30 to 2.37 p. m., 7 cc 2 per cent caffein injected from the burette at the rate of 1 cc per minute; 2.37 p. m., flow of liquid ceased, veins were engorged and bled freely, injection was continued by means of a syringe; 2 cc 2 per cent caffein injected in two minutes; injections discontinued as convulsions appeared; 2.50 p. m., rabbit raised itself but fell over; 3.10 p. m.,

rabbit assumed normal attitude, walked about the floor without manifesting any signs of the effects of caffein; 4.30 p. m., walked about, gait normal, condition seemed to be good.

February 1: 2 p. m., condition good, appetite good, total amount of caffein injected, 9 cc 2 per cent solution, or 114 mg per kilo.

Rabbit 558. Gray, female. Weight, 1,590 grams.

January 31: 3 p. m., given 8 cc 2 per cent caffein in eight minutes; 3.10 p. m., violent convulsions; 3.20 p. m., rabbit was stretched out on his abdomen, extremities extended, urinated; 4.30 p. m., looked normal; was able to walk about.

February 1: 2 p. m., condition good, appetite good.

Rabbit 292. Belgian hare, male. Weight, 1,770 grams.

February 18: 4.26 to 4.39 p. m., 12.5 cc warm caffein solution (0.141 gram per kilo) injected into ear vein, convulsion followed when this quantity was injected, tonic rigidity of limbs followed soon after; 4.52 p. m., condition unchanged, rabbit on floor, limbs stretched out, and lying on abdomen.

Rabbit 294. Belgian hare, female. Weight, 1,350 grams. Carrot diet for about 10 days before the experiment.

February 19: 12.20 p. m., 5 cc 2 per cent caffein (74 mg per kilo) injected into ear vein in five minutes, edema of the ear, other ear used, 3.5 cc injected in 10 minutes, repeated convulsions; 1.25 p. m., rabbit still alive, frequent attacks of convulsions; 2.30 p. m., found dead. Total amount injected in 15 minutes, 8.5 cc, or 0.126 gram per kilo.

It will be observed in the preceding experiments that symptoms of severe intoxication were present in all of the six rabbits, but only two of these (Nos. 294 and 556) died from the effects of caffein. Of those which survived, three received doses of 100 to 114 mg caffein per kilo, and another (No. 292) received 141 mg of caffein per kilo. The death of rabbits Nos. 294 and 556 may be regarded therefore as a case of exceptionally low resistance to caffein.

SERIES B.

Doses of 160 to 200 mg caffein per kilo were employed in these experiments. The rate of injection was 1 cc per minute, with the exception of Experiment 254, in which 10.8 cc 2 per cent caffein were introduced in 17 minutes and 25 seconds.

Rabbit 562. Gray female. Weight, 1,650 grams. Diet, oats.

February 1: Injection began at 3 p. m., injected 10 cc in 12 minutes; 3.01 p. m. to 3.09 p. m., 3 cc injected, convulsions; 3.09 p. m. to 3.14 p. m., 3 cc injected, followed by violent convulsions, marked opisthotonos; 4.30 p. m., rabbit died; total quantity injected, 16 cc.

Rabbit 561. Gray female. Weight, 1,450 grams. Diet, oats.

February 1: Injection began at 11.40 a. m.; 11.48, rabbit struggled, 7 cc 2 per cent caffein injected; 11.50, convulsions, 10 cc 2 per cent caffein total amount injected; 11.55 a. m., injections stopped; injections resumed 11.58, violent convulsions, injections discontinued, total quantity received, 14.5 cc 2 per cent caffein solution; 1.30 p. m., found dead, did not urinate, 25 cc urine found in the bladder.

Rabbit 560. Gray male. Weight, 1,620 grams. Diet, oats.

February 1: Injection began 11 a. m.; 11.10 a. m., 7 cc 2 per cent caffein injected, rabbit struggled; 1 cc was injected during the next three minutes, rabbit struggled but there were no convulsions, injection stopped; resumed at 11.15 a. m. and continued 10 minutes, 8 cc 2 per cent caffein introduced during this time; total amount caffein injected, 16 cc; reflexes markedly increased; 12 noon, tetanic convulsions off and on until 2 p. m., then remained stretched out on abdomen, extremities extended.

February 2: 9 a. m., found dead.

Rabbit 559. Gray female. Weight, 1,875 grams. Diet, oats.

January 31: 4 p. m., convulsions after injection of 9 cc 2 per cent caffein in 14 minutes; 4.08 p. m., convulsions after injection of 7 cc caffein in 8 minutes; 4.10 to 4.12 p. m., injected 2 cc more, rabbit lying stretched out on abdomen, extremities extended; total amount of caffein injected, 18 cc (190 mg per kilo).

February 1: 2 p. m., condition good, walked about, appetite good, passed 155 cc dark, reddish-brown urine since 5.30 p. m. previous day.

Rabbit 279. Gray and white female. Weight, 1,320 grams.

February 24: 10.09 a. m., 6 cc 2 per cent caffein passed rapidly into jugular vein; 10.15 a. m., involuntary twitching of muscles of legs, but no other symptoms; 10.23 to 10.26, 3 cc of 2 per cent caffein injected; 10.27 to 10.28, 2 cc 2 per cent caffein injected, convulsions; 10.29, convulsions stopped; 10.32, convulsions; 11 a. m., rabbit lying on its side, anterior extremities paralyzed, posterior extremities contracted, no clonic convulsions, breathed deeper and more

slowly than normal; 11.10 a. m., rabbit died, had no convulsions immediately before death; amount of caffein injected, 11 cc 2 per cent solution, or 0.166 gram per kilo.

Rabbit 254. Belgian hare, female. Weight, 1,285 grams. Diet, oats.

November 12: 1.30$^{1}/_{3}$ to 1.47¾ p. m., received 10.8 cc 2 per cent caffein from burette into ear vein, after injection of 6.2 cc dyspnœa, 6.7 cc struggling, convulsions; at 1.50½ p. m., released from holder, paralysis especially marked in the anterior extremities; 1.50 p. m., recovered, survived; total amount injected, 10.8 cc 2 per cent caffein in 17 minutes and 25 seconds, or 0.16 gram caffein per kilo.

Rabbit 255. Belgian hare, male. Weight, 1,105 grams. Diet, oats.

November 12: 2.31¾ to 2.35¼ p. m., received 3.7 cc; from 2.37$^{1}/_{6}$ to 2.46$^{1}/_{6}$ p. m., 5 cc injected; after injection of 6.1 cc convulsions followed by dyspnœa, then continuous struggling; when 8.3 cc were injected rabbit had another convulsion; 2.47 p. m., tonic contraction of anterior extremities; amount injected, 8.7 cc (158 mg per kilo) in 15 minutes and 35 seconds.

Rabbit 567. Gray female. Diet, oats.

February 6: Injection began at 4.11 p. m.; 4.18, convulsions after injection of 5 cc 2 per cent caffein; 4.21, convulsion after total injection of 8 cc; 4.24 p. m., injection resumed and 2 cc more introduced; 4.28 p. m., convulsions, injected 2 cc more; total caffein injected, 12 cc, or 162 mg per kilo; 4.40 p. m., rabbit paralyzed in posterior extremities; 5 p. m., found dead.

In the eight experiments comprising series B rabbits Nos. 567, 254, 279, and 255, which may be designated as Group II, received doses of 162, 160, 166, and 158 mg, respectively. Nos. 562, 561, 560, and 559, which may be designated as Group I, received about 200 mg caffein per kilo. In Group II, which received the smaller doses, one (No. 254) survived. This may be regarded as exceptional, since, as was shown in the experiments of the preceding series, even smaller doses may be fatal. About 160 mg per kilo is, therefore, the smallest surely fatal dose. This might be regarded as a contradiction of the results obtained for rabbit No. 559, but it will be noticed that in this case diuresis was very marked. The results of experiments Nos. 294 and 255 are of interest in this connection, since they indicate that a moderate difference in the rate of injection is without any effect on the toxicity of caffein. The greater resistance to caffein of rabbit No. 559 is in all probability due, therefore, to increased diuresis.

SERIES C.

In these experiments the minimum toxic dose was determined. The conditions were the same as in the experiments of the other series.

Rabbit 293. Belgian hare, female. Weight, 1,610 grams. Diet, oats.

February 18: 3.40 to 3.43 p. m., 4 cc 2 per cent warm caffein solution injected into ear vein, convulsions when 3 cc were injected, repeated attacks; 4 p. m., raised itself on legs, but fell over immediately and lay stretched on abdomen.

February 19: 9 a. m., rabbit looked normal, apparently recovered.

Rabbit 227. White male. Weight, 2,320 grams.

October 26: 3.29¼ to 3.37½ p. m., injected into ear from burette 6.7 cc 2 per cent caffein, no symptoms; experiment discontinued; survived.

Rabbit 563. Gray female. Weight, 1,650 grams. Diet, oats.

February 6: Injection began at 1.02 p. m., injected 3.5 cc 2 per cent caffein (42 mg per kilo) in four minutes, 0.6 cc more within the next two and one-half minutes, total amount injected 4.1 cc; 1.10 p. m., hypersensitive, some disturbance of muscular coordination; restlessness; 1.35. p. m., reflexes decreased, urinated and walked about, gait normal. Under observation for several days; no symptoms noted.

Rabbit 564. Gray female. Weight, 1,515 grams.

February 6: Injection began at 1.26 p. m., 3.5 cc 2 per cent caffein (46 mg per kilo) injected at the rate of 1 cc per minute; 1.30 p. m., reflexes increased; 1.34 p. m., marked paresis of the extremities, rabbit stretched out on abdomen, legs abducted and partly extended, able to hop about but gait disturbed, no untoward symptoms noticed, under observation for several days after experiment.

Rabbit 565. Gray female. Weight, 1,545 grams. Diet, oats.

February 6: Started to inject at 3.40 p. m., received 2.5 cc 2 per cent caffein intravenously in two minutes or 32 mg per kilo, under observation all afternoon, no symptoms.

Rabbit 566. Gray female. Weight, 1,900 grams. Diet oats.

February 6: Injection began at 3.05 p. m., received 3 cc 2 per cent caffein intravenously in three minutes or 31 mg per kilo, no symptoms observed.

These experiments show that a dose of about 50 mg per kilo when injected intravenously produces mild symptoms, such as increased reflexes. In the four experiments with this amount of

caffein these effects were observed in each case. In the experiments in which smaller quantities, 30 mg per kilo, were given intravenously there was no manifestation of symptoms. A dose not over 50 mg per kilo may, therefore, be regarded as the minimum toxic dose when injected intravenously under the conditions stated.

SERIES D.

A 0.5 per cent caffein solution was used in these experiments in order to test the effect of concentration on its toxicity; the rate of injection was 1 cc per minute.

Rabbit 569. Gray male. Weight, 1,475 grams. Diet, oats.

February 6: 11.50 a. m. to 12.01 p. m., injected 10 cc 0.5 per cent caffein; 12.03 to 12.12 p. m., injected 10 cc of 0.5 per cent caffein; 12.13 to 12.26 p. m., injected 10 cc of 0.5 per cent caffein, total amount injected, 30 cc; 12.20, passed 35 cc of urine; 12.30, increased reflexes, but no convulsions; 4 p. m., reflexes increased.

February 11: Alive, condition good.

Rabbit 574. Gray female. Weight, 1,555 grams. Diet, oats.

February 8: 10.25 to 10.33 a. m., injected 4 cc of 0.5 per cent caffein in salt solution, injection discontinued for five minutes; 10.38 to 11.10, injected 30 cc, total amount of caffein solution received, 34 cc; 11.55 a. m., very sensitive; reflexes markedly increased.

February 9: Alive, condition good.

Rabbit 571. Gray female. Weight, 1,530 grams. Diet, oats.

February 7: Injection 3.18 to 3.50 p. m., received 30 cc in 32 minutes, not hypersensitive; 3.55, restlessness and weakness of extremities; 4.10 p. m., control of anterior extremities impaired, distinctly paretic but tried to walk about, died the same afternoon.

Rabbit 568. Gray male. Weight, 1,605 grams. Diet, oats.

February 7: Injection 10.53 to 11.01 a. m., injected 10 cc 0.5 per cent caffein; 11.03, injection resumed after two minutes interval; 11.14, received 10 cc 0.5 per cent caffein intravenously in 11 minutes; 11.16, injection resumed; 11.35, received 12 cc 0.5 per cent caffein, total amount of caffein solution received, 32 cc; 12.30 p. m., urinated 14 cc of bloody urine; 12.55 p. m., convulsions and death a few minutes later. Autopsy showed congestion of viscera, but no other lesions.

Rabbit 570. Gray female. Weight, 1,225 grams. Diet, oats.

February 7: 2.06 to 2.35 p. m., injected 24.5 cc 0.5 per cent caffein, reflexes increased but no convulsions, paresis especially marked in the anterior extremities; 3 p. m., passed urine which was normal in appearance, reflexes not increased but rabbit was weak.

February 9: Found dead. *Autopsy*: Liver, spleen, and kidneys congested; large intestines hemorrhagic; omentum congested and showed the presence of small caseous nodules; liver showed adhesion to diaphragm; viscera presented the appearance of intraabdominal infection.

Of the five rabbits of this series three died as a result of the administration of caffein. The other two which survived showed mild symptoms only, such as increased reflexes, but no evidence of severe poisoning such as was observed after the injection of the same doses of caffein in series A when a 2 per cent solution of caffein was injected. Convulsions were noticed in one case only (No. 568); paresis in two cases (Nos. 570 and 571). The nervous symptoms even in this group, therefore, were much milder than in series A. The percentage of death, however, was greater than in series A, in which the concentration of caffein was four times as great. It is quite probable that the strain on the heart due to the sudden increase in volume of the blood and its dilution might be an important factor in increasing the toxicity of caffein. It is conceivable that doses just sufficiently large to depress the normal heart may cause paralysis of an already overstrained organ.

SERIES E.

In the two experiments of this series the rate of injection as a possible factor influencing the toxicity of caffein was tested. A 2 per cent caffein solution was injected at the rate of 1 cc in two and one-half to three minutes.

Rabbit 572. Gray male. Weight, 1,770 grams. Diet, oats.

February 8: Injection began at 3 p. m., discontinued at 3.37 p. m., and resumed at 3.38 p. m.; rabbit was restless; injection finished at 3.52 p. m. Total quantity received, 17.4 cc 2 per cent caffein intravenously in 52 minutes; struggled intermittently during the injection; anterior legs paralyzed.

February 9: Found dead.

Rabbit 573. Gray male. Weight, 1,810 grams. Diet, oats.

February 8: Started to inject at 1.35 and discontinued at 2.27 p. m.; received 18 cc 2 per cent caffein intravenously in 52 minutes; reflexes markedly increased soon after; 2.45, passed bloody

urine; 4.30 p. m. reflexes increased; no other symptoms.

February 9: 9 a. m., found dead.

It will be observed that some retardation of the onset of symptoms was caused by slower injection, but the final result was the same as when the injections were made more rapidly. It is quite probable, therefore, that a much slower rate of injection may lessen considerably the toxicity of caffein.

From the results of the experiments by intravenous injection summarized in the table, it appears that the minimum toxic dose for rabbits of a 2 per cent caffein solution, injected at the rate of 1 cc per minute, is about 50 mg per kilo. Twice the dose induces severe symptoms and may be fatal; 160 mg per kilo are surely fatal. If the rate of injection is diminished, the toxicity of caffein is lessened, but this effect is not marked unless the injections are very slow. Dilution of the caffein solution suppresses to some extent the nervous symptoms, but the toxicity, on the contrary, seems to be increased.

TABLE 5.—*Intravenous injections.*

No.	Weight.	Caffein per kilo	Symptoms.	Duration of life.	Diet.	Remarks.
	Grams.	*Mg.*				
SERIES A.						
194	1,310	114	Present	Survived	Oats	White female.
556	1,635	134	10 minutes	20 minutes	do.	Gray female.
557	1,580	114	Present	Survived	do.	Do.
558	1,590	100	do.	do.	do.	Do.
292	1,770	141	do.	do.	do.	Do.
194	1,350	126	do.	10 minutes	Carrots	Do.
SERIES B, GROUP I.						
562	1,650	200		1½ hours	Oats	Gray female.
561	1,450	200		do.	do.	Do.
560	1,620	200	Present	Less than 24 hours	do.	Do.
559	1,875	190	do.	Survived	do.	Do.
SERIES B, GROUP II.						
279	1,320	166		1 hour		Gray and white female.
254	1,285	160		Survived	Oats	Gray female.
567		162		About 45 minutes		Do.
255		158		Died		
SERIES C.						
293	1,610	500	Present	Survived	Oats	Gray female.
227	2,320	570	None	do.		White male.
563	1,650	500	Present	do.	do.	Gray female.
564	1,515	460	do.	do.		Do.
565	1,545	320	None	do.	do.	Do.
566	1,900	310	do.	do.	do.	Do.
SERIES D.						
569	1,475	100	Present	Survived	Oats	Gray male.
574	1,555	112	do.	do.	do.	Gray female.
571	1,530	100	do.	About 2 hours	do.	Do.
568	1,605	100		20 minutes	do.	Gray male.
570	1,225	100		Less than 20 hours	do.	Do.
SERIES E.						
572	1,770	200	Present	About 24 hours	Oats	
573	1,810	200		do.	do.	

SUMMARY.

The results of the experiments on rabbits show considerable variation in the toxicity of the single dose. Individuals differed so widely in their resistance to this drug that the same experiments had to be repeated many times with each method of administration before satisfactory conclusions could be drawn. This is strikingly illustrated in the experiments by intravenous injection in which a dose of nearly 0.2 gram per kilo was not fatal. Similar instances of exceptional resistance or of sensitiveness to caffein were observed when it was given in other ways. A comparison of the toxicity of caffein administered by different methods in this investigation shows well-marked differences in its activity, although they are not quite so striking as similar experiments with other alkaloids reported by several observers. The toxicity of caffein in these experiments on the rabbit indicates that it is greatest when given by vein and least when given by mouth. The ratio of the minimum toxic doses by these two methods of introduction of caffein was about 7.1; the relation of the minimum fatal dose was about 3.1. The toxicity when given subcutaneously is

about 15 to 20 per cent greater than when given by mouth. The difference between the intramuscular and subcutaneous injection is even more marked. The toxicity of caffein when injected into the muscles is about midway between that administered by the subcutaneous and intraperitoneal routes, and is about half that injected intravenously. Meltzer and Auer,[58] who experimented with a number of drugs found that the intramuscular method of administration is as effective as the intravenous, fluorescin forming the only exception according to their observations. In the experiments of Sollman and Brown[81] with ergot, the effect was quite different from those obtained by Meltzer and Auer[58] with the drugs they used. It is quite possible that the result obtained with ergot is merely illustrative of a difference in the behavior of various substances in this regard. This appears probable on account of the difference in the rate of absorption for various substances. Thus, according to Achard, Gaillard, and Ribot (Compt. rend. Soc. biol., 1907, *62*: 90), absorption from the peritoneal cavity varies with the concentration of the solution and the size of the molecule. The smaller the molecule and the greater the concentration the more rapid the absorption. That the rate of absorption from the intramuscular tissues is unequal and varies for different substances appears from the experiments of Meltzer and Auer.[58] The difference was very striking between intramuscular and subcutaneous administration of curara or adrenalin; the results were somewhat different with morphin and with fluorescin. As shown in their protocols, the onset of the symptoms after the intramuscular injection of morphin was sooner than after subcutaneous injection, but in time the difference diminishes and disappears altogether. The absorption of fluorescin is much faster when the intramuscular path is used than when given subcutaneously, but the writers state that the rate falls far behind that of the intravenous administration. The difference in toxicity we observed between feeding by mouth and subcutaneous injection, although distinct, was not very great. It was much less than Maurel[55] obtained with the hydrobromid of caffein in the rabbit. Whether this difference between his results and ours is due to the use of the pure alkaloid in our experiments and the hydrobromid employed by Maurel can not be stated at present with any degree of accuracy. It is hoped that the work in progress in the laboratory will throw some light on the subject in the near future. But Maurel's[56] experiments show that various substances behave differently in this regard. Thus the toxicity of strychnin, he states, is three times as great when given subcutaneously as when given by mouth and six times that of the minimum fatal dose by vein. It may be remarked, however, that examination of his data shows that his doses are much too large for the rabbit. In experiments with other drugs little or no difference between the two modes of administration was noticed. Thus, digitalin was but slightly more active when given subcutaneously than by mouth, while the toxicity of emetin hydrochlorid was just the same, whichever one of these methods of introducing the substance was used. Differences in the toxicity of substances have also been observed between subcutaneous and intravenous modes of administration, but here, too, the differences for various substances were unequal.

EXPERIMENTS ON GUINEA PIGS.

The toxicity of caffein was studied in a large number of individuals. The experiments were conducted on full-grown animals and were carried out at different seasons of the year in a variety of ways. Special attention was given to diet as a possible factor influencing resistance to caffein, and the effect of different modes of administration on toxicity. Some animals were therefore fed oats, some carrots, others received both hay and oats. Caffein was introduced subcutaneously, intraperitoneally, and by mouth.

SUBCUTANEOUS INJECTION.

Series A.

Preliminary experiments carried out on three guinea pigs, which received 360, 300, and 290 mg of caffein per kilo subcutaneously have shown that such doses were rapidly fatal. Two of the animals were seized with convulsions half an hour after the introduction of caffein and died during the attack. The other had tetanus two minutes after the injection of caffein. Repeated attacks followed, which terminated in the death of the animal two and a half hours later. The fatal and toxic doses must therefore be considerably under 0.3 gram of caffein per kilo when introduced by this path and smaller doses were therefore injected. The results are shown in the experiments of the next series.

Series B.

Experiments with 2 decigrams per kilo constituted this series.

Guinea pig 20. Female. Weight, 497 grams. Diet, oats.

April 2: 5 cc 2 per cent caffein injected subcutaneously at 11.30 a. m.; 1.50 p. m., spasm of short duration. Died at 3 p. m., three and one-half hours after injection.

Guinea pig 38. Brown male. Weight, 570 grams. Diet, carrots and oats week previous to injection.

February 11: 3.50 p. m., 6 cc 2 per cent caffein injected subcutaneously in back (210 mg per kilo); 4.15, reflexes increased, had convulsion of short duration when disturbed; 4.45 p. m., on handling, repeated convulsion and paralysis; 5 p. m., guinea pig lying on his side, respiration difficult and labored.

February 11: 5.05 p. m., guinea pig found dead, 2 hours and 15 minutes after injection.

Guinea pig 37. Male. Weight, 820 grams. Diet, carrots and oats during week preceding the injection of caffein.

February 11: 3.35 p. m., 8.5 cc 2 per cent caffein injected subcutaneously in the back; 5 p. m., pig very sensitive, anterior extremities paralyzed when handled, frequent spasms of posterior extremities, no symptoms noticed before 5 p. m., although watched all the time; 5.05 p. m., guinea pig on his legs and looked normal. No attack on handling.

February 12: 9 a. m., found dead; died within 18 hours.

Guinea pig 13. Female. Weight, 618 grams. Diet, oats.

March 29: 2.45, 6 cc 2 per cent caffein injected subcutaneously (0.194 grams per kilo).

March 30: Died at 4 p. m., 25 hours after injection.

Guinea pig 36. Male. Weight, 850 grams. Fed oats and carrots for one week previous to injection.

February 11: 3.30 p. m., 8.5 cc 2 per cent caffein injected subcutaneously into back; 5 p. m., somewhat more sensitive than normal, no other symptoms, no effect on handling; 5.05 p. m., no symptoms.

February 12: 9 a. m., found dead, about 18 hours after injection.

The results of these experiments, as observed in five guinea pigs, indicate that two decigrams of caffein per kilo of animal produce symptoms within a half to about two and a quarter hours after injection. Death followed in two guinea pigs 70 minutes to 1 hour after the first manifestations of symptoms. Two others died during the night, while one lived 25 hours after the injection of caffein. Even 2 decigrams caffein per kilo weight might therefore be fatal to the guinea pig. Experiments carried out later have shown, however, that the resistance to caffein is appreciably greater in some guinea pigs. This is indicated by the following experiments, in which doses of 0.2 to 0.24 gram caffein per kilo were administered by the same path.

SERIES C.

Guinea pig 66. Yellow and dark brown male. Weight, 510 grams. Diet, oats.

October 4: 5 cc 2 per cent caffein (0.2 gram per kilo) injected subcutaneously in the back at 3 p. m.; 5 p. m., no symptoms.

October 5: 9 a. m., alive; condition good.

October 9: Found dead. *Autopsy*: Congestion of liver, kidney, and small intestine.

Guinea pig 65. White and black male. Weight, 510 grams. Diet, oats.

October 4: 5 cc 2 per cent caffein (0.2 gram per kilo) injected subcutaneously in the back at 3 p. m.; 5 p. m., no symptoms.

October 5: 9 a. m., condition good.

Guinea pig 60. White and gray female. Weight, 320 grams. Diet, oats.

October 3: 2.25 p. m., 3.5 cc 2 per cent caffein (0.219 gram per kilo) injected subcutaneously in the back; 3.40 p. m., convulsion with recovery; 3.50 p. m., frequent spasms with paralysis, especially of anterior extremities; 5.30 p. m., tetanus when removed from cage and put on floor.

October 4: 8.50 a. m., found dead. *Autopsy*: Congestion of small intestines, lungs, liver.

Guinea pig 57. White and gray female. Weight, 350 grams. Diet, oats.

October 3: 2.15 p. m., 3.5 cc 2 per cent caffein injected subcutaneously in the back (0.2 gram per kilo); 3.40 p. m., convulsions with recovery; 5.30 p. m., no marked symptoms.

October 4: 8.50 a. m., alive, active.

October 6: Found dead at 9 a. m. *Autopsy*: Congestion of lungs and liver; kidneys petechiated; severe gastro-enteritis.

Guinea pig 68. Yellow male. Weight, 785 grams. Diet, oats.

October 6: 11.35 a. m., 7.8 cc 2 per cent caffein (0.2 gram per kilo) injected subcutaneously; 12 noon, reflexes increased markedly; 4.20 p. m., reflexes the same as at 12 noon.

October 7: 9 a. m., dead. *Autopsy*: Lungs congested; liver congested and fatty; spleen congested, kidney showed hemorrhagic spots; gastric mucosa necrotic; small portion of small intestine inflamed.

Guinea pig 69. White male. Weight, 585 grams. Diet, oats.

October 6: 11.40 a. m., 5.8 cc 2 per cent caffein injected subcutaneously; 12 noon, reflexes increased, but not as much as in No. 68; 4.20 p. m., guinea pig hypersensitive, reflexes increased more than at 12 noon.

October 7: 9 a. m., alive.

October 15: 9 a. m., found dead.

Guinea pig 61. Brown and black female. Weight, 330 grams. Diet, oats.

October 3: 4 p. m., 4 cc 2 per cent caffein (240 mg per kilo) injected subcutaneously; 5.30 p. m., reflexes increased; runs, but drags posterior extremities.

October 4: 8.50 a. m., found dead.

Guinea pig 62. White, yellow, and black female. Weight, 335 grams. Diet, oats.

October 3: 4.05 p. m., 4 cc 2 per cent caffein (238 mg per kilo) injected subcutaneously in the back; 5 p. m., convulsions; 5.20 p. m., convulsions, alternating with paralysis of anterior and posterior extremities.

October 4: 8.50 a. m., found dead.

Guinea pig 70. White and brown male. Weight, 545 grams. Diet, oats.

October 7: 3 p. m., 6.5 cc 2 per cent caffein (238 mg per kilo) aqueous solution injected subcutaneously; 3.50 p. m., reflexes increased.

October 9: 9 a. m., found dead.

Guinea pig 71. Brown and white male. Weight, 540 grams. Diet, oats.

October 7: 3 p. m., 6.5 cc 2 per cent caffein solution (0.24 gram per kilo) injected subcutaneously; 3.45 p. m., reflexes increased, tetanus.

October 9: 9 a. m., found dead.

Guinea pig 72. Brown and white male. Weight, 560 grams. Diet, oats.

October 7: 3 p. m., 6.5 cc 2 per cent caffein (0.232 gram per kilo) aqueous solution administered by subcutaneous injection; 3.35 p. m., reflexes increased.

October 10: found dead. *Autopsy*: Nos. 70, 71, 72 showed congestion of organs.

The reaction to caffein in the experiments of this series (C) showed considerable variation. The appearance of symptoms, as well as the final outcome of the experiments, differed markedly in a number of cases, notwithstanding the fact that the conditions were the same; thus the administration of 0.2 gram per kilo to guinea pigs, all of which received the same diet, induced no symptoms in two of the animals (Nos. 66 and 65), while marked symptoms were observed in the other four; in two of these the symptoms appeared in one hour and a quarter after injection, and in two others (Nos. 68 and 69), mild symptoms only appeared in 20 or 25 minutes. The last two were under observation for 4 hours longer, but there was no visible change in their condition. The duration of life in all of these guinea pigs, as indicated in the table, likewise varied. Two (Nos. 60 and 68) died during the night after they received caffein, one survived (No. 65), and three others (Nos. 57, 66, and 69) lived 2½, 5, and 9 days, respectively. Experiments with larger doses likewise showed differences in the behavior of these animals toward caffein, but they were not quite so marked. As shown in the table, symptoms appeared in from 35 minutes to 1.5 hours after injection. The duration of life was less than 1 day in two pigs, about twice as long in two others, and in one case between 2 and 3 days.

A comparison made with results obtained in the preceding series shows a striking difference in the resistance to caffein. As 2 decigrams per kilo proved more rapidly fatal to the guinea pig than the larger doses employed in the later experiments, this difference in the resistance to caffein may be due to several factors. As pointed out in the experiments on rabbits, age might be an important factor influencing the toxicity of caffein. Unfortunately, no accurate data were available on the age of the guinea pigs, but they were all apparently full grown, although they differed in weight considerably. The difference in their ages was in all probability not very great. Moreover, it will be observed that the resistance in series B and C differed in animals of approximately the same weight. This is evident on comparing experiments Nos. 20, 38, and 13 of series B with Nos. 65, 66, and 69 of the next series. Again, further inspection and analysis of these tables show no difference in the toxicity, although there may be considerable difference in the weight, from which it may be concluded that the animals were of about the same age or that this plays no part in the resistance to caffein in the guinea pig.

Diet is another factor which should be taken into consideration in this connection. The recent work of Hunt[39] indicates that this may influence the resistance of animals to some poisons. Our experiments, however, fail to show any difference in the toxicity of the caffein in guinea pigs, whether fed oats, carrots, or both, for different results were obtained on the same diet, and there seemed to be little or no difference in the toxicity of caffein when the diet was different. Other explanations suggest themselves to account for the results obtained. Seasonal changes have been assigned by a number of investigators as a cause of variation in the resistance to drugs. According to Focke,[24] frogs are more susceptible to digitalis in the spring than in the summer, while Moschkowitsch[61] and Edmunds[21] reported the very opposite results. Schmiedeberg's[80] observations on strophantin in frogs were in harmony with those of Edmunds[21] and Moschkowitsch.[61] Similar results were reported with guinea pigs. Harrington's[34] experiments

indicate that stimulation of the vagus is less effective from October to January than from February to April, when they are also much more susceptible to operative procedure. Hunt found that the resistance of guinea pigs to aceto nitril is about twice as great in the summer months as it is in January and February.

Race might also be thought of as an important factor in this connection. Since the guinea pigs used at different seasons of the year were of several varieties, there is no reason to suppose, however, that the varieties experimented upon in the summer were more resistant than those used in the winter and spring. It is highly probable, therefore, that the greater resistance to caffein of the guinea pigs of series C than those of series B was due to seasonal variation.

Doses of 0.20 to 0.24 gram caffein per kilo weight, therefore, may be regarded as the minimum fatal dose for the guinea pig, depending upon the season. Since 0.2 gram per kilo proved to be rapidly fatal in series B, this quantity was perhaps not the minimum fatal dose for the guinea pig at the season during which the experiments were made. Additional tests with smaller doses were therefore carried out during February and March. The results are shown in series D.

SERIES D.

Guinea pig 49. Male. Weight, 510 grams. Diet, oats for 1 month previous to experiment.

March 17: 3 p. m., 4 cc 2 per cent caffein (0.16 gram per kilo) were injected subcutaneously; 4.40 p. m., reflexes increased; 5.40 p. m., no symptoms.

March 18: 9 a. m., found dead, died in less than 18 hours. *Autopsy*: Hemorrhage into abdominal cavity; liver and spleen unduly congested; intestines injected; hemorrhagic area at point of injection.

Guinea pig 40. Male. Weight, 630 grams. Diet, oats and carrots one week previous to injection.

February 12: 11 a. m., 5 cc 2 per cent caffein (0.158 gram per kilo) injected subcutaneously into back.

February 13: 1 p. m., still alive.

February 14: 9 a. m., found dead.

Guinea pig 45. Female. Weight, 435 grams. Diet, oats for about one month previous to injection.

March 17: 3 p. m., 3.5 cc of 2 per cent caffein injected subcutaneously in the back (0.160 gram per kilo); 4.35 p. m., no symptoms; 5.40 p m., no symptoms.

Guinea pig 39. Male. Weight, 820 grams. Diet, oats and carrots.

February 12: 11 a. m., 6 cc (0.15 gram per kilo) 2 per cent caffein injected subcutaneously in back.

February 14: 9 a. m., alive; seemed to be in good condition; found dead at 1 p. m.

Guinea pig 41. Weight, 660 grams. Diet, oats and carrots one week previous to injection.

February 12: 11 a. m., 5 cc (0.15 gram per kilo) 2 per cent caffein injected subcutaneously.

February 14: 2 p. m., pig alive; apparently normal.

February 18: Guinea pig still alive and apparently in good condition.

Guinea pig 46. Female. Weight, 470 grams. Diet, oats about one month previous to experiment.

March 17: 3.15 p. m., 4 cc (0.170 gram per kilo) 2 per cent caffein injected into back subcutaneously; 4.35 p. m., reflexes increased, tremors on handling marked; 5.40 p. m., no change, symptoms about as before.

March 18: 2.30 p. m., no symptoms.

The experiments of this series (D) likewise showed a considerable difference in the resistance of the individual guinea pigs. Nos. 41, 45, and 46 survived; the rest of the pigs died within 18 hours to 2 days after the administration of caffein. Since an autopsy was held on one only, it is impossible to assign a cause for the variation in the toxicity of caffein in these guinea gigs, as the diet and the other conditions under which the experiments were conducted were the same. It was found in the experiments on cats and rabbits that the presence of morbid processes tends to increase the toxicity of caffein. The observations of Ophüls[66] are of interest in this connection. He found spontaneous lesions of the kidney and liver in a large proportion of guinea pigs examined. The greater susceptibility to caffein of guinea pigs Nos. 39, 40, 49, is probably due therefore to some pathological change which increased its toxicity. About 0.2 to 0.24 gram per kilo may therefore be regarded as the minimum lethal dose for the normal guinea pig when caffein is introduced subcutaneously, the minimum toxic dose being about 150-160 mg per kilo.

Experiments were also conducted to determine the largest dose which does not produce any visible effects. In a number of tests with from 100 to 120 mg caffein per kilo (series E, see Table 6, p. 51) no manifestation of nervous or muscular disturbance nor any departure from the normal in respiratory activity was observed. Such quantities may be regarded as the largest doses which are surely safe for these animals. It is quite possible, therefore, that the greater variation in the

toxicity of caffein observed in these experiments is due to morbid conditions. Moreover, there is some evidence that caffein increases the toxicity of certain poisons, as shown by Hale[33] for acetanilid. Is it not possible that caffein may similarly be affected by poisons circulating within the body? Indeed the recent work of Loeb[23] makes this supposition highly probable. This investigator found that caffein and adrenalin injected together produce myocarditis in the rabbit. It is conceivable that the combined action of caffein and some preexisting poison may cause changes which terminate in the death of the animal. The delayed death of guinea pigs after the administration of caffein observed in this and other series may probably be accounted for in this way.

Experiment 57 lends some support to this view. The condition of the kidneys and the presence of a severe gastro-enteritis are sufficient to account for the death of this case. Again the frequent association of gastro-enteritis and congestion of the organs in caffein intoxication found in different animals makes it highly probable that these lesions were caused by caffein.

INJECTION INTO THE PERITONEAL CAVITY.

The experiments were carried out with different doses. All the guinea pigs in this series were kept on a uniform diet, consisting of oats. Most of them were of average size and there were no wide variations in their weights. The experiments of series A with the smallest doses were conducted in March and April; all the other experiments it will be noticed were made in October.

SERIES A.

Guinea pig 41. Weight, 700 grams. Diet, oats.

April 1: 3.30 p. m., 4.5 cc 2 per cent caffein (130 mg per kilo) injected into peritoneal cavity. 5.35 p. m., symptoms present but no tetanus.

April 2: Found dead about 2 p. m., duration of life about 22 hours. *Autopsy*: Subcutaneous hemorrhage at the point of inoculation; serious exudate on visceral and parietal peritoneum with marked inflammation of peritoneum; portions of intestines showed slight enteritis.

Guinea pig 49. Male. Weight, 370 grams. Diet, oats.

April 1: 3.15 p. m., 2.5 cc 2 per cent caffein (135 mg per kilo) injected into the peritoneal cavity; 5.30 p. m., symptoms present; reflexes increased, but no tetanus. Guinea pig survived.

Guinea pig 47. Female. Weight, 550 grams. Diet, oats since about February 4.

March 17: 3.30 p. m., 3.5 cc 2 per cent caffein (127 mg per kilo) injected into peritoneal cavity; 4.35 p. m., increased irritability present, but not marked; 5.40 p. m., symptoms about the same as before.

March 18: 2.30 p. m., condition good; no symptoms. Survived.

Guinea pig 50. Female. Weight, 290 grams. Diet, oats.

April 1: 3.30 p. m., 2 cc 2 per cent caffein (138 mg per kilo) injected into peritoneal cavity; 5.35 p. m., symptoms present; reflexes much increased, but no tetanus. Survived.

SERIES B.

Guinea pig 51. Yellow female. Weight, 415 grams.

October 1: 9.50 a. m., 3 cc (144 mg per kilo) 2 per cent caffein injected into peritoneal cavity; 4.30 p. m., no symptoms, although under observation all day.

October 3: 2 p. m., alive.

Guinea pig 52. White male. Weight, 450 grams.

October 1: 9.45 a. m., 3.5 cc, 2 per cent caffein (155 mg per kilo), injected into peritoneal cavity; 4.30 p. m., no symptoms developed since injection.

October 3: 2 p. m., alive.

Guinea pig 58. Brown and white male. Weight, 490 grams.

October 1: 9.45 a. m., 4 cc, 2 per cent caffein (163 mg per kilo), injected into peritoneal cavity; 4.30 p. m., no symptoms developed since injection.

October 3: 2 p. m., alive.

October 8: Found dead. *Autopsy*: Congestion of lungs, spleen, liver, kidneys, and small intestines.

SERIES C.

Guinea pig 59. Gray and white. Weight, 375 grams. Diet, oats.

October 3: 2 p. m., 3.75 cc (0.2 gram per kilo) injected into peritoneal cavity; 2.15 p. m., reflexes increased but not markedly; 4 p. m., reflexes still more increased; no other symptoms; 5.30 p. m., no symptoms.

October 4: 8.50 a. m., guinea pig alive and active.

Guinea pig 58. Brown and white. Weight, 380 grams. Diet, oats.

October 3: 2 p. m., 3.8 cc caffein (0.2 gram per kilo), 2 per cent solution, injected into peritoneal cavity; 2.10 p. m., hind legs extended, then tetanus; attack lasted a few seconds, after which pig raised himself on his legs, but reflexes remained much exaggerated; 4 p. m. to 5.30 p. m., no symptoms of caffein intoxication.

October 4: 8.50 a. m., guinea pig alive and active.

Guinea pig 56. Gray and white male. Weight, 440 grams. Diet, oats.

October 1: 11.30 a. m., received 4.6 cc of 2 per cent caffein solution (0.2 gram per kilo) into abdominal cavity; 11.45 a. m., stiffness and rigidity of posterior extremities, reflexes increased; 12.30 p. m., hind legs paralyzed, reflexes increased; 4.35 p. m., no symptoms, guinea pig in good condition.

October 3: Still alive in good condition.

October 14: Died. *Autopsy*: Anterior lobe of right lung hepatized. Small portion of small intestine edematous. Other organs normal.

Guinea pig 55. White and yellow male. Weight, 690 grams. Diet, oats.

October 1: 11.30 a. m., received 6.5 cc of 2 per cent solution caffein (188 mg per kilo) into peritoneal cavity; 11.40 a. m., stiffness in all extremities, reflexes markedly increased; 12.30 p. m., reflexes increased, anterior and posterior extremities paralyzed; 3 p. m., found dead.

SERIES D.

Guinea pig 67. Gray and yellow male. Weight, 330 grams. Diet, oats.

October 5: 11.25 a. m., 4 cc of 2 per cent caffein injected into peritoneal cavity (240 mg per kilo); 11.30 a. m., tetanus—survived, convulsions off and on. Death at 2.55 p. m. *Autopsy*: Severe gastroenteritis; kidney petechiated; congestion of lungs and liver.

Guinea pig 63. Gray and white male. Weight, 340 grams. Diet, oats.

October 5: 11.20 a. m., 4 cc of 2 per cent caffein (235 mg per kilo) injected into peritoneal cavity.

October 14: Alive and in good condition.

Guinea pig 64. Brown and black female. Weight, 305 grams.

October 5: 11.35 a. m., 3.8 cc 2 per cent solution caffein (250 mg per kilo) injected into peritoneal cavity; 11.40 a. m., tetanus—survived, convulsions off and on, died at 4.15 p. m. *Autopsy*: Findings exactly the same as in No. 67.

Examination of the results of the experiments by intraperitoneal injections showed that 0.2 gram caffein per kilo was toxic in two guinea pigs (Nos. 59 and 58). Severe symptoms were observed within 15 minutes in No. 56 and within one hour in No. 55 after the administration of approximately the same dose of caffein. One of these died within three and one-half hours; the other, No. 56, made a good recovery from the acute effects. This amount of caffein may be regarded, therefore, as the minimum toxic dose for the guinea pig when injected into the peritoneal cavity. This is corroborated by the experiments of series B in which smaller doses failed to show any muscular, nervous, or respiratory symptoms, nor were there any after effects noticed, as all of them survived and were kept under observation for some time. The guinea pigs of series A, however, seem to contradict these results. It will be remarked that appreciably smaller doses induced symptoms in all of them, and one case terminated fatally. The seasonal variation, as already pointed out, is in all probability likewise responsible for the difference in the resistance between the guinea pigs of series A and B. Tests were made also to determine the minimum fatal dose. For this purpose the experiments of series D were performed. The resistance of No. 63 in this series is quite striking. We are unable to explain such a discrepancy in the results obtained under practically uniform conditions. The minimum fatal dose of caffein, when injected into the peritoneal cavity, is therefore about 240 to 250 milligrams per kilo. These amounts, it will be observed, were rapidly fatal, in striking contrast to the results obtained when such doses were injected subcutaneously. This is probably due to a better absorption from the peritoneal cavity than from the subcutaneous tissues.

ADMINISTRATION BY MOUTH.

All the guinea pigs in these experiments were kept on a diet of hay and oats and were of large size. The tests were made with different doses of caffein in order to determine the limits of toxicity when the drug was administered by mouth.

Guinea pig 129. White and black male. Weight, 855 grams. Diet, oats and hay.

June 6: 2.20 p. m., 12 cc of 2 per cent caffein (0.28 gram per kilo) by mouth; 3 p. m., reflexes increased; 5 p. m., reflexes still more increased; no other symptoms.

June 7: 9 a. m., found dead; guinea pig passed 75 cc urine, which was almost colorless. *Autopsy*:

Heart and blood vessels injected; lungs congested; small intestines congested; other organs apparently normal.

Guinea pig 130. Black and brown male. Weight, 800 grams. Diet, oats and hay.

June 6: 2.30 p. m., 12 cc of 2 per cent caffein (0.3 gram per kilo) administered by mouth; 3 p. m., reflexes increased; 5 p. m., increase of reflexes greater than at 3 p. m.

June 7: 9 a. m., found dead; only a few cubic centimeters of urine passed since 4 p. m. *Autopsy*: Heart and blood vessels injected; lungs congested; small intestines congested slightly.

Guinea pig 181. White and yellow male. Weight, 860 grams. Diet, oats and hay.

June 6: 2.40 p. m., 12 cc 2 per cent caffein administered by mouth; 3 p. m., reflexes increased; 5 p. m., reflexes still more marked.

June 7: 9 a. m., found dead, pig passed about 5 cc urine since 4 p. m. of previous day. *Autopsy*: Same as in No. 130.

Guinea pig 136. White and black male. Weight, 1,000 grams. Diet, oats and hay.

June 9: 4 p. m., 7.5 cc 2 per cent caffein solution injected subcutaneously into the back; 4.50 p. m., reflexes increased.

June 10: 9.30 a. m., more sensitive than normal guinea pigs, but reflexes not quite so marked as at 5 p. m. previous day, about 15 cc urine passed since caffein was injected, reduction of Fehling's solution considerable, no albumin.

June 13: Alive and in good condition. Appetite good. (NOTE.—Parallel test with urine from two guinea pigs which did not receive caffein failed to show reduction of Fehling's solution.)

Guinea pig 137. White and brown male. Weight, 925 grams. Diet, oats and hay.

June 9: 4 p. m., 7 cc 2 per cent solution caffein injected subcutaneously; 4.50 p. m., reflexes increased.

June 10: Reflexes less marked than at 5 p. m. previous day, but is more sensitive than normal guinea pig, about 10 cc urine passed since injection of caffein, moderate amount of reduction of Fehling's solution.

June 13: Guinea pig alive, appetite good, condition good.

June 16: 9 a. m., found dead.

Guinea pig 135. White and black male. Weight, 955 grams. Diet, hay and oats.

June 9: 3 p. m., 7.5 cc 2 per cent caffein solution given by mouth through stomach tube; 4.50 p. m., reflexes increased.

June 10: Reflexes less than on previous day and less marked than in No. 136, a few cubic centimeters dirty brown urine collected but could not be tested for reduction.

June 13: Condition good, appetite good.

June 16: 9 a. m., found dead.

Guinea pig 134. White and brown male. Weight, 740 grams. Diet, hay and oats.

June 9: 2.55 p. m., 6 cc warm 2 per cent caffein solution given by mouth through stomach tube; 4.50 p. m., reflexes increased.

June 10: 9.30 a. m., reflexes much less than day before, increase slight, a few cubic centimeters of urine passed since injection of caffein, looked brown and dirty, could not be tested for reducing substances.

June 13: Guinea pig alive, appetite good, condition good.

June 14: 9 a. m., found dead.

Guinea pig 128. White and black male. Weight, 1,075 grams. Diet, hay and oats.

June 7: 10 a. m., 11 cc 2 per cent caffein by mouth through stomach tube; 11.10 a. m., no symptoms, no urine passed; 1 p. m., increased reflexes, about 15 cc (estimated) urine passed; 4 p. m., reflexes increased, still more urine passed (about 20 cc); 4.50 p. m., tetanus, frequent attacks, then paralysis and death at 4.58 p. m. *Autopsy*: Lungs congested; blood vessels of heart injected; intestines slightly congested; fatty liver.

Guinea pig 126. White and gray male. Weight, 980 grams. Diet, oats and hay.

June 7: 9.40 a. m., 9.8 cc 2 per cent caffein given by mouth through stomach tube; 10 a. m., no symptoms; 11.10 a. m., no urine passed, reflexes increased; 1 p. m., more sensitive than before; 4 p. m., increase of reflexes more marked, no urine passed; 4.45 p. m., about 15 cc urine collected; 5 p. m., no change.

June 8: 9 a. m., reflexes about the same as 5 p. m. previous day, no urine passed since 4.45 p. m. previous day, considerable reduction of Fehling's solution, much more than urine of guinea pig

No. 127; 11.05 a. m., convulsions; 12 noon, still alive and stretched out on abdomen; died at 1 p. m. *Autopsy*: Lungs badly congested; heart and blood vessels injected; blood vessels of kidney and of small intestines injected; liver engorged with blood; a few necrotic spots in stomach.

Guinea pig 127. White, black, and brown male. Weight, 760 grams. Diet, oats and hay.

June 7: 9.50 a. m., 7.6 cc 2 per cent caffein by mouth through stomach tube; 10 a. m., no symptoms; 11.10 a. m., reflexes increased, no urine passed; 1 p. m., very sensitive; 4 p. m., sensitiveness increased, about 20 cc urine passed; 5 p. m., no change.

June 8: 9 a. m., reflexes about the same as 5 p. m. previous day; 9.30 a. m., guinea pig passed 30 cc urine since he received caffein, urine showed a moderate amount of reduction; 12 noon, convulsions; died at 2.30 p. m. *Autopsy*: Lungs congested; blood vessels of heart and of intestines injected; numerous necrotic spots in stomach; other organs apparently normal.

Examination of the protocols shows that the absorption of caffein from the gastro-intestinal canal was quite rapid, symptoms having been observed as early as 20 minutes after its introduction. The duration of life, it will be remarked, varied with the size of the dose. When approximately 3 decigrams per kilo were fed, all the animals died in the night. They lived, therefore, less than 18 hours. Two decigrams per kilo were likewise fatal, but the duration of life was longer. To decide whether or not this is the smallest fatal dose, smaller amounts were fed. It seemed at first that about 150 mg per kilo was the smallest toxic dose, and about 200 mg per kilo the minimum fatal dose. Macroscopic examination of the organs, however, threw some doubt on this supposition, for well-marked lesions were noticed in all of the guinea pigs which received 0.2 gram per kilo. It is quite possible, therefore, that the minimum fatal dose may be somewhat higher, as we have reason to believe that, at least in some pathologic conditions, the susceptibility to caffein is increased. The presence of fatty changes in the liver of No. 128 and the rapid death in this case lends especial support to this view. Hence, the minimum fatal dose is probably greater than 0.2 gram per kilo for the normal guinea pig. The doses employed for the tests on guinea pigs Nos. 129, 130, and 131 may be considered therefore the minimum fatal dose for these animals. It will be also remarked that macroscopical examination of the organs of these animals failed to reveal the presence of severe lesions. That the minimum toxic dose is probably much smaller than 0.28 gram per kilo is indicated by the experiments on guinea pigs Nos. 135 and 134, in which 0.15 gram caffein per kilo induced mild symptoms in from two to three hours. Both of these, however, and also No. 137 died four to six days after the drug was fed. As already pointed out, caffein may be a factor in the delayed death of guinea pigs which received moderate doses of it. That this supposition may also be true for guinea pigs Nos. 134, 135, and 137 is indeed made probable by the observation that after moderate amounts of caffein symptoms may persist in the guinea pig for about 24 hours, and also by the fact that the secretion of urine in these animals was very scanty, as shown in the preceding record of the experiments; this means slow elimination of caffein and its products of decomposition. It is conceivable that the presence of toxic amounts of caffein in the body for a considerable length of time would induce changes that ultimately lead to the death of the animal or that morbid processes are set up by the combined action of caffein and some preexisting poison. Since some guinea pigs, however, survived the doses indicated, it is more probable that such changes would be brought about by caffein in the presence of a preexisting poison. The death of these pigs, and also of No. 137 several days later, is difficult to account for on any other theory than the one suggested. Were it not for the fact that controls, that is, animals of the same lot which had not received caffein survived all of the experimental animals, changed conditions of environment or accident might be considered the cause of death in the guinea pigs of the last series.

TABLE 6.—*Subcutaneous injection of guinea pigs.*

Number of pig.	Weight.	Caffein per kilo	Appearance of symptoms in	Duration of life.	Diet.	Month.	Remarks.
			SERIES A.				
	Grams.	*Mg.*					
18	500	0.300	2 minutes	2 hours 40 minutes	Carrots	March	Female.
15	548	.290		30 minutes	Oats	do.	Do.
14	442	.360	15 minutes	do.	do.	do.	Do.
			SERIES B.				
20	497	0.200	2 hours 20 minutes	3 hours 30 minutes	Oats	April	Female.
38	570	.210	25 minutes	2 hours 15 minutes	Carrots	February	Male.
37	820	.200	1 hour 25 minutes	Less than 18 hours	Carrots and oats.	do.	Do.
13	618	.194	25 hours		Oats	March	Female.
36	850	.200	1 hour 30 minutes	18 hours	Carrots and	February	Male.

					oats.		
SERIES C.							
66	510	0.200	None	5 days	Oats	October	Male.
65	510	.200	do.	Survived	do.	do.	do.
60	320	.219	1 hour 15 minutes	Within 18 hours	do.	do.	Female.
57	350	.200	do.	About 2½ days	do.	do.	Do.
68	785	.200	25 minutes	Less than 22 hours	do.	do.	Male.
69	585	.200	20 minutes	9 days	do.	do.	Do.
61	330	.240	1 hour 30 minutes	Less than 24 hours	do.	do.	Female.
62	335	.238	1 hour	do.	do.	do.	Do.
70	545	.238	50 minutes	About 2 days	do.	do.	Male.
71	540	.240	45 minutes	do.	do.	do.	Do.
72	560	.232	35 minutes	About 3 days	do.	do.	Do.
SERIES D.							
49	510	0.160	1 hour 40 minutes	Less than 18 hours	Oats	March	Male.
40	630	.158		Less than 2 days	Oats and carrots.	February	Do.
45	435	.160	None	Survived	Oats	March	Female.
39	820	.150		2 days	Oats and carrots.	February	Male.
41	660	.150		Survived	do.	do.	
46	470	.170	1 hour 20 minutes	do.	Oats (?)	March	Female.
SERIES E.							
19	556	0.100		Survived	Oats	April	
42	490	.120	None	do.	do.	February	
43	430	.116	do.	do.	do.	do.	
44	535	.112	do.	do.	do.	do.	
97	330	.100	do.	do.	do.	November	
98	520	.100	do.	About 3 days	Carrots	do.	

TABLE 7.—*Injection into peritoneal cavity; guinea pigs.*

Number of pig.	**Weight.**	**Caffein per kilo**	**Appearance of symptoms in**	**Duration of life.**	**Diet.**	**Month.**	**Remarks.**
	Grams.	*Mg.*					
SERIES A.							
41	700	0.130	2 hours 15 minutes	22 hours	Oats	April	Male.
49	370	.135	2 hours	Survived	do.	do.	Do.
47	550	.127	1 hour	do.	do.	March	Female.
50	290	.138	2 hours	do.	do.	April	Do.
SERIES B.							
51	415	0.144	None	Survived	Oats	October	Female.
52	450	.155	do.	do.	do.	do.	Male.
53	490	.163	do.	do.	do.	do.	Do.
SERIES C.							
59	375	0.200	15 minutes	Survived	Oats	October	Gray and white.
58	380	.200	10 minutes	do.	do.	do.	
56	440	.200	15 minutes	14 days	do.	do.	Male.
55	690	.188	1 hour	3 hours 30 minutes	do.	do.	Do.
SERIES D.							
67	330	0.240	5 minutes	30 minutes	Oats	October	Male.
63	340	.235		Survived	do.	do.	Do.
64	305	.250	25 minutes	4 hours 40 minutes	do.	do.	Female.

TABLE 8.—*Caffein by mouth; guinea pigs.*

SERIES A.							
		Caffein	**Appearance**				

Number of pig.	Weight.	per kilo	of symptoms in	Duration of life.	Diet.	Month.	Remarks.
	Grams.	*Mg.*					
129	855	0.280	40 minutes	Less than 8 hours	Hay and oats	June	Male.
130	800	.300	30 minutes	Less than 18 hours	do.	do.	Do.
131	860	.280	20 minutes	do.	do.	do.	Do.
135	955	.150	1 hour 50 minutes	6 days	do.	do.	Do.
134	740	.160	3 hours	4 days	do.	do.	Do.
[1]137	925	.150	50 minutes	6 days	do.	do.	Do.
[1]136	1,000	.150	do.	Survived	do.	do.	Do.
126	980	.200	20 minutes	27 hours	do.	do.	Do.
127	760	.200	1 hour	28 hours	do.	do.	Do.
128	1,075	.200	3 hours	7 hours	do.	do.	Do.

[1] Subcutaneous injection for comparison.

SUMMARY.

A survey of the results obtained in experiments on guinea pigs shows that the mode of introduction of caffein exerts but little influence on its toxicity. On careful analysis it will be observed that the rate of absorption after the administration of caffein by mouth, subcutaneously, or intraperitoneally is about the same for the time of appearance of symptoms. The persistence of the symptoms of caffein intoxication observed in these experiments for 24 hours after administration points to slow elimination, which may be expected, owing to the fact that the guinea pigs passed but little urine and caffein is not diuretic for these animals. The prolonged presence of caffein in the body probably exerts a harmful influence or after effect, which may account for the delayed death of some animals many days after a single dose of caffein was given. Among the factors which undoubtedly influence toxicity, season should be considered, while the presence of a diseased condition undoubtedly tends to decrease the resistance of the guinea pig to caffein. Diet was without any influence on the toxicity of the single dose of caffein.

EXPERIMENTS ON CATS.

These experiments were performed on well-fed animals which were kept under observation for several days before the tests with caffein were made. The diet consisted of meat exclusively. In some cases the urine was examined for albumin and sugar before caffein was given. No tests with caffein were made if large amounts of albumin were found. It may be remarked that sugar was never found in cats before the administration of caffein, but that considerable amounts of it were found in some cases after it was given. Studies by various modes of administration were made, by subcutaneous injection, intraperitoneally, or by mouth. Attention was also directed to the resistance to caffein in young cats, several experiments on kittens being made with this object in view.

SUBCUTANEOUS INJECTION.

Rost stated that caffein is eliminated in the urine unchanged after its introduction into the body and that the amounts found varied with different species of animals. In the rabbit the amount eliminated was about 21 per cent; in the dog about 8 per cent; and in the cat somewhat less than 2.5 per cent. It would appear, therefore, that the cat decomposes caffein more readily than the rabbit or dog; its resistance consequently ought to be greater than that of the other animals. Moderately large doses were accordingly employed in the preliminary experiments (series A), but the results obtained, as shown in the protocols, indicated that caffein is fully as toxic for the cat as for the rabbit or dog. The doses were then decreased and experiments were performed in order to ascertain the smallest toxic as well as the smallest fatal dose.

SERIES A.

Three decigrams of caffein per kilo were administered in these experiments. The results are shown in the following protocols:

Cat 4. Black and white. Weight, 1,440 grams.

May 26: 10.05 a. m., 22 cc 2 per cent caffein (0.3 gram per kilo) injected subcutaneously; 11.10 a. m., copious salivation, cat irritable, muscular stiffness present, but no tetanus; 11.45 a. m., cat restless, convulsions, attacks of short duration, no paralysis observed after the convulsions, pupils dilated; 4.45 p. m., cat quiet, slight paralysis present.

May 27: Cat exhausted.

May 28: Found dead.

Cat 5. Black and white male. Weight, 1,396 grams.

June 3: 10 a. m. 21 cc of 2 per cent caffein (0.3 gram per kilo) injected subcutaneously; 12 noon, found dead.

Although there was considerable difference in the duration of life following the injection of the same dose of caffein per kilo, the final outcome was the same, as both cats died from the effects of the drug. One died within 2 hours and the other lived more than 30 hours after its administration. Three decigrams of caffein per kilo is, therefore, surely fatal to these animals. Tests made with smaller doses are shown in the following experiments:

SERIES B.

In these experiments the doses employed ranged between 0.20 and 0.25 gram caffein per kilo.

Cat 3. Black and white female. Weight, 2,854 grams. Well fed.

June 4: 10.30 a. m., 35 cc 2 per cent caffein (0.25 gram per kilo) injected subcutaneously; 11 a. m., found dead.

Cat 6. Black and white. Weight, 1,645 grams.

June 3: 20 cc 2 per cent caffein (0.243 gram per kilo) injected subcutaneously at 3 p. m., cat grew very irritable in a few minutes; about 4 p. m. reflexes decidedly increased; 5 p. m., cat paralyzed.

June 4: Cat found dead.

Cat 8. Weight, 1,735 grams.

October 7: 4 p. m., 22 cc 2 per cent caffein (0.25 gram per kilo) injected subcutaneously in the back; 4.30 p. m., cat irritable, salivation profuse, convulsions; died at 5.30 p. m.; no urine passed after caffein was given.

Cat 9. Weight, 1,960 grams.

October 7: 3.45 p. m., 25 cc 2 per cent caffein (0.25 gram per kilo) injected subcutaneously in the back; 4.45 p. m., cat very irritable, repeated attacks of convulsions, salivation copious; died at 5.30 p. m.; cat did not urinate after injection of caffein.

Cat 12. Striped kitten. Weight, 1,185 grams.

October 9: Urine examined, no albumin, no sugar; 1.45 p. m., 12 cc 2 per cent caffein administered; 5 p. m., cat alive, no symptoms except salivation and general irritability.

October 10: 10.30 a. m., found dead. About 15 cc urine collected, but no examination made.

Cat 14. Black. Weight, 1,855 grams.

October 8: 1.40 p. m., 18.5 cc 2 per cent caffein (0.2 gram per kilo); 3 p. m., cat became restless about 10 minutes after caffein was injected; cried persistently and moved about in cage, no convulsions, cat urinated about 15 cc, cat defecated.

October 9: 9 a. m., cat found dead in cage. Urine gave very heavy reduction of Fehling's solution (much more than was obtained from urine of rabbits); 20 cc urine analyzed contained 4.65 per cent sugar. *Autopsy*: Lungs deeply congested; liver marked fatty infiltration and degeneration; spleen normal; kidneys pale and anemic; intestines normal; stomach normal.

Cat 15. Striped. Weight, 2,145 grams.

October 8: 2 p. m., 22 cc (0.2 gram per kilo) 2 per cent caffein injected subcutaneously; 2.30 p. m., cat irritable, restless, trying to get out of cage, crying persistently; 2.40, convulsions lasting about two minutes, then cat raised itself and made attempts to get out of cage, no salivation, cat urinated about 10 cc and defecated.

October 9: 9 a. m., cat found dead in cage, about 10 cc of urine contained enormous quantities of sugar. *Autopsy*: Lungs severely congested; liver showed marked fatty degeneration; spleen normal; kidneys slightly pale and anemic; intestines mildly congested; stomach normal.

Cat 19. White. Weight, 1,100 grams.

October 20: 13 cc of 2 per cent caffein (0.236 gram per kilo). About 15 minutes later cat became irritable, reflexes increased, persistent crying, stiffness of extremities, diarrhea present; 4.30 p. m., stiffness of muscles, coordination much disturbed, walked with great difficulty; 4.30 p. m., no new symptoms, persistent crying continued.

October 21: Found dead.

Cat 20. White kitten. Weight, 790 grams.

October 20: 11.35 a. m., 10 cc 2 per cent caffein (0.25 gram per kilo) given subcutaneously; 12 noon, convulsions followed by paralysis; 1.30 p. m., still breathing, apparently in comatose condition, lay on its side, dyspnœa, profuse salivation; 4 p. m., convulsions and death.

The results of the experiments of series B show that a dose of even 0.2 caffein per kilo is very toxic for the cat. Symptoms appeared in one animal 40 minutes after the injection of caffein. Some of them were found dead 18 hours after injection, which means that the duration of life was probably a great deal less since there was evidence that they had been dead for some time. Death

occurred quite soon after larger doses were injected. Cat No. 3 died 30 minutes after it received caffein. The amounts employed in these experiments can not be considered therefore as the minimum fatal doses. Smaller doses were then tried, as shown in the experiments of the next series.

SERIES C.

Experiments were performed on five cats which received from 140 to 155 mg per kilo, as follows:

Cat 24. Striped. Weight, 1,300 grams.

October 25: 10 a. m., 50 cc urine, albumin moderate amount—no sugar; 10 cc caffein injected subcutaneously at 12 noon; 12.30, irritable, cried persistently, no appetite; 4 p. m., no convulsions, but persistent crying.

October 27: Cat was still alive.

Cat 17. Weight, 2,620 grams.

October 12: 9.30 a. m., 65 cc urine collected; more than a trace of albumin present, no reduction of Fehling's solution; 3 p. m., 20 cc 2 per cent caffein (150 mg per kilo) injected subcutaneously; 3.15 p. m., irritable and restless.

October 13: 9 a. m., about 15 cc urine collected, reduction of Fehling's solution marked; osazone test also positive.

Cat 23. Black and white. Weight, 1,645 grams.

October 25: 10 a. m., 140 cc urine collected (since October 23), small amount of albumin present, no sugar.

October 27: 9 a. m., no albumin; no sugar in urine; 11.50 a. m., 12.5 cc caffein injected subcutaneously (0.15 gram per kilo); 1 p. m., convulsions and death.

Cat 7. Striped kitten. Weight, 1,285 grams.

October 11: Urine collected, no albumin, no sugar; 9.50 a. m., 10 cc 2 per cent caffein injected subcutaneously in the back; 10.10, violent convulsions lasting about 30 seconds; 10.20, convulsions of shorter duration; 10.30 convulsions; 10.35, convulsions lasting a few seconds; urine passed about 10.20, contained a moderate amount of albumin, but there was no reduction of Fehling's solution; 10.45, profuse salivation and paralysis; died about 10.50.

Cat 39. Yellow. Weight, 2,285 grams.

April 13: 2.40 p. m., 16 cc 2 per cent caffein (0.14 gram per kilo) injected subcutaneously in the back; 3.45 p. m., cat died.

Of the five experiments of this series three died after doses of 140, 150, and 155 mg per kilo. The other two showed symptoms of toxicity, but survived a dose of 150 mg per kilo which indicated that the minimum fatal dose was probably reached. To test this supposition smaller doses were administered, as shown in the following experiments.

SERIES D.

Ten cats were used for this series of experiments, and the doses administered varied between 103 and 139 mg per kilo. The results shown in the appended table (p.58) indicate that about 120 to 140 mg of caffein per kilo may induce mild symptoms in some cases. The conclusion may be safely drawn therefore that 150 mg per kilo is approximately the minimum fatal dose for the cat when the drug is given subcutaneously. That smaller doses are, however, by no means to be regarded as always safe is shown in the following experiments.

SERIES E.

Cat 43. Weight, 3,225 grams.[D]

September 14: 10.20 a. m., 20 cc 2 per cent caffein (0.124 gram per kilo) injected into the back; 11 a. m., tetanus and death. *Autopsy*: Lungs congested; liver congested and showed hemorrhagic spots in capsules and fatty degeneration; kidneys slightly congested; other organs normal.

Cat 48. Black female. Weight, 3,050 grams.

September 14: 18 cc 2 per cent caffein (0.118 gram per kilo) injected subcutaneously in the back; 10.30 a. m., violent convulsions and death. *Autopsy*: Lungs congested in spots showing numerous petechia; liver congested; spleen congested; other organs normal.

The diminished resistance to caffein of cats Nos. 43 and 48 might be due to the pathologic changes found on autopsy, for evidence is not wanting that the toxicity of drugs might be greatly altered under pathological conditions. Hunt[40] has shown that resistance to acetonitril is considerably diminished in chronic alcoholism. This seems to be true also of other drugs under abnormal conditions. Smaller doses of atropin[78] are required in lead poisoning than under normal conditions to produce the same results. The following experiment is of interest in this connection, for in this case a much smaller dose than was given in experiments Nos. 43 and 48 produced the typical symptoms of caffein poisoning and proved to be fatal.

Cat 47, black and white male. Weight, 4,220 grams.

September 15: Received subcutaneously 18 cc 2 per cent caffein (0.084 gram per kilo); no symptoms observed for about six hours.

September 16: No symptoms.

September 17: Weight, 4,250 grams; injected 18 cc 2 per cent caffein (0.084 gram per kilo); tetanus and death after two hours. *Autopsy*: Severe hemorrhagic pneumonia; kidneys pale, other organs normal.

Since two controls survived the same dose in proportion to the body weight of the animal without showing any symptoms, the assumption is justified that the lower resistance to caffein was due to the presence of pneumonia, thus affording additional support to the view that the toxicity of caffein may be increased in disease.

INJECTION INTO THE PERITONEAL CAVITY.

These experiments were carried out on full-grown and on young subjects. As in previous experiments, doses of different sizes were employed. A dose of 0.2 gram per kilo was tried first and then reduced gradually to 0.1 gram per kilo.

Cat 10. Female. Weight, 2,970 grams.

October 9, 1909: 1.30 p. m., 30 cc 2 per cent caffein (0.2 gram per kilo) injected into the peritoneal cavity; urine examined for albumin and sugar, negative; cat found dead at 2.30 p. m. No urine in the bladder.

Cat 16. Black female. Weight, 2,420 grams.

October 9, 1910: Urine examined for albumin and sugar, negative; 2.30 p. m., 22 cc 2 per cent caffein (0.183 gram per kilo) injected into the peritoneal cavity; found dead at 3 p. m.

Cat 99. Well-fed gray female. Weight, 3 kilos.

June 22, 1911: 3.40 p. m., 15 cc 2 per cent caffein injected into peritoneal cavity; salivation and marked irritability within one hour after injection.

June 24: Alive, appetite good.

Cat 98. Well-fed black male. Weight, 4,100 grams.

June 22: 3.45 p. m., 20.5 cc 2 per cent caffein (0.1 gram per kilo) injected into peritoneal cavity; very irritable a few minutes after injection, no other symptoms.

June 24: No symptoms, appetite good.

Cat 93. Black and white. Weight, 1,450 grams.

June 22: 3 p. m., 30 cc 2 per cent caffein (0.137 gram per kilo) injected into peritoneal cavity; salivation, no other symptoms; under observation until 6 p. m.

June 23: 9 a. m., no urine, cat showed no symptoms.

June 24: 9 a. m., no symptoms, took nourishment as usual.

Cat 87. Well-fed white female. Weight, 2,615 grams.

June 23: 2.45 p. m., 19 cc 2 per cent caffein (0.145 gram per kilo) injected into peritoneal cavity; became irritable and restless.

June 24: 9 a. m., no symptoms, took nourishment as usual.

Cat 97. Gray. Age, 3 months. Weight, 500 grams. Diet, meat.

June 24: 2.25 p. m., 5 cc 2 per cent caffein (0.2 gram per kilo) injected into peritoneal cavity; 4 p. m., no symptoms.

June 29: Died.

Cat 96. Gray and white. Age, 3 months. Weight, 575 grams. Diet, meat.

June 24: 2.20 p. m., 4 cc 2 per cent caffein (0.139 gram per kilo) injected into peritoneal cavity; 3.55 p. m., no symptoms.

June 30: Died.

Cat 95. Black. Age, about 3 months. Weight, 860 grams. Diet, meat.

June 24: 10.15 a. m., 8.6 cc 2 per cent caffein injected into peritoneal cavity, salivation immediately after injection; 10.25, convulsions and paralysis; died 10.45 a. m. *Autopsy*: Macroscopical examination of the organs, negative.

Cat 94. Black and white. Weight, 790 grams. Age, about 3 months. Diet, meat.

June 24: 10 a. m., 8 cc 2 per cent caffein injected into peritoneal cavity; 4 p. m. under continual

observation since injection, cat very irritable, respiration more rapid than normal, diarrhea present.

Examination of the above protocols show that a dose of 2 decigrams per kilo was fatal within one hour to one cat and that a somewhat smaller dose killed another individual in 30 minutes. Amounts under 0.15 gram per kilo were just sufficient to induce mild symptoms, such as increased irritability and salivation, which disappeared within a few hours. In no case were the effects noticeable on the following day. The experiments on young kittens are especially interesting, as they proved, contrary to expectation, to be distinctly more resistant than full grown individuals. The death of Nos. 97 and 96 within five and six days, respectively, can not be ascribed to caffein, since some of the controls also died. Moreover, it will be remarked in this connection that no symptoms appeared in three of the four young kittens after the administration of a dose which was rapidly fatal to adult cats. The rapid death of No. 95 after the same dose forms an exception which can not be accounted for, as macroscopical examination at autopsy proved negative.

ADMINISTRATION BY MOUTH.

Two decigrams per kilo were given at first, but it was found that this amount was surely fatal. The dose was therefore reduced to 0.125 gram per kilo. In all of these experiments caffein was given by means of a soft rubber catheter slipped over the stem of a funnel which served as a stomach tube. A 2 per cent aqueous solution was used throughout these tests except in one case in which caffein was given mixed with the food.

Cat 92. Black and white female. Weight, 1,750 grams.

June 10: 12.05 p. m., 14 cc 2 per cent caffein (0.16 gram per kilo) given by mouth; cat was quiet when tied on holder, struggled only a little when tube was put into stomach; 12.30 p. m., cat vomited, no other symptoms.

June 13: Condition good, appetite good.

Cat 87. White female. Weight, 2,620 grams. Diet, meat.

June 5: 2.15 p. m., 20 cc 2 per cent caffein (0.15 gram per kilo) solution administered by mouth through stomach tube; 2.30 p. m., cat irritable, but no other symptoms; 5 p. m., condition about the same, except that it was more irritable and showed some stiffness of the extremities.

June 13: Alive and in good condition, appetite good, not irritable.

Cat 91. White female. Weight, 3,050 grams.

June 10: 12 noon, 23 cc (0.15 gram per kilo) of 2 per cent caffein administered by mouth, cat struggled violently; 1.30, salivation; 1.40 p. m., convulsions; died at 2 p. m. *Autopsy*: Congestion of lungs, liver, and spleen; heart vessels injected; other organs normal.

Cat 88. Black and white female. Weight, 3,260 grams. Diet, meat.

June 5: 2.20 p. m., 25 cc of 2 per cent caffein (0.15 gram per kilo) given by mouth; 2.45 p. m., cat irritable, no other symptoms (cat did not vomit after the administration of caffein); 4 p. m., cat found dead. *Autopsy*: Liver very much congested; heart contracted; body was still warm at the time of autopsy.

Cat 90. White and yellow female. Weight, 2,685 grams. Diet, meat.

June 5: 3.15 p. m., 27 cc of 2 per cent caffein (0.2 gram per kilo) given by mouth through stomach tube, about half an hour later cat became irritable and began to salivate; at 4.30 p. m. salivation became more marked, dyspnœa was well developed, and the cat was quite restless and had tremors; 5 p. m., short spasms of posterior extremities, but lay quietly in the cage most of the time; 5.20 p. m., convulsions of short duration and death, muscular relaxation followed immediately after convulsions, no vomiting, diarrhea observed after administration of caffein, and cat passed about 10 cc of urine.

June 6: 9 a. m., found dead.

Cat 89. White and black female. Weight, 2,860 grams. Diet, meat.

June 5: 3.15 p. m., 28.6 cc (0.2 gram per kilo) of 2 per cent caffein given by mouth through stomach tube, no vomiting observed, nor any other symptoms; 3.30, found dead. *Autopsy*: Organs normal; liver congested.

Cat 82. Gray female. March 3, weight 2,450 grams; June 6, weight 2,750 grams. Diet, 150 grams of meat daily.

June 7: Given 0.4125 gram of caffein in 150 grams of meat, did not eat.

June 8: Given 0.4125 gram of caffein in 150 grams of meat, refused to eat.

June 9: Given 150 grams of meat without caffein, ate all of it.

June 10: No food given.

June 11: No food given.

June 12: Given 0.4125 gram caffein in 150 grams of meat (150 mg per kilo), ate all of it.

June 13: Found dead. *Autopsy*: Lungs congested, liver congested; other organs apparently normal.

Cat 100. Gray female. Weight, 2,740 grams. Diet, meat.

July 17: 3 p. m., 17 cc of 2 per cent caffein (124 mg per kilo) given by mouth through stomach tube at 3.20 p. m.; 5 p. m., very irritable, but no other symptoms.

July 18: Under observation all day, no symptoms.

Cat 93. Black and white female. Weight, 1,640 grams.

July 17: 3.30 p. m., 10 cc (0.125 gram per kilo) of 2 per cent caffein given by mouth through stomach tube.

July 18: Under observation all day, no symptoms.

From the results of the experiments of this series it appears that 0.15 gram caffein per kilo may be fatal within a few hours after its administration, even if the drug is mixed with a moderate amount of meat. Experiments 87 and 92 show, however, that this amount may be borne by some individuals without any serious consequences, as the cats were under observation for some time after they received caffein, and no untoward symptoms were noticed in either of them during this time. It may be remarked that cat No. 92 vomited shortly after it received caffein. It is practically certain, therefore, that this amount of caffein in proportion to the weight of the animal will in the great majority of cases prove fatal, and perhaps in a smaller percentage of individuals it is surely toxic if it does not escape absorption. Smaller doses may cause irritability in some individuals, but symptoms referable to nervous symptoms of muscles were absent, as in experiments Nos. 93 and 100. The minimum fatal dose of caffein for the cat when given by mouth is, therefore, 0.15 gram per kilo.

TABLE 9.—*Subcutaneous injection; cats.*

Number.	Weight.	Caffein per kilo	Symptoms.	Duration of life.
	Grams.	*Gram.*		
SERIES A.				
4	1,440	0.30	65 minutes	Over 29 hours.
5	1,396	.30		About 2 hours.
SERIES B.				
3	2,854	0.25		30 minutes.
6	1,645	.243	Few minutes	1 hour 30 minutes.
8	1,735	.25	30 minutes.	1 hour.
9	1,960	.25	1 hour	1 hour 45 minutes.
12	1,185	.20	3 hours	Less than 18 hours.
14	1,855	.20	1 hour 20 minutes	Do.
15	2,145	.20	40 minutes.	Do.
19	1,100	.236	15 minutes	Do.
20	790	.25		4 hours 30 minutes.
SERIES C.				
24	1,300	0.153	1 hour	Survived.
17	2,620	.15	15 minutes	Do.
23	1,645	.15		1 hour 10 minutes.
7	1,285	.155	20 minutes	1 hour.
9	2,285	.14		65 minutes.
SERIES D.				
13	730	0.139	Restlessness	
21	1,165	.138	None	
25	965	.103	do.	
26	1,605	.125	do.	
27	1,625	.125	do.	
28	2,335	.128	do.	Received 2 doses: survived.
40	2,710	.129	do.	Do.
41	1,785	.123	do.	Do.
42	2,315	.112	do.	Do.
38	2,325	.120	Mild	Died after second dose.
SERIES E.				
43	3,225	0.124		40 minutes.
48	3,050	.118		Died soon after.
47	4,220	.084		Survived.
[2]47	4,250	.084		2 hours.

[1] Pathological conditions. [2] Two days after first injection.

TABLE 10.—*Injections into peritoneal cavity; cats.*

SERIES A.

Number.	Weight.	Caffein per kilo	Symptoms.	Duration of life.
	Grams.	*Gram.*		
99	3,000	0.100	Mild	Survived.
98	4,100	.100	do.[1]	Do.
93	1,450	.137	Very mild	Do.
87	2,615	.145	do.	Do.
97	505	.200	None	5 days.
96	575	.139	do.	6 days.
95	860	.200	15 minutes	30 minutes.
94	790	.200	Diarrhea	Survived.
10	2,970	.200		1 hour.
16	2,420	.183		30 minutes.

[1] In few minutes.

TABLE 11.—*Administration of caffein by mouth; cats.*

SERIES A.

Number.	Weight.	Caffein per kilo	Symptoms.	Duration of life.
	Grams.	*Gram.*		
91	3,050	0.15	1 hour 40 minutes	2 hours.
88	3,260	.15		1 hour 40 minutes.
92	1,750	.16	25 minutes	Survived.
87	2,620	.15	3 hours	Do.
90	2,685	.20	1 hour 15 minutes	Less than 18 hours.
89	2,860	.20		75 minutes.
82	2,450	.15		Less than 24 hours.
100	2,740	.124	1 hour 40 minutes	Survived.
93	1,640	.125		Do.

SUMMARY.

The toxicity of caffein in cats is shown to be the same when given by mouth as when injected subcutaneously, the minimum fatal doses in both cases being 0.15 gram per kilo. When introduced by the intraperitoneal route, caffein is, on the contrary, distinctly less toxic. After the administration of 0.137 and 0.145 gram caffein per kilo (Nos. 93 and 87) salivation in one cat (No. 93) and irritability and muscular stiffness in the other were the only effects noticed. These symptoms were no longer observed the next day and the cats appeared to be perfectly normal. Experiments with larger doses indicate that the minimum fatal dose by this method of administration is about 0.2 gram per kilo.

EXPERIMENTS ON DOGS.

The experiments were carried out on well-fed adult dogs and on puppies, kept under observation for some time before the drug was administered. Only those manifesting no signs of abnormality were used for these tests. Caffein was given by mouth mixed with 10 to 20 grams of meat, or subcutaneously in 2 per cent aqueous solution. The young animals received caffein dissolved in milk. The determination of the minimum toxic or fatal doses when the drug was fed presented considerable difficulty, as in many instances the ingestion of the drug was closely followed by vomiting.

ADMINISTRATION BY MOUTH.

SERIES A.

The effective dose in these experiments showed considerable variation. One dog (No. 38) died after a dose of 0.12 gram caffein per kilo, while some subjects survived doses of 0.2 and 0.23 gram per kilo. In the 12 experiments given in Table 12, page 62, it will be noticed that from 0.12 to 0.152 gram per kilo proved fatal to three dogs, while three others survived the same amounts in proportion to the body weight. The results were the same with larger doses. It may be observed in this connection that in the case of the five dogs in which vomiting was noticed some time during the 24 hours following the administration of caffein, four survived, No. 38 being the exception. The greater toxicity of caffein in this case is in all probability due to some morbid process, the presence of which was indicated by the high temperature of this subject.

That vomiting may avert a fatal issue after larger doses of caffein is made further probable by experiment on dog No. 48, for which, in the absence of vomiting, a dose of 0.2 gram of caffein per kilo proved fatal. On this supposition the discrepancy in the results obtained in this series may be

readily explained. The smallest doses which proved fatal in these experiments were 0.145 and 0.152 gram per kilo. No. 38, which died from a dose of 0.12 gram per kilo, may be considered as an exception, as this subject was not normal. Experiments with caffein on dogs were made at various other times in this laboratory but failed to show that smaller doses of caffein, even when vomiting did not occur after its administration, were fatal, although toxic effects were observed. The conclusion is therefore justified that the minimum fatal dose of caffein for the normal dog is about 0.15 gram per kilo when given by mouth.

SUBCUTANEOUS INJECTION.

SERIES B.

To determine the toxicity of caffein more accurately, especially for comparison with animals of other species, the subcutaneous method of administration was also used. The injections were made with a syringe of 20 cc capacity, the contents of which were introduced into contiguous areas. The results of experiments on six dogs indicate that approximately 150 to 160 mg per kilo is the minimum fatal dose, since such doses proved fatal to two out of the three animals receiving this amount, while three others which received doses of from 143 to 160 mg per kilo survived.

EXPERIMENTS ON PUPPIES.

SERIES C.

In these experiments the resistance of young growing puppies to caffein was studied. Caffein was given by mouth to all the subjects except one, to which it was administered subcutaneously. The protocols, only a few of which are given, and the tabulated data of the experiments (p. 62) show that the age of the animal has a decided influence on the toxicity of caffein.

Dog 11. Weight, 1,260 grams.

August 2: At 10 a. m. given 12.5 cc of 2 per cent of caffein through stomach tube; 2 p. m., had convulsions, diarrhea, salivation, and stiffness of limbs.

August 3: Found dead 9 a. m. *Autopsy*: Thoracic viscera apparently normal; stomach immensely distended and filled with a white, cheesy mass and some fluid; round worms plentiful in stomach and small intestine; mucosa of entire intestine congested; contents of lower intestine congested; liver pale; spleen flabby; kidney congested.

Dog 10. Weight, 1,650 grams.

July 26: 9.30 a. m., 29 cc of 2 per cent caffein added to 60 cc of milk offered, but refused, and was therefore fed by mouth through stomach tube; 10.25 a. m., no symptoms; 11.30 a. m., restlessness, extremities stiff, post. extremities spread apart, dog shows well-marked symptoms of caffein poisoning; 12.10 p. m., symptoms more severe, extremities extended and spread out, is lying flat on belly so that nose touches floor of the cage; 12.40 p. m., found dead; was alive at 12.10 p. m. *Autopsy*: Lungs showed hemorrhagic foci in all lobes; heart apparently normal; liver fatty; stomach and intestines filled with round worms; spleen and kidney apparently normal.

Dog 9. Weight, 3,000 grams.

July 25: 350 mg caffein per kilo; 5 p. m., lying down most of the time, occasionally walks about in stall; restlessness present, but not marked; 5.30 p. m., vomit which looked frothy and mucilaginous noticed on the floor of the stall; no meat particles noticed in vomit, though searched for; whines occasionally.

July 26: 9 a. m., looks well; no signs of the effect of caffein given the day previous.

Dog 8. Yellow female. Weight, 3,100 grams.

July 22: 10.50 a. m., received 1.1 grams of caffein in 10 grams of meat (354 mg caffein per kilo); 3 p. m., vomited mucus; gait clumsy; refused to eat; continually drinking water; very restless; 4 p. m., convulsions set in at 3.55 p. m.; tonic rigidity of the posterior extremities; profuse salivation; convulsions were both tonic and clonic in character, and resembled those seen in rabbits in caffein intoxication; a striking feature was the duration of the spasm, which began at 3.55 p. m. and kept up for more than two hours.

July 23: Found dead at 9 a. m.

The data recorded in the table and in the protocols of the experiments of series C show that four out of the seven animals experimented upon died in less than 24 hours after caffein was fed; three of these received 300 to 354 mg caffein per kilo, and one received 200 mg caffein per kilo. No. 8 vomited four hours after caffein was given. No vomiting was observed in the other three dogs. From 0.300 to 0.350 gram of caffein per kilo may be regarded, therefore, as surely fatal to young growing puppies. That this is in all probability the minimum lethal dose appears from the following experiments: No. 9, which received 350 mg per kilo, vomited one hour after and survived, which indicates that some of it was probably not absorbed. The amount which entered the circulation was therefore less than 350 mg per kilo. Since No. 15, which received 250 mg

caffein subcutaneously, likewise survived, the probabilities are that 300 to 350 mg per kilo were the minimum fatal doses for these animals. Moreover, No. 12, which received 200 mg caffein per kilo, survived, no vomiting having been observed. The case of No. 11, in which the same amount of caffein in proportion to body weight proved fatal, may be explained perhaps by the findings of the autopsy.

The results obtained in these experiments justify the conclusion that young growing dogs can stand larger doses of caffein than full-grown and older dogs.

Attention may also be called here to the difference in the symptoms produced by caffein in very young and in adult dogs. It was often noticed in these experiments that the symptoms in older subjects when given toxic doses of caffein set in rather abruptly and ended in instantaneous death. We failed to observe this phenomenon after the administration of large amounts of caffein to very young dogs, in which tonic and clonic convulsions alternating with paresis were observed. These symptoms set in rather gradually and lasted several hours (see experiment No. 8), resembling the rabbit in this regard.

SUMMARY.

The toxicity of caffein for adult dogs is about the same, whether given by mouth or injected subcutaneously. The resistance of puppies to caffein is much greater than that of adults.

TABLE 12.—*Administration by mouth; dogs.* (*Series A.*)

No.	Weight.	Caffein per kilo	Results.	Remarks.
	Kilos.	*Gram.*		
47	13.60	0.144	Survived	Vomiting observed.
55	12.75	.200	do.	Stiffness of muscles; no other symptoms.
56	7.95	.200	Found dead next day	
52	13.60	.147	Survived	
57	6.50	.230	do.	Vomited after 1 hour; convulsions after 1 hour and 45minutes.
39	23.10	.120	do.	Increased frequency of respiration, thirst, loss of appetite, vomited rest of day when he drank water, salivation, restlessness, passed feces frequently.
48	11.50	.174	do.	Vomiting observed.
48	12.00	.200	Found dead next day	No vomiting observed. Second dose was given 8 days after first.
54	13.40	.200	Survived	Vomiting observed.
49	13.15	.152	Found dead next day	
38	14.50	.120	do.	Symptoms after 1½ hours: Dog had a temperature of 104° F. before caffein was given; vomited 3 hours after caffein was fed.
18	10.80	.145	do.	

TABLE 13.—*Subcutaneous injection; dogs.* (*Series B.*)

No.	Weight.	Caffein per kilo	Results.	Remarks.
	Kilos.	*Gram.*		
62	9.30	0.161	Survived	Restlessness and vomiting one-half hour after injection.
61A	14.00	.160	Found dead next day	
63	12.00	.150	Survived	Restlessness 1 hour after injection.
64	14.00	.150	do.	Restlessness and thirst 45 minutes after injection.
59	7.20	.160	Died 1 hour and 20 minutes after injection.	Marked restlessness, but no convulsion.
61	14.60	.143	Survived	Symptoms observed in 1½ hours.

TABLE 14.—*Administration by mouth to puppies.* (*Series C.*)

No.	Weight.	Caffein per kilo.	Results.	Remarks.
	Kilos.	*Gram.*		
8	3.10	0.354	Found	Vomited in 4 hours after feeding;

			dead next day	restlessness, loss of appetite, thirst, incoordination of muscles, convulsions.
9	3.15	.350	Survived	Muscular incoordination and stiffness, restlessness, vomited 1 hour after caffein was given.
10	1.60	.350	Died in 3 hours	Convulsion; no vomiting.
11	1.26	.200	Found dead	Salivation; convulsions.
12	1.28	.200	Survived	No symptoms.
15	1.20	.250	do.	Subcutaneous injection.
16	3.50	.300	Died in 1 hour	Convulsions 45 minutes after caffein was fed.

CHRONIC CAFFEIN INTOXICATION.

The object of this study was to ascertain the effect of repeated dosage when caffein was given daily or at longer intervals. The experiments were tried on rabbits and on dogs. As in the experiments on acute intoxication, the animals were under observation for some time in the laboratory before the administration of caffein was begun in order to ascertain the presence or absence of abnormality. The relation of diet to toxicity received some attention, but the question was not studied exhaustively in the present investigation.

EXPERIMENTS ON RABBITS.

Full-grown adult as well as young rabbits were employed. The diet consisted either of carrots or of oats; water was given ad libitum. The rabbits were kept in metal cages in a well-lighted and well-ventilated room. Unnecessary handling or any other procedure tending to fatigue or to cause discomfort to the animals was very carefully avoided, since we had found that such treatment was likely to decrease the resistance of the rabbit to caffein. The caffein was administered by feeding by mouth and through a stomach tube, or by the subcutaneous method. In a good many cases it was given daily, in some at longer intervals.

SERIES A.

The experiments of this series formed a preliminary study for the purpose of testing the effect of moderate doses. One decigram of caffein per kilo was given daily for several days; when administered at longer intervals the dose was increased to 150 to 200 mg per kilo. It was found that the smaller doses did not produce any symptoms; even the weights of the animals were not influenced. Doses of medium size given on two successive days were likewise without any noticeable effect (Nos. 182, 183, 123, 101). When a third dose of this size was given within 48 or 24 hours it proved fatal (Nos. 123, 182, and 183). Exceptionally, however, moderately large doses (for rabbits) may be given for three consecutive days without fatal issue, as in rabbit No. 101. When given at intervals of two to three days, larger doses, as may be seen from the protocols, can be administered without causing acute death (Nos. 173, 181, 201).

The results of the tests of this series point to the absence of any accumulation and to the possible elimination of moderate doses of caffein and its products of decomposition within 24 hours or thereabouts. When the doses are larger the time of its elimination is apparently longer, as shown by the fact that repetitions of the dose the next day may be fatal, but when a longer interval is allowed it may be given without causing death. It will be observed that only one rabbit of this series survived, but it was extremely emaciated. This condition has been observed in a number of cases after caffein had been given for several days. Even when the drug was withdrawn the animals continued to lose weight. This may be explained by the condition of the gastro-intestinal canal as found at autopsy. The presence of inflammation of the mucous membrane of the stomach and intestines, with ulceration of the mucous membrane of the pylorus in one of the rabbits (No. 173) of the series, in all probability caused diminution or loss of appetite, which of itself would tend to cause loss of flesh and strength and finally death. Protocols of the experiments follow.

Rabbit 173. Carrots were fed from October 1 to 18 and oats for the remainder of the experiment.

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Oct. 9	1,980	0.141	Oct. 16	2,005	0.220
Oct. 11	1,905	.190	Oct. 18	1,845	
Oct. 13	1,930	.207	Oct. 20	1,740	.230

October 21: Paralysis of posterior extremities.

October 22: 9 a. m., found dead.

The urine was examined before and after the administration of caffein. No symptoms were observed after the administration of caffein (5 doses in 11 days), nor was albumen or sugar found in the urine after any of the experiments on this rabbit. *Autopsy*: Pyloric mucosa exhibited several ulcers; small intestines showed slight inflammation; liver deeply congested; kidneys showed marked inflammation of cortex; other organs practically normal.

Rabbit 181. Diet, carrots September 29-October 17, then oats.

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Oct. 4	1,425	0.105	Oct. 11	1,370	0.175
Oct. 5	1,450	.100	Oct. 13	1,385	.180
Oct. 6	1,270	.100	Oct. 16	1,345	.200
Oct. 7	1,210	.100	Oct. 17	1,030	
Oct. 8	1,375	.130	Oct. 18	1,230	
Oct. 9	1,305	.153	Oct. 20	1,215	

Rabbit was markedly emaciated and weak. No albumen or sugar found in the urine as a result of caffein feeding.

Rabbit 182. Diet of carrots from September 29.

Received caffein subcutaneously as follows:

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Oct. 4	1,765	0.100	Oct. 8	1,685	.135
Oct. 5	1,880	.100	Oct. 9		.150
Oct. 6	1,750	.100	Oct. 11	1,605	.174
Oct. 7	1,710	.100			

October 12: 11 a. m., 23 hours after caffein was given, convulsions with recovery; rabbit died at 1.30 p. m. No sugar was found in the urine at any time after the administration of caffein. Albumen was present only in one specimen.

Rabbit 183. Diet of carrots from September 29.

Received caffein subcutaneously as follows:

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Oct. 4	1,385	0.100	Oct. 8	1,310	0.153
Oct. 5	1,460	.100	Oct. 9	1,390	.142
Oct. 6	1,385	.100	Oct. 11	1,390	.187
Oct. 7	1,240	.122			

October 12: 9 a. m., found dead. No albumen was found in the urine. Only one sample contained sugar.

Rabbit 123. White, female. Diet, oats.

Received caffein subcutaneously as follows:

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Apr. 14	2,350	42	Apr. 20	2,126	141
Apr. 16	2,250	90	Apr. 21	1,965	152
Apr. 17	2,325	86	Apr. 22	1,876	160

Rabbit died 30 minutes after last injection of caffein. *Autopsy*: Stomach exhibited marked inflammation of mucosa. Slight enteritis. Liver and kidneys were deeply congested and dark colored.

Rabbit No. 101, white male. Diet, oats.

Received caffein subcutaneously as follows:

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Mar. 18	2,025	0.100	Mar. 24	1,815	.166
Mar. 19	1,970	.100	Mar. 25	1,830	.185
Mar. 20	2,009	.100	Mar. 26	1,710	.176
Mar. 22	1,855	.100	Mar. 29	1,734	.219
Mar. 23	1,738	.114	Apr. 1	1,606	.224

April 5: Found dead. *Autopsy*: Marked inflammation of gastric mucosa. Considerable enteritis affecting the whole extent of the intestines; liver congested and friable; kidneys deeply congested in cortical and medullary portions; spleen congested, but of normal size; lungs and heart normal.

Four days, 0.1 per kilo; 10 doses in 14 days.

Rabbit 201. Diet of carrots begun October 1; October 19, oats.

Subcutaneous injections as follows:

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Oct. 9	1,000	0.150	Oct. 16	1,065	.225
Oct. 11	1,015	.180	Oct. 18	850	
Oct. 13	1,065	.187	Oct. 20	890	.111

Under observation six hours October 20; no symptoms.

October 23: Died; was much emaciated but did not show any symptoms; emaciation set in when caffein was withdrawn; urine never contained sugar or albumen; symptoms observed after second dose only.

Series B.

The question whether caffein is cumulative in the rabbit, suggested in the preceding experiments, was the subject of further investigation in Series B. Caffein was given by mouth or

subcutaneously. Carrots formed the exclusive diet, a measured amount being given. The rabbits were kept under observation for two weeks, except Nos. 370 and 373, records of which were made only for four days before the administration of caffein was begun. Caffein was given by mouth in experiments of Groups I and III. Rabbits 292, 293, and 295 received daily 20 cc water by mouth for four days previous to the administration of caffein, while in the rabbits of Group II the caffein treatment was preceded by the injection of 0.8 per cent salt solution subcutaneously. The object in both cases was to ascertain whether or not the method of the administration of caffein has any influence on the animal, but observation made from day to day failed to show any effect of such treatment. About 1 decigram of caffein per kilo was administered daily, with occasional intermissions. Later in the course of the experiment the doses were increased, 0.15 gram per kilo being the maximum dose given. Rabbit 293 died after the third dose with symptoms of typical caffein poisoning. The administration of the same dose of caffein was continued 10 days longer in Nos. 292 and 295. It was omitted on the seventh, fourteenth, and fifteenth days of the experiment. On the eighteenth day of the experiment the dose was increased to 150 mg per kilo and was repeated 2 days later. No. 295 was found dead the next day. No. 292 survived. Rabbits 313 and 315 may be considered together, as they were treated alike in every respect. The initial dose of 100 mg caffein per kilo was finally increased to 122 mg. After the twelfth dose the emaciation was well marked and the rabbits were very weak. No. 313 was found dead 2 days, and No. 315 3 days, after the last dose of caffein was given. It should be remarked in this connection that symptoms of caffein poisoning were never observed in these rabbits. Death was not due, therefore, primarily to caffein, but the rapid loss of flesh and strength observed during the last few days suggests that it was due to malnutrition apparently brought about by caffein.

The results obtained by subcutaneous injection of caffein are given in the table as Group II. The initial dose of 100 mg per kilo was injected daily. No. 298 died after the second dose. Nos. 223 and 296 received this amount daily for 6 days. An intermission of 2 days followed, at the end of which the same dose was given again. The next day it was increased to 150 mg per kilo, but no effect was observed; 48 hours later this dose was repeated. No. 223 was found dead, but its mate survived. Symptoms of acute caffein intoxication were not observed in any of these rabbits. It would seem, therefore, that caffein is not cumulative. This supposition, however, appears somewhat contradictory in view of the fact that out of the eight rabbits of this series six died, nor could any cause of death be ascribed other than caffein. Also the first results of Experiments 293, 370, and 373 might be considered as indicating that cumulation, though to a moderate extent, does take place, since in these cases reflexes developed after the drug was given for some time. But this view is contradicted by the results of Experiment 371, in which 150 mg per kilo given 5 days after the daily dosage of caffein was suspended likewise caused increased reflexes. Cumulation, therefore, does not account for the effects noted in the other rabbit. It will be observed that rabbit No. 370, as well as Nos. 371 and 373, had diarrhea for several days. It is quite possible that the weakened condition rendered the rabbits more sensitive to the action of the drug. This is made highly probable by the observations recorded in the experiments on acute intoxication with caffein in which death occurred after small doses. In such cases some pathological condition was often disclosed by the autopsy. The results of this series corroborate, therefore, those of Series A, and indicate again the absence of cumulative action. The results obtained are in all probability due to malnutrition and other conditions brought about by congestion of the viscera and consequent injury to the gastro-intestinal canal.

TABLE 16.—*Chronic caffein intoxication of rabbits; Series B on cumulation.*

	Group I.			Group II.			Group III.	
Data.	**No. 292.**	**No. 293.**	**No. 295.**	**No. 296.**	**No. 223.**	**No. 298.**	**No. 315.**	**No. 313.**
Diet (grams carrots in 2 days)	1,000	1,000	975	930	905	880	355	300
Caffein administered (cc) and weight (grams):								
Mar. 5	1,410	1,470	1,045	1,040	1,070	955	770	770
Mar. 7	1,415	1,360	1,140	1,090	1,095	1,000	715	690
Mar. 9	1,350	1,270	1,070	1,000	1,055	1,005	655	665
Mar. 11	1,505	1,465	1,190	1,230	1,285	1,250	755	760
Mar. 16	1,580	1,460	1,230	1,165	1,170	1,145	730	745
Mar. 17	1,515	1,415	1,080	1,040	1,115	1,105	720	685
Mar. 19	1,565	1,570	1,280	1,195	1,235	1,220	710	735
Mar. 21	7	7	6				4	4
	1,585	1,530	1,265	1,150	1,215	1,260	755	700
Mar. 22	7	7	6	(1)	(1)	(1)	4	4
	1,440	1,315	1,175	1,100	1,045	1,150	675	635
Mar. 23	7	7	6	(1)	(1)	(1)	4	4
	1,335	1,140	1,110	1,145	1,190	1,230	715	700

Mar. 24	7		6	(1)	(1)	(1)	4	4
	1,310	(2)	1,090	1,115	1,170	1,250	680	650
Mar. 25	7		6	(1)	(1)	(1)	4	4
	1,375		1,035	1,125	1,215	1,215	695	685
Mar. 26	7		6				4	
	1,255		1,095	1,105	1,155	1,150	675	695
Mar. 27								
Mar. 28	7		6	5.5	6	6	4	4
	1,355		1,115	1,120	1,160	1,155	595	685
Mar. 29	7		6	6	6	5	4	4
	1,385		1,150	1,155	1,165	955	695	675
Mar. 30	7		6	6	6		4	4
	1,330		1,075	1,035	1,095	Dead.	630	610
Mar. 31	7		6	6	6		4	4
	1,325		1,170	1,110	1,140		690	605
Apr. (1)	7		6	6	6		4	4
	1,335		1,050	1,050	1,120		625	620
Apr. 2	7		6	6	6		4	4
	1,390		1,125	1,090	1,155		695	625
Apr. 3								
						..	200	...
Apr. 4								
	1,300		1,005	1,105	1,080		585	580
Apr. 5	7		6	6	6		4	4
	1,385		1,090	1,130	1,090		655	630
Apr. 6	9		7.5	7.5	8			
	1,260	..	1,010	1,050	1,110		560	530
Apr. 7								
								(3)
Apr. 8	9		7.5	7.5	8			
	1,260		1,000	1.090	1.965		Dead.	
Apr. 9	Survived.		Dead.	Surv.	Dead.			Dead.

[1] On these days 5 *cc* of salt solution was administered subcutaneously.

[2] Dead Mar. 23.

[3] Found dead 9 a. m.

TABLE 17.—*Chronic intoxication of rabbits, series B, Group IV, on cumulation.*

RABBIT, 370.

Date.	Weight.	Carrots.	Water.	Urine.	Caffein by stomach.	Symptoms.
	Grams.	*Grams.*	*cc.*	*Mg per kilo.*		
Aug. 7	2,155	450	50	280		
Aug. 8	2,030	450	25	185		
Aug. 9	2,105	290	0	275		
Aug. 10	2,095	450	30	335		
Aug. 11	2,105	450	65	360	50	
Aug. 12	2,125	450	65	220	50	
Aug. 13	2,120	350	25	265	50	
Aug. 14	2,170	450	35	275	75	
Aug. 15	2,175	350	(?)	200	75	
Aug. 16	2,170	360	65	250	75	
Aug. 17	2,175	310	35	170	100	
Aug. 18	2,095	180	40	285	100	Severe diarrhea.
Aug. 19	2,120	400	(?)	285	125	Do.
Aug. 20	2,120	400	(?)	310	125	Better.

Aug. 21	2,120	400	70	250	125	Do.
Aug. 22	2,040	400	45	265	150	Diarrhea bad.
Aug. 23	2,030	370	35	220	150	Diarrhea better.
Aug. 24	1,950	215	40	120	150	Do.
Aug. 25	1,885	195	35	60	200	Reflexes.
Aug. 26						Found dead at 9.

RABBIT, 373.

Aug. 7	2,240	450	50	230		
Aug. 8	2,150	150	30	300		
Aug. 9	2,120	205	0	150		
Aug. 10	2,150	450	15	245		
Aug. 11	2,195	450	5	285	50	
Aug. 12	2,160	450	65	325	50	
Aug. 13	2,120	300	45	190	50	
Aug. 14	2,195	450	40	265	75	
Aug. 15	2,215	350	35	200	75	
Aug. 16	2,205	310	45	225	75	
Aug. 17	2,240	400	40	265	100	
Aug. 18	2,255	350	30	320	100	
Aug. 19	2,115	185	(?)	170	125	Severe diarrhea.
Aug. 20	2,115	280	35	195	125	Diarrhea better.
Aug. 21	2,050	175	75	115	125	Slight diarrhea.
Aug. 22	2,060	180	75	130	150	
Aug. 23	2,005	200	75	125	150	Reflexes.
Aug. 24	1,990	200	75	150	150	Slight diarrhea.
Aug. 25	1,950	255	55	132	175	Severe diarrhea.
Aug. 26	1,870	205	80	140	None	Do.
Aug. 27	1,830	200	50	140	do.	Do.
Aug. 28	1,950	400	25	265	do.	Slight diarrhea.
Aug. 29	1,825	400	0	315	do.	Very weak and in poor condition
Aug. 30	1,850		10	140	do.	
Aug. 31	1,835					

RABBIT, 370.

Aug. 7	2,240	450	50	300		
Aug. 8	2,260	450	50	225		
Aug. 9	2,310	430	(?)	300		
Aug. 10	2,295	450	50	305		
Aug. 11	2,320	450	50	335	50	
Aug. 12	2,280	450	70	400	50	
Aug. 13	2,300	350	70	255	50	
Aug.	2,265	425	55	154	75	

14						
Aug. 15	2,260	250	40	125	75	
Aug. 16	2,295	155	70	Lost	75	
Aug. 17	2,180	105	70	120	100	Severe diarrhea.
Aug. 18	2,150	125	70	100	100	Diarrhea better.
Aug. 19	2,075	210	(?)	192	100	Diarrhea severe.
Aug. 20	2,075	280	70	180	100	Do.
Aug. 21	2,165	260	50	225	None	Diarrhea better.
Aug. 22	2,105	400	50	275	do.	
Aug. 23	2,080	300	0	145	do.	Diarrhea severe.
Aug. 24	2,105	250	15	245	do.	Do.
Aug. 25	2,055	320	10	176	150	Reflexes.
Aug. 26	2,040	190	75	250	150	Died at 1 p. m., without having showed any symptoms other than reflexes.

SERIES C.

The subjects used in these experiments were rabbits of medium size and were apparently young or at any rate were not very old. The series was planned for the study of the possible effect of diet on the toxicity of caffein when given for some time, and therefore oats were substituted for carrots, which had been fed in the previous work, as already stated. Caffein was given by mouth in the usual way, in 1 per cent solution, 100 mg. per kilo daily. Fourteen rabbits were used for these tests. Their weights were recorded daily and observations made at frequent intervals during the day.

The only change noticed in all of the experiments of this series was progressive loss of weight which set in from 3 to 8 days after the administration of the drug was begun. The duration of life varied considerably. No. 382 died after the first dose. No. 389 lived 2 days, No. 386, 3 days, and No. 385, 5 days; No. 390 lived 7 days and No. 404 lived 20 days after the administration of caffein was begun. The duration of life in all the others was from 11 to 16 days. The findings at autopsy are interesting and suggestive as regards the possible explanation of the effects of repeated dosage of caffein. In eight of the rabbits there was involvement of the mucous membrane of the stomach or intestines or of both. Since the same condition of the gastro-intestinal canal was observed in previous experiments with caffein when injected subcutaneously, the mere passing of the tube into the stomach is obviously not the cause of this condition. The fatal outcome due is therefore, as was suggested above, to inanition brought about by the condition of the gastro-intestinal canal. Moreover parallel experiments carried out on rabbits in the same way with alcohol survived this treatment much longer. Obviously then the passing of the soft rubber catheter is not the cause of this condition of the gastro-intestinal canal nor the diet. Rabbits were fed oats exclusively for several months in this laboratory and thrived. The presence of pneumonia in the other rabbits of this series may be regarded as accidental, as it is inconceivable that one or two doses of caffein, as was the case in Nos. 382 and 389, could predispose the lungs to infection. The results of these experiments therefore are in harmony with those of the preceding two series, indicating that caffein does not accumulate in the body, and that the toxicity of caffein, whether of the single dose or of repeated doses is the same, on a diet of carrots or of oats. These results also show that caffein is much more toxic with repeated dosage. As stated in the historical part of this bulletin the same view was held by Gourewitch.[28]

Rabbit 386. Belgian female.

Given 1 cc of 1 per cent caffein for each 100 grams, through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	1,300	13.0	Aug. 19	(?)	(?)
Aug. 18	1,215	12.0			

August 20: Found dead 9 a. m. *Autopsy*: Lungs slightly congested; liver engorged and friable; gall cyst well filled.

Rabbit 389. Black male.

Given 1 cc of 1 per cent caffein for each 100 grams, through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.

	Grams.	cc.		Grams.	cc.
Aug. 17	1,070	10.0	Aug. 18	1,025	10.0

August 19: Found dead 9 a. m. *Autopsy*: Lungs severely congested and partially hepatized; liver was engorged; other organs appeared normal.

Rabbit 382. Belgian female.

On August 17 weighed 1,035 grams; received 1 cc of 1 per cent caffein for each 100 grams; 10 cc of 1 per cent caffein given in all.

August 18: Found dead 9 a. m. *Autopsy*: Lungs congested and hepatized; liver engorged; stomach showed numerous petechial hemorrhages on mucosa; kidneys slightly congested; intestines appeared normal.

Rabbit 385. Belgian female.

Given 1 cc of 1 per cent caffein for each 100 grams, through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	cc.		Grams.	cc.
Aug. 17	780	8.0	Aug. 20	715	7.0
Aug. 18	760	7.5	Aug. 21	700	7.0
Aug. 19	755	7.5			

August 22: Found dead 9 a. m. *Autopsy*: Lungs exhibited pneumonic lesions, with inflammation of adjacent pleura; a fibro-plastic exudate present around lung; liver showed a coccidial infestation; stomach distended with ingesta; mucous membrane characterized by a catarrhal inflammation; contents of small intestine liquid in nature and bile stained; large intestine somewhat impacted; liver and kidneys seemingly normal.

Rabbit 404. White male.

Given 1 cc 1 per cent caffein for each 100 grams.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	cc.		Grams.	cc.
Aug. 20	1,465	14.5	Aug. 30	1,320	13.0
Aug. 21	1,475	14.5	Aug. 31	1,330	13.5
Aug. 22[1]			Sept. [1]	1,335	13.5
Aug. 23	1,475	14.5	Sept. 2	1,315	13.0
Aug. 24	1,400	14.0	Sept. 3	1,350	13.5
Aug. 25[2]	1,405	14.0	Sept. 4	1,335	13.5
Aug. 26	1,415	14.0	Sept. 5	1,350	13.5
Aug. 27	1,400	14.0	Sept. 6	1,380	14.0
Aug. 28[1]			Sept. 7	1,375	14.0
Aug. 29	1,310	13.0	Sept. 8	1,325	13.0

[1] Not fed. [2] Reflexes.

September 9: Found dead 9 a. m. *Autopsy*: Both lungs showed extensive pneumonia, with adhesions to pleura; pleuritis and pericarditis very marked; large amount of fibrous exudate in pleural cavity; pyloric end of stomach slightly congested; liver congested; other organs normal.

Rabbit 393. Belgian.

Given 1 cc of 1 per cent caffein to each 100 grams, through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	cc.		Grams.	cc.
Aug. 17	950	9.5	Aug. 25	835	8.5
Aug. 18	910	9.0	Aug. 26	780	8.0
Aug. 19	895	9.0	Aug. 27	765	7.5
Aug. 20	910	9.0	Aug. 28[1]		
Aug. 21	905	9.0	Aug. 29	710	
Aug. 22[1]			Aug. 30[2]		
Aug. 23	825	8.0	Aug. 31[1]		
Aug. 24	870	8.5			

[1] Not fed. [2] Condition very poor; not fed.

September 1: Found dead. *Autopsy*: Lungs congested and adhering to the pleura; extensive inflammation of pleura; liver slightly enlarged and congested; mucosa of stomach and small intestines slightly congested; other organs normal.

Rabbit 390. Belgian, male.

Given 1 cc of 1 per cent caffein to each 100 grams through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.

	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	1,490	15.0	Aug. 21	1,265	12.5
Aug. 18	1,370	14.0	Aug. 22[1]		
Aug. 19	1,365	13.5	Aug. 23	1,120	11.0
Aug. 20	1,340	13.5			

[1] Not fed.

August 24: Found dead 9 a. m. *Autopsy*: Heart and lungs appeared normal; abdominal viscera showed no apparent pathologic change other than coccidial infection of the liver and fullness of the blood vessels.

Rabbit 392. Maltese, female.

Given 1 cc of 1 per cent caffein to each 100 grams through stomach tube.

Date.	**Weight.**	**Treatment.**	**Date.**	**Weight.**	**Treatment.**
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	1,265	12.5	Aug. 26	1,140	11.5
Aug. 18	1,275	12.5	Aug. 27	1,140	11.5
Aug. 19	1,240	12.5	Aug. 28[1]		
Aug. 20	1,220	12.0	Aug. 29	1,115	11.0
Aug. 21	1,245	12.5	Aug. 30	1,080	11.0
Aug. 22[1]			Aug. 31	1,020	10.0
Aug. 23	1,180	12.0	Sept. 1	995	10.0
Aug. 24	1,190	12.0	Sept. 2	930	9.0
Aug. 25	1,155	11.5			

[1] Not fed.

Died at 3 p. m. September 2. *Autopsy*: The stomach and small intestines showed numerous small hemorrhagic spots; a thick coating of mucus surrounded the contents of the stomach; the other organs were apparently normal.

Rabbit 403. Black.

Given 1 cc of 1 per cent caffein for each 100 grams.

Date.	**Weight.**	**Treatment.**	**Date.**	**Weight.**	**Treatment.**
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 20	1,640	16.5	Aug. 26	1,390	14.0
Aug. 21	1,640	16.5	Aug. 27	1,330	13.5
Aug. 22[1]			Aug. 28[1]		
Aug. 23	1,490	15.0	Aug. 29	1,130	11.5
Aug. 24	1,515	15.0	Aug. 30	1,055	10.5
Aug. 25	1,475	15.0			

[1] Not fed.

August 31: Found dead at 3 p. m. *Autopsy*: Extensive gastroenteritis; liver enlarged and congested; spleen slightly congested; peritoneum thickened and congested; other organs normal.

Rabbit 884. Black, female.

Given 1 cc of 1 per cent caffein for each 100 grams through stomach tube.

Date.	**Weight.**	**Treatment.**	**Date.**	**Weight.**	**Treatment.**
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 16	1,195	12.0	Aug. 25	990	10.0
Aug. 17	1,205	12.0	Aug. 26	960	9.5
Aug. 18	1,140	11.5	Aug. 27	955	9.5
Aug. 19	1,180	12.0	Aug. 28[1]		
Aug. 20	1,145	11.5	Aug. 29	870	9.0
Aug. 21	1,145	11.5	Aug. 30[2]	850	8.5
Aug. 22[1]			Aug. 31	810	8.0
Aug. 23	1,005	10.0	Sept. 1	740	7.5
Aug. 24	1,035	10.5			

[1] Not fed. [2] Poor condition, mucus from rectum.

September 2: Found dead at 9 a. m. *Autopsy*: The mucosa of stomach showed numerous hemorrhagic spots; the first portion of the small intestines was slightly congested; the other organs were apparently normal in appearance.

Rabbit 383. Belgian, female.

Given 1 cc of 1 per cent caffein for each 100 grams through stomach tube.

Date.	**Weight.**	**Treatment.**	**Date.**	**Weight.**	**Treatment.**
	Grams.	*cc.*		*Grams.*	*cc.*

Aug. 16	995	10.0	Aug. 22[1]		
Aug. 17	1,005	10.0	Aug. 23	875	9.0
Aug. 18	990	10.0	Aug. 24	855	8.5
Aug. 19	895	9.0	Aug. 25	850	8.5
Aug. 20	945	9.5	Aug. 26	785	8.0
Aug. 21	965	9.5	Aug. 27	710	7.0

[1] Not fed.

August 28: Found dead at 9 a. m. *Autopsy*: Lungs, heart, and spleen apparently normal; liver infected with coccidia; stomach apparently normal; walls of small intestines injected; colon marked congestion and hemorrhagic; kidneys hemorrhagic.

Rabbit 387. Belgian male.

Given 1 cc of 1 per cent caffein for each 100 grams through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	1,260	12.5	Aug. 26	1,185	12.0
Aug. 18	1,340	13.0	Aug. 27	1,255	12.5
Aug. 19	1,335	13.0	Aug. 28[1]		
Aug. 20	1,300	13.0	Aug. 29	1,115	11.0
Aug. 21	1,325	13.0	Aug. 30	1,135	11.5
Aug. 22[1]			Aug. 31	1,175	12.0
Aug. 23	1,205	12.0	Sept. 1	1,050	10.5
Aug. 24	1,200	12.0	Sept. 2	900	9.0
Aug. 25	1,285	12.5			

[1] Not fed.

September 3, found dead. *Autopsy*: Stomach and small intestines showed numerous hemorrhagic spots; thick coating of mucus surrounded the contents of the stomach; bladder was greatly distended with urine; the other organs were apparently normal.

Rabbit 388. Belgian male.

Given 1 cc of 1 per cent caffein for each 100 grams, through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	1,080	10.0	Aug. 23	1,020	10.0
Aug. 18	1,115	11.0	Aug. 24	985	10.0
Aug. 19	1,150	11.5	Aug. 25	960	9.5
Aug. 20	1,130	11.5	Aug. 26	900	9.0
Aug. 21	1,120	11.0	Aug. 27	875	9.0
Aug. 22[1]			Aug. 28[1]		

[1] Not fed.

August 29, found dead 9 a. m. *Autopsy*: Heart and lungs normal; liver and kidneys engorged; stomach normal; intestines showed a catarrhal inflammation, though not severe; spleen normal; walls of colon somewhat injected.

Rabbit 391. Belgian.

Given 1 cc of 1 per cent caffein to each 100 grams through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	940	9.5	Aug. 24	805	8.0
Aug. 18	950	9.5	Aug. 25	800	8.0
Aug. 19	955	9.5	Aug. 26	765	7.5
Aug. 20	935	9.5	Aug. 27[2]	690	7.0
Aug. 21	945	9.5	Aug. 28[1]		
Aug. 22[1]			Aug. 29	565	5.5

[1] Not fed. [2] Poor condition.

August 30, found dead 9 a. m. *Autopsy*: Heart injected; lungs normal; liver affected slightly with coccidiidea; stomach normal in appearance; small intestines normal, but colon considerably inflamed; kidneys slightly engorged; other organs normal.

Rabbit 402. Black female.

Given 1 cc of 1 per cent caffein to each 100 grams.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 20	2,030	20.0	Aug. 27	1,765	17.5

Aug. 21	1,950	19.5	Aug. 28[1]		
Aug. 22[1]			Aug. 29	1,630	16.5
Aug. 23	1,955	19.5	Aug. 30	1,540	15.5
Aug. 24	1,905	19.0	Aug. 31	1,510	15.0
Aug. 25	1,890	19.0	Sept. 1	1,425	14.0
Aug. 26	1,780	18.0			

[1] Not fed.

September 2, found dead 9 a. m. *Autopsy*: The lungs were badly congested, the posterior lobe of the right lung showing hepatization; the liver was considerably enlarged and congested; the mucous membrane of the stomach and small intestines was congested and showed numerous hemorrhagic spots; the kidneys showed slight congestion; all other organs normal.

SERIES D.

The evidence brought forth in the preceding pages regarding cumulation of caffein naturally suggests the question whether or not the body acquires a tolerance for it. This question has already been answered in the affirmative by Gourewitch,[28] but owing to the method he used for the identification of caffein and the few experiments made his results are not conclusive. The experiments of series A, B, and C might be regarded as indicating that tolerance for caffein is not acquired by the rabbit. It was noticed, however, that the rabbit apparently does tolerate increasingly larger doses under certain conditions, as the following experiments show:

Rabbit 223. Belgian hare, male.

October 22: Weight, 1,520 grams; 15 cc 2 per cent caffein injected subcutaneously at 2 p. m.

November 1: 10.30 a. m., weight, 1,510 grams; 17 cc 2 per cent caffein injected subcutaneously (225 mg per kilo), reflexes observed, but no tetanus.

November 4: 10.30 a. m., weight 1,535 grams; 19 cc 2 per cent caffein injected subcutaneously at 2.40 p. m.; 4.40 p. m., no symptoms.

November 8: Weight, 1,425 grams; 20 cc 2 per cent caffein (285 mg per kilo) injected at 11.45 p. m.; 5 p. m., no symptoms.

November 17: Weight, 1,325 grams; 22 cc 2 per cent caffein injected at 2.55 p. m. (329 mg per kilo), no symptoms.

November 18: Rabbit in good condition.

Rabbit 224. Belgian hare, female. Diet, carrots.

October 18: Weight, 1,935 grams; 11.20 a. m., 15 cc 2 per cent caffein (155 mg per kilo) injected.

November 1: Weight, 1,780 grams; 20 cc 2 per cent caffein (224 mg per kilo) injected subcutaneously, reflexes increased, muscle tremors present, but no other symptoms.

November 4: Weight, 1,710 grams; 21.5 cc 2 per cent caffein (252 mg per kilo) injected.

November 8: Weight, 1,435 grams; 22.5 cc 2 per cent caffein or 314 mg per kilo injected at 11.40 p. m.; 5 a. m., no symptoms.

November 17: Weight, 1,340 grams; 24 cc 2 per cent caffein (358 mg per kilo) injected subcutaneously.

November 18: 9 a. m., rabbit died.

Rabbit 226. Gray male. Diet, carrots.

October 28: Weight, 1,045 grams; 10 cc 2 per cent caffein injected subcutaneously at 1.50 p. m.; 4.30 p. m., tremors observed, but no other symptoms.

October 29: Rabbit in good condition.

November 1: Weight, 950 grams; 10.55 a. m., 11 cc 2 per cent caffein injected subcutaneously (231 mg per kilo).

November 4: Weight, 930 grams; 2.50 p. m., 12 cc 2 per cent caffein injected subcutaneously (258 mg caffein per kilo).

November 6: Weight, 945 grams; 11.45 a. m., 15 cc 2 per cent caffein (313 mg per kilo) injected subcutaneously.

November 17: Rabbit still alive; weight, 890 grams.

The results of these experiments indicate that when sufficient time is allowed between two successive injections, susceptibility to caffein is not increased. The rabbit, on the contrary, seems to acquire a tolerance for the drug, for the fourth dose was 15 per cent larger than the minimum fatal dose of caffein. This is in all probability due to the better elimination of caffein and its products of decomposition and to recovery from the deleterious effects of each dose, made possible by long intervals between injections.

The results of these experiments may be briefly summed up by stating that subminimum doses of caffein given to the rabbit daily or at intervals (not too long) do not produce any symptoms such as were observed in acute caffein intoxication, namely, increased reflexes and convulsions, or increased rate of respiration, thus showing that it is not cumulative. But evidence of undoubted summation of effect was adduced to show that if the administration of subminimum doses of caffein be continued daily for a period of 11 to 18 days the result is fatal. Tolerance, however, may be acquired, although to a limited extent only, provided sufficiently long intervals between injections are allowed to give time for repair of the injury done by the drug and to develop a mechanism for its better decomposition and elimination. Furthermore, the evidence just given indicates that the elimination of subminimum doses of caffein and its products of decomposition is probably accomplished within 24 hours or thereabouts. That the elimination of larger doses is not accomplished in this interval is made probable by the following experiment:

Gray rabbit 455. Female. Diet, oats.

October 12: Weight, 1,185 grams; 3.30 p. m., 11.5 cc 2 of per cent caffein injected into the lumbar muscles; 3 p. m., reflexes increased.

October 13: 10 a. m., rabbit weighed 1,070 grams; no symptoms of caffein poisoning, reflexes normal; 10.30 a. m., 10 cc 2 per cent caffein injected into the lumbar muscles; 11.30 a. m., rabbit jumped off the table, had convulsions, and died.

EXPERIMENTS ON DOGS.

Having gained some information respecting the effects of repeated doses of caffein on rabbits, it was of interest to find out how carnivora reacted to the drug when similarly administered. A number of dogs were used for the purpose. Considerable variation in the mode of experimentation, as will appear later, was allowed.

Since the condition of the animal, its age, environment, or diet might be factors influencing toxicity, tests were made on full-grown and on young growing dogs whose food was varied. The subjects of the experiment were kept under observation for a few days to several weeks before the administration of caffein was begun, in order to determine whether or not any morbid condition existed, as well as to ascertain whether the new environment had any effect on these animals. Caffein was given chiefly by mouth, but the subcutaneous method was also employed during a portion of the experimental period in some dogs. The initial dose, which varied for different individuals, was maintained for a variable length of time. It was then progressively increased, in most cases until the death of the animal. With larger doses the intervals between successive injections were also increased.

SERIES A.

Six dogs were used in this series. Caffein was administered by mouth for periods of six days to five weeks. It was given daily or at intervals of two, and sometimes of three, days. In a few instances the drug was withheld for four or even for seven days, and its administration was resumed at the end of this time. The initial dose in these experiments varied approximately between 40 and 140 mg per kilo. The doses were then increased gradually, and thus the maximum resistance of the subject to caffein was tested. The diet consisted either exclusively of meat or largely of carbohydrates with a minimum amount of meat to give flavor to the food.

Dog 11. Female.

Diet consisted of rice, 250 grams; cane sugar, 250 grams; meat, 50 grams; cracker meal, about 100 grams. Caffein was given by mouth daily or at intervals of one day, when the dose did not exceed 1.5 grams. Before the dose was increased to 2 grams, or approximately 0.213 gram caffein per kilo, an interval of two days was allowed. Symptoms were noticed the next day. An interval of two days was therefore allowed again at the end of which the same dose was repeated. It will be remarked that there were no symptoms this time, and the general condition of the dog seemed to be good. Two grams of caffein were, therefore, given daily during the next two days without any untoward effects; the dose was then increased to 2.5 grams. Even after this enormous quantity no symptoms were observed except slight tremors. When this dose was repeated 26 hours later, it proved fatal. No albumin or sugar was found in the urine, although the dog was fed on a very liberal carbohydrate diet. The following is a complete record of the experiment.

April 20: Urine acid, no albumin, no sugar.

April 21: Urine free from sugar.

April 22: Urine free from sugar. 1 gram caffein given in the afternoon.

April 23: 9 a. m., dog was very thirsty, drank a large quantity of water, urine did not reduce Fehling's solution.

April 24: 2.30 p. m., 1 gram caffein, no sugar in urine.

April 25: 1 gram caffein administered.

April 26: Weight, 10.6 kilos, urine collected in the morning, no sugar; 4.10 p. m., 1.5 grams caffein.

April 27: 1.5 grams caffein; 1.30p. m., diet as before, no sugar in urine.

April 28: Weight, 10.2 kilos, no caffein, no sugar in urine.

April 30: Weight, 10.4 kilos, no sugar in urine; 4.20 p. m., 2 grams caffein.

May 1: Urine examined, sugar absent, weight 10 kilos, vomited, sick, tremors observed, drank 500 cc water at one time, appetite poor.

May 2: No caffein, drank 150 cc water.

May 3: Urine, no sugar, moderate quantity of albumen present; 12 noon, 2 grams caffein given by mouth, weight 10.3 kilos; 2 p. m., urine, sugar negative, condition of dog good, no symptoms of caffein intoxication.

May 4: 10 a. m., about 10 cc thick, dark-colored mucilaginous urine found in collecting bottle; albumin a little more than a trace, decidedly less than on May 3, no sugar, condition of dog pretty good except for slight muscular tremors; 4 p. m., 2 grams caffein by mouth (as usual).

May 5: Urine not examined, no symptoms; 4 p. m., 2 grams caffein.

May 6: Urine not examined; 2.30 p. m., 2.5 grams caffein given by mouth; 4 p. m., slight tremor, no other symptoms.

May 7: No examination of urine, no symptoms observed; 4 p. m., 2.5 grams caffein.

May 8: 9 a. m., found dead, urine collected since last dose of caffein was given did not contain any sugar or albumin, the amount of caffein fed to this dog was 18 grams in 18 days. *Autopsy*: Post-mortem examination showed marked enteritis with hemorrhagic spots on the mucosa; liver and kidneys congested and dark colored; lungs congested; thyroid gland was greatly enlarged and congested.

Dog 28.

April 30: Weight, 6.8 kilos; the diet consisted of 250 grams rice, 250 grams sugar, 100 grams cracker meal, and 100 grams of meat. On May 3 his weight was 7 kilos. He received 1 gram of caffein by mouth at 12 noon. At 2 p. m. he vomited and tremors were observed. The next day, May 4, tremors were still present though less pronounced. Examination of the urine for sugar and albumin was negative; on May 4, 1 gram caffein was given again and repeated on May 5. On this date his general condition was not good—dog had no appetite and refused to take caffein. As the dog lost 10 per cent of his weight he was put on a meat diet exclusively and the dose of caffein was reduced to 0.5 gram. He became sick after the second dose, and the administration of caffein was therefore discontinued. It was resumed after five days and the caffein was administered in increasing amounts, i. e., on May 18, 0.5 gram; May 19, 0.5; May 20, 1; May 21, 1 gram in two doses of 0.5 each, given at intervals of one hour; May 22, 1 gram. Dog became irritable, but no other symptoms were observed. The administration of caffein was omitted the next day. On the following day when the same dose of caffein was given there was again marked irritability and tremors. The experiment was therefore discontinued.

Dog 22. Male bulldog.

June 24: Dog weighed 13.7 kilos. Diet consisted of meat exclusively; 1 gram caffein was given by mouth; diarrhea developed; no caffein was given for three days.

June 28: Dog weighed 13.6 kilos, 1.5 grams caffein given at 10 a. m.

June 30: 1.75 grams caffein administered.

July 2: Dog weighed 13.5 kilos; 2 grams caffein or 0.15 gram per kilo, caused well-marked thirst, but did not produce any other symptoms.

Dog 20. Female.

May 12: Weight, 7.7 kilos. Fed liberal carbohydrate diet, consisting of rice, 100 grams; sugar 100 grams; meat and cracker meal, a sufficient quantity to flavor the food.

May 14: Weight, 7.7 kilos. Examination of urine for albumin and sugar gave negative results. Urine was acid to litmus.

May 17: Weight, 7.4 kilos. Three hours after it was fed the dog received 0.5 gram caffein by mouth. The test of the urine the next day for sugar was negative, but a trace of albumin was present. It will be noticed that the doses were increased gradually and that symptoms were observed only after the fourth dose of 0.1 gram per kilo. Later meat was substituted for the carbohydrate diet and the administration of caffein was stopped for four days. At the end of this period 100 mg caffein per kilo was fed daily for five days, and the dose was then very gradually increased. Diarrhea occurred twice, but no other symptoms, the second attack having lasted a few days. The following is a complete record of the experiment:

May 19: 0.5 gram caffein 11.45 a. m.

May 20: 0.75 gram caffein 12.45 a. m.

May 21: 0.75 gram caffein 12 noon; no sugar, no albumin in urine.

May 22: 0.75 gram caffein; urine, same condition found; no symptoms.

May 23: Weight, 7.5 kilos; no caffein.

May 24: 0.75 gram caffein; tremors very marked.

May 25: No caffein.

May 26: 0.75 gram caffein.

May 27: 0.75 gram caffein.

May 28: 0.75 gram caffein.

May 29: 1 gram caffein in two doses of 0.75 and 0.25 gram.

May 30: No caffein.

May 31: No caffein; meat diet exclusively.

June 1: No caffein; meat diet exclusively.

June 2: No caffein; no sugar, no albumin in urine.

June 3: Weight, 7.6 kilos; 0.75 gram caffein; no sugar in urine.

June 4: Weight, 7.3 kilos; 0.75 gram caffein; no sugar in urine.

June 5: Weight, 7.5 kilos; 0.8 gram caffein; drank 500 cc water; ate 200 grams meat.

June 6: Weight, 7.4 kilos; 0.8 gram caffein; 500 cc urine; drank 500 cc water; ate 200 grams meat; no symptoms.

June 7: Weight, 7.7 kilos; 0.8 gram caffein 10 a. m.; 400 cc urine, 500 cc water, 200 grams meat.

June 8: Weight, 7.5 kilos; 0.9 gram caffein, 450 cc urine, 1 p. m.; 200 grams meat, 500 cc water.

June 9: Weight, 7.6 kilos; 0.9 gram caffein, 1 p. m.; 500 cc water, 200 grams meat and bone dust; diarrhea and restlessness all afternoon.

June 10: Weight, 7.6 kilos; 1 gram caffein, 500 cc water, 200 grams meat, 480 cc urine.

June 11: Weight, 8 kilos; 1 gram caffein, 470 cc urine, 500 cc water, 200 grams meat.

June 12: Weight, 7.8 kilos; 1 gram caffein, 710 cc urine, 500 cc water, 200 grams meat.

June 13: 450 cc urine, 500 cc water, 300 grams meat.

June 14: Weight, 7.9 kilos; 1.2 grams caffein, 500 cc water, 300 grams meat, 490 cc urine.

June 15: Weight 7.8 kilos, 500 cc water, 300 grams meat, 550 cc urine.

June 16: Weight 8.0 kilos, 1.2 gram caffein, 500 cc water, 300 grams meat, bone dust added to check diarrhea.

June 17: 500 cc water, 300 grams meat, 450 cc urine, diarrhea continues, bone dust added.

June 18. Weight 7.8 kilos, 1.3 gram caffein, 300 grams meat, 500 cc water, 300 cc urine.

June 19: Dog very thirsty, drank 1 liter of water and ate 350 grams of meat; 960 cc urine passed during the past 24 hour.

June 21: Weight 7.5 kilos, 1.5 grams caffein given at 10 a. m. At 2 p. m. convulsions and death. This dog received a total of 21.15 grams caffein in 25 doses during a period of 35 days, which amounts to an average of 85 mg per kilo daily.

Dog 19. Female fox terrier.

May 13: Weight 6.4 kilos. Diet consisted of rice, 100 grams; sugar, 100 grams; and a sufficient quantity of meat and cracker meal to give flavor to the food. Examination of the urine showed a trace of albumin but no sugar. The urine was acid to litmus. Two days later the urine was alkaline to litmus. There was still a small amount of albumin but no sugar.

May 17: 0.5 gram caffein was given by mouth. Examination of the urine collected the next day still showed the presence of albumin and the absence of reducing substances. The dog had tremors. Caffein was, therefore, not administered.

May 19: 0.5 gram caffein was given by mouth.

May 20: 0.75 gram caffein was fed at 12.45 p. m. The dog vomited during the night and tremors were observed the next morning. The urine collected was examined for albumin and sugar, but neither was found.

May 21: 12 noon, 0.75 gram caffein was fed. The dog weighed 6 kilos, which therefore represented a loss of 0.4 kilo. Grew abnormally thirsty and lost appetite, but no other symptoms of caffein poisoning were observed.

May 22: The dog was again given 0.75 gram caffein at 12 noon. The examination of the urine for

albumin and sugar gave negative results. The dog died at 4.15 p. m. The fatal dose for this dog was therefore 0.125 gram caffein per kilo, and the total amount of caffein ingested in six days amounted to 3.25 grams, or 0.54 gram per day, which makes 90 mg per kilo.

Dog 21. White female bull.

This dog was kept on a diet exclusively of meat, and was given water ab libitum. From 0.5 to 0.6 gram of caffein was administered daily for seven days; the doses were then increased and were given at longer intervals. No symptoms of the effects of caffein were observed until a dose of 1.5 gram was fed, when diarrhea was noticed on the next day. In the following record the details of the experiment are given:

Date.	Weight.	Caffein.	Date.	Weight.	Caffein.
	Kilos.	*Grams.*		*Kilos.*	*Grams.*
June 7	12.5	0.5	June 16	12.7	0.8
June 8	12.5	.5	June 18	12.9	1.0
June 9	12.5	.5	June 21	13.4	1.2
June 10	12.3	.6	June 24	13.3	1.5
June 11	12.3	.6	June 25	[1]	.0
June 12	12.3	.6	June 27	13.5	1.5
June 13	12.3	.6	June 30	13.5	1.75
June 14	12.3	.8			

[1] Diarrhea.

July 2: 11.30 a. m., 2.0 gram caffein fed by mouth; 1.30 p. m., tetanus, dog died. The total amount of caffein fed to dog No. 21 out of the 25 days of the experiment was 14.45 grams, or an average of 578 mg per day, which amounts to about 42 to 43 mg per kilo of body weight.

Notwithstanding the diversity in the method of experimentation, there was a striking uniformity in some of the results obtained. All the experiments of the series showed absence of cumulative action of caffein. The experimental evidence presented indicates that moderately large doses may be given at intervals of about 24 hours without inducing any symptoms of nervous or any other disturbance. This is illustrated in the tests on dog 11, which were preliminary in character. In this subject 100 to 150 mg of caffein per kilo were ingested daily for several days without showing any changes. Later in the course of the experiment, after larger doses were given, mild symptoms only, such as tremors, were observed. Additional evidence of the absence of cumulative action of caffein was furnished by the results of the following experiments:

Dog 23 received 142 mg of caffein per kilo on three successive days. His general condition indicated that these amounts of caffein were toxic, but he survived. In another series of tests, made after he was allowed to rest a few days, he again failed to show any cumulation of the drug, as he survived this time a series of tests of longer duration than the first.

A much better illustration of the absence of cumulative action of the drug is furnished by the experiments on dog No. 20. In this case 100 to 125 mg of caffein per kilo, given on 10 consecutive days, did not cause any marked effects. Diarrhea and restlessness were the only symptoms observed. These experiments therefore show that the elimination and decomposition of caffein are apparently effected by the body within twenty-four hours or thereabouts.

Experiments on dog 19, however, form an exception—the third dose of 125 mg caffein per kilo having proved fatal. The very low protein content of the diet of this dog suggests itself as a possible cause of the lower resistance to caffein of this subject. But it may be observed that the same diet was furnished to dog 20, which stood such amounts of caffein much longer. The presence of a trace of albumin in the urine of dog 19 is likewise inadmissible as a cause of the difference in the toxicity of caffein in this dog, for the urine of dog 20 likewise contained a trace of albumin. The alkaline reaction of the urine, together with the fact that the first dose of only 60 mg of caffein per kilo induced symptoms of toxicity, suggests the presence of an abnormal condition which in all probability was the cause of the death of this subject under the conditions indicated.

In a large number of experiments on caffein performed in this laboratory it has been observed that symptoms due to caffein often disappeared when the administration of the same dose of the drug was continued. Thus dog 19 vomited when the amount of caffein was increased to 125 mg per kilo. When this amount was repeated the next day there was no vomiting. Similar observations were made on dogs 11 and 23, also on other dogs. No. 22 developed diarrhea at first; when the administration of caffein was resumed several days later, however, there was no diarrhea. In other experiments performed in this laboratory, symptoms of nervous irritability induced by caffein disappeared on continued treatment.

It was interesting, therefore, to inquire whether resistance to caffein would be increased by the continued administration of progressively larger amounts of the drug. When doses of 150 and over were fed, the intervals allowed were usually longer than 24 hours. Two and sometimes three days were permitted to elapse between two successive doses. This was done in order to allow time for recovery from possible changes induced by larger doses of caffein, and thus prevent the summation of effect. In the experiments considered, therefore, Nos. 11, 23, 20, and 19, the toxicity of caffein does not seem to be greater than in the experiments on acute caffein intoxication in the dog. It was thought, however, that the large initial doses or the quick change

to large doses when the amounts used in the beginning were small, might have something to do with failure to induce a marked degree of tolerance. The experiment on dog 21 was therefore carried out by giving from 40 to 60 mg per kilo for eight days, and then increasing the dose, but tolerance could not be induced, as is shown in the protocol to the experiment.

Series B.

According to the studies of Chittenden,[16] low protein diet improves the general metabolism of the body, fatigue is diminished, and bodily vigor, therefore, correspondingly increased. The expectation is, therefore, justified that the defense of the organism against deleterious substances introduced into the body is much improved by such a diet, thus increasing its resistance to poisons. Hunt's experiment on this subject, also quoted by Chittenden, lends support to this view. He found that mice fed on carbohydrates chiefly, or on foods containing only a small amount of protein, were more resistant to acetonitril. It was interesting, therefore, to inquire whether the toxicity of caffein differs under similar conditions of diet.

A fixed diet of the same calorific value was provided for all dogs of this series, but the protein content for three of the animals was approximately one-third of the amount usually fed to dogs. Caffein was at first administered subcutaneously, but all the dogs on a low protein diet developed abscesses at the site of injection, while none of those on high protein diet showed a local reaction. Feeding by mouth was then begun and continued throughout the experiment in each case. The initial dose was 50 mg per kilo, which was given daily for seven to nine days. It was then increased progressively by 25 mg per kilo; 75 mg per kilo were administered for one to two days, 100 mg for two to three days, 125 mg for one to two days, 150 mg for one to two days, and a single dose of 175 mg. It will be remarked that sometimes an interval of one day had to be allowed during which no caffein was fed.

Dog 30. Black and tan hound, male.

The dog was under observation for about eight weeks before the experiment was begun and had received a high protein diet. He was then given 50 mg caffein for nine consecutive days. On the tenth day the dose was increased to 75 mg per kilo. As no symptoms developed, this dose was increased to 100 mg per kilo, and was fed one day apart. It was then raised to 125 mg per kilo. For the first time since the drug was fed, symptoms appeared; they were noticed a few hours after feeding and persisted during the next day. Although the appetite was good, no caffein was given on this day. On the following day this dose was repeated. As the symptoms were not serious, 150 mg per kilo were given daily for the next three days, until 175 mg per kilo was reached. This dose proved fatal within six hours. Record of experiment follows:

October 9: Weight, 9 kilos, on full nitrogen diet, received daily 0.724 gram nitrogen per kilo or 87 calories per kilo, received 18 grams meat per kilo, 4 grams lard per kilo, 3 grams carbohydrates per kilo, bone dust, ad libitum.

November 3: Weight, 9.10 kilos.

November 10: Weight, 9 kilos.

November 20: Weight, 9.55 kilos.

November 29: Weight, 8.70 kilos.

December 6, 7, 8, and 9: Received subcutaneously 22 cc 2 per cent caffein. Condition good, site of injection normal.

December 10, 11, 12, 13, and 14: Received 0.4375 gram caffein by mouth equal to 0.050 gram per kilo, no symptoms, appetite and general condition good.

December 15: 11.30 a. m., received 0.6563 gram caffein by mouth, or 0.75 gram per kilo, no symptoms, appetite good, condition excellent.

December 16: 11 a. m., received 0.870 gram caffein by mouth, or 0.1 gram per kilo, weight 8.70 kilos, no symptoms.

December 17: No caffein given.

December 18: Received 0.870 gram caffein, or 0.1 gram per kilo, no symptoms.

December 20: 2.45 p. m., received 1.0875 grams caffein, or 0.125 per kilo; 4 p. m., ate food readily, seemed very uncomfortable and sick.

December 21: 9 a. m., stiffness in muscles, but no other symptoms, appetite good, no caffein given.

December 22: 11 a. m., received 1.0875 grams caffein, or 0.125 gram per kilo; 3 p. m., depressed in spirits and sick, but no other symptoms observed.

December 23: 11.30 a. m., received 1.305 grams caffein, or 0.150 gram caffein per kilo; 1.30 p. m., apparently quite sick, but no other symptoms, had good appetite.

December 24: 10 a. m., received 0.175 gram caffein per kilo; 4 p. m., when about to be fed fell over and died; no autopsy.

The total amount of caffein given dog 30 was 11.3458 grams, administered for a period of eighteen days. The average daily amount per kilo was therefore 72 mg. The feces became offensive when the amounts of caffein were increased to 75 mg per kilo. It will be observed that in this dog the appetite was uniformly good until the day of his death. Whether or not this is the cause of his resistance to caffein will be discussed later.

Dog 32. White, male, young.

Although he was growing rapidly this dog's weight was constant, but he looked anemic. He received a high protein diet until December 3, when the rations were increased by one-third. This dog was under observation from October 26 to December 6 when the administration of caffein was begun. He then received 50 mg caffein per kilo daily for nine days consecutively without showing any effects, when the dose was increased to 75 mg per kilo, then to 100 mg per kilo. This dose was further increased to 150 mg per kilo without causing symptoms, which was repeated the next day. No symptoms having been observed after such amounts of caffein, 175 mg per kilo were fed. This dose, however, proved fatal within two hours. Record of experiment follows:

October 26: Weight, 6.90 kilos.

November 3: Weight, 6.90 kilos.

November 10: Weight, 6.90 kilos.

November 20: Weight, 6.90 kilos.

November 29: Weight, 6.55 kilos.

December 3: Put into cage, diet increased one-third.

December 6, 7, 8, 9: Weight 6.30 kilos; 12.30 p. m., received 16 cc 2 per cent caffein by subcutaneous injection in back, no symptoms of any kind noticed, site of injection normal.

December 10, 14: 0.05 gram caffein per kilo.

December 15: Received 0.4725 gram caffein by mouth, no symptoms.

December 16: Received 0.655 gram caffein, 0.100 gram per kilo.

December 17: No caffein given.

December 18: Received 0.655 gram caffein daily, 0.100 gram per kilo, no symptoms.

December 20: Received 0.8188 gram caffein, 0.125 gram per kilo, no symptoms, appetite good.

December 21: Received 0.9825 gram caffein, 0.150 gram per kilo, somewhat uncomfortable, no other symptoms.

December 22: Received 0.9825 gram caffein, 0.150 gram per kilo, no symptoms except some uneasiness.

December 23: 9 a. m., no symptoms, appetite good; 11.30 a. m., received 1.146 grams caffein, 0.1759 gram per kilo; 1.30 p. m., died while making an effort to get out of cage, tonic contraction of limbs observed before death.

The amount of caffein received during the entire experimental period was 9.2223 grams, or an average per day approximately of 80 mg per kilo, and therefore 10 per cent more than dog No. 30 received. It will be observed that the appetite in dog No. 32 was likewise uniformly good, and that he received a very high protein diet which was also of a very high calorific value.

Autopsy (dog 32).—Stomach presented a severe inflammation of the mucosa, especially in the fundus and pyloric portions. The gastritis was more marked in pyloric portion, and the inflammatory condition extended along the whole course of small intestines, which presented numerous hemorrhagic areas, and a thick catarrhal exudate on the mucosa. The large intestine contained quite a large number of parasites, probably round worms. The liver was enlarged and the gall cyst well filled. The spleen was also considerably engorged, kidneys appeared normal, other organs all appeared normal.

Dog 31. Black spaniel, male.

This dog had been under observation one month previous to the experiments with caffein. The usual initial dose was then administered for nine days. There were no signs of local irritation when the drug was given subcutaneously, but symptoms of toxicity were present. These disappeared, however, when the drug was administered by mouth. The dose was therefore increased to 75 mg per kilo. This, as will be seen, proved fatal within six hours. High nitrogen diet, same as No. 30.

November 3: Weight 10.250 kilos.

November 10: Weight, 10.25 kilos.

November 20: Weight, 10.30 kilos.

December 1: Put in cage.

December 6, 7, 8, 9: Weight, 10.20 kilos; received 26 cc 2 per cent caffein subcutaneously, site of injection normal.

December 6: Very restless and excited, whined when handled as though muscles were sore, appeared to be sick.

December 10-14: Condition good, received 0.51 gram caffein by mouth daily, no noteworthy symptoms, appetite continues good, somewhat restless at intervals.

December 15: 11.30 a. m., received 0.765 gram caffein per mouth (0.075 gram per kilo); 2 p. m., depressed in spirit, seemed sick and uncomfortable; 4.15 p. m., when about to feed, animal jumped up, then fell back dead.

Autopsy (dog 31): Lungs congested; heart filled with blood and contained small amount of blood-stained fluid in pericardial sac. Liver deeply congested, soft and friable; gall bladder distended with bile; kidneys showed inflammation of cortex; spleen pale, normal in size and consistency; stomach practically empty, the mucosa of the pyloric portion exhibited severe gastritis, with thick catarrhal exudate. This catarrhal inflammation extended through the duodenum; remaining portion of small intestine showed mild inflammation; large intestine appeared practically normal. The total amount of caffein received by dog 31 during 10 days was 5.395 mg, or a daily average of 53.9 mg per kilo. This unusually low resistance to caffein (which was practically the only case in all the experiments on dogs presented in this research) suggests the presence of some abnormal condition. The bloody exudate in the pericardial cavity indicating pericarditis, which is likely to induce secondary changes of cardiac muscle, may be considered as a possible cause of the increased toxicity of caffein in this case.

Dog 29. Male fox terrier, black.

This dog was kept on a low nitrogen diet for nearly five weeks before the feeding of caffein was begun. The administration of 50 mg of caffein per kilo was then carried on for eight days without showing any symptoms of toxicity. The usual increase of dose was then given—75 mg per kilo—which was followed by a manifestation of symptoms. Further increase, however, to 100 mg per kilo had no visible effect. Nevertheless it was considered advisable to suspend the feeding of caffein for one day. The same amounts were then repeated on two consecutive days. No symptoms having been observed, 125 mg per kilo were given. As symptoms of toxicity and especially loss of appetite were observed, the dog was not given any caffein the next day. Since his appetite had now improved, the experiment with larger doses was resumed. Death followed after the second dose of 150 mg per kilo. Protocol follows:

Weight, 9.90 kilos. One-third nitrogen diet. Receives 0.269 gram nitrogen per kilo (88.269 calories per kilo).

November 3: Weight, 9.85 kilos.

November 10: Weight, 9.55 kilos.

November 12: Weight, 9.40 kilos.

November 29: Weight, 9.85 kilos.

December 6: Weight, 9.90 kilos; 11.35 a. m., received 25 cc 2 per cent caffein solution by subcutaneous injection in back; 4 p. m., no symptoms, appetite good.

December 7-9: Received 25 cc caffein 2 per cent solution—subcutaneous injection, no symptoms, area of injection inflamed and swollen.

December 10, 13: Site of injection showed increased inflammation, received 0.495 gram caffein (50 mg per kilo) in 30 grams meat daily without showing any symptoms.

December 14: 12 noon, received 0.7425 gram caffein by mouth (0.075 per kilo); 2.30 p. m., restless and uneasy.

December 15: 11.30 a. m., received 0.7425 gram caffein by mouth; 2 p. m., depressed in spirits, although continues to have good appetite.

December 16: Weight, 9.50 kilos; 3.15 p. m., received 0.9509 gram caffein by mouth; 4.50 p. m., no symptoms.

December 17: Animal rested.

December 18: Received 0.950 gram caffein by mouth, no symptoms.

December 19: Received 0.9509 gram caffein by mouth, no symptoms.

December 20: 2.45 p. m., received 1.1875 grams caffein (0.125 gram per kilo); 4 p. m., restless and quite sick; ate only a little food.

December 21: 9 a. m., still uncomfortable, allowed to rest, no caffein given, gradually recovered appetite.

December 22: 11 a. m., received 1.875 grams caffein; 3 p. m., seemed sick, but showed no other symptoms, appetite fair.

December 23: 9 a. m., showed no symptoms from the day before, ate food gradually, seemed sick; 11.30 a. m., received 1.425 grams caffein (0.150 gram per kilo); 1.30 p. m., looked and behaved as if very sick, no other symptoms; 3.45 p. m., in attempting to get out of box fell over on back, had convulsions, whined, dyspnœa, died within 30 seconds.

Autopsy: Stomach exhibited mild inflammation; small intestine inflamed and hemorrhagic areas on mucosa; liver engorged and friable; spleen normal; kidneys slightly congested; other organs appeared normal. The total amount of caffein fed to Dog 29 was 12.135 grams, which was given in 18 days. The average daily amount per kilo was therefore 67.68 mg.

Dog 28. Male fox terrier.

Low nitrogen diet was begun about four weeks before the feeding of caffein; 50 mg of caffein was then fed for seven consecutive days. Partial loss of appetite was observed after the first dose. As the experiment progressed the desire for food steadily diminished, and the feces became fetid. Symptoms of intoxication manifested themselves early in the experiment, and vomiting occurred after the fourth dose. The dog was then put on a diet exclusively of meat. After an intermission of 10 days 109 mg caffein per kilo were given. Since there were no symptoms, the following day the amount was increased to 125 mg per kilo. This dose proved fatal within 16 to 20 hours.

This dog was stout and strong, weight 12.25 kilos, received daily 0.269 gram nitrogen per kilo (88.269 calories per kilo).

November 3: Weight, 11.75 kilos.

November 10: Weight, 11.95 kilos.

November 20: Weight, 11.20 kilos. All through this period had been kept in a cold, poorly ventilated room, put in a warm room, with bedding and good ventilation.

November 29: Weight, 11.95 kilos.

December 1: Put in a cage; weight, 11.95 kilos.

December 6: Weight, 11.95 kilos; 11.45 a. m., received 0.050 gram caffein per kilo; then received 30 cc 2 per cent caffein (0.6 gram) in practically one subcutaneous injection; 4.30 p. m., ate only part of food.

December 7: 10.25 a. m., received 30 cc 2 per cent caffein by subcutaneous injection (0.6 gram, or 50 mg, per kilo); 1.45 p. m., seemed sensitive to touch, no desire for food, depressed in spirit.

December 8: 11.40 a. m., received 30 cc 2 per cent caffein by subcutaneous injection (50 mg per kilo); 1 p. m., depressed in spirit, hind legs seemed somewhat stiff, no desire for regular food, site of injection inflamed.

December 9: 10.50 a. m., received 30 cc 2 per cent caffein by subcutaneous injection (50 mg per kilo); 2.30 p. m., had vomited, no desire for regular food.

December 10: Inflammation of site of injection, and swelling very pronounced; 2 p. m., received 0.5975 gram caffein, or 50 mg per kilo, with 30 grams of meat, refused regular food.

December 11, 12: Received 0.5975 gram caffein by mouth, no symptoms except refusal of regular food, feces fetid.

December 13-22: Put on meat diet exclusively, high temperature, no caffein, weight 10 kilos, appetite good, feces fetid.

December 22: 12 a. m., weight 11 kilos, received 1.2 grams caffein by mouth (0.109 gram per kilo); 4 p. m., no symptoms.

December 23: 11.30 a. m., received 1.375 grams caffein (0.125 gram per kilo) had vomited food of the day before, but could notice no caffein or capsules in vomit; 4.30 p. m., no symptoms, seemed in good spirits, appetite good, had no meat to feed with, so was given low nitrogen feed, of which he ate about one-fourth.

December 24: 9 a. m., found dead, stiff, and cold. The most striking effect of caffein in this dog is the increased intestinal putrefaction. The feces were still fetid 10 days after the administration of caffein was stopped.

Autopsy, dog 28: Stomach partially filled with an undigested food mass; mucosa showed severe inflammation; small intestines presented a hemorrhagic enteritis along whole extent; large intestine also exhibited mild inflammation; liver was engorged; spleen appeared normal; kidneys slightly congested in cortical portion; other organs appeared normal.

Dog 24. White and tan male: Was put on low protein diet six weeks before experiments with caffein were begun. The initial dose of 50 mg per kilo was then administered on eight consecutive days. The only symptoms observed during this period of caffein administration were those of intestinal putrefaction. Fetid feces were noticed already after the first dose of caffein was injected. When the second dose of 75 mg of caffein was repeated, mild symptoms appeared, but none have been observed even with increased amounts of caffein.

One-third nitrogen diet. Received daily 0.269 gram nitrogen per kilo (88.269 calories per kilo).

October 26: Weight 11.15 kilos. Food consisted of 5 grams cracker meal per kilo; meat, 3 grams per kilo; lard, 2 grams per kilo; tapioca, 10.69 grams per kilo. Kept in a cold, damp room with poor ventilation until November 20.

November 3: Weight, 11 kilos.

November 10: Weight, 10.75 kilos.

November 20: Weight, 10.55 kilos; changed to a warm room, with bedding and good ventilation.

November 29: Weight, 10.85 kilos.

December 1: Put into a cage.

December 6: Weight, 10.90 kilos; 11.25 a. m., received 28 cc 2 per cent caffein subcutaneously in side, below the shoulders, area washed with alcohol and ether, approximately 50 mg per kilo administered, no symptoms.

December 7: 10.15 a. m., received 28 cc 2 per cent caffein injected subcutaneously; feces soft and very fetid; 1 p. m., depressed in spirit, eyes dull.

December 9: 10.45 a. m., received 25 cc 2 per cent caffein solution subcutaneously, feces still fetid, site of injection inflamed and swollen, no other symptoms.

December 10: Inflammation of area of injection more pronounced; 2 p. m., given 0.5449 gram caffein and 30 grams of meat; 4 p. m., fed, no symptoms, feces fetid.

December 11: 12 a. m., given 0.5459 gram caffein and 30 grams of meat, no symptoms, feces fetid.

December 12, 13: Given 0.5459 gram caffein daily, without noticing any symptoms.

December 14: 12 a. m., received 0.817 gram caffein (75 mg per kilo); 2.30 p. m., restless and uncomfortable, no other symptoms.

December 15: 11.30 a. m., received 0.8175 gram caffein by mouth; 2 p. m., depressed in spirit, acted as though sick, no other symptoms.

December 16: Weight, 11 kilos; 11 a. m., received 0.100 gram caffein per kilo (1.100 grams) by mouth, no symptoms.

December 17: Rested.

December 18: 2.30 p. m., received 1.100 grams caffein by mouth; 4 p. m., no symptoms.

December 19: 12 noon, received 1.100 grams caffein by mouth; 4.15 p. m., no symptoms.

December 20: 2.45 p. m., given 1.375 grams caffein (0.125 gram per kilo); 3.45 p. m., vomited—one of the capsules being found intact, the other broken open; 4 p. m., given regular diet, containing 1.3757 grams caffein in capsules, ate most of this during the night, whined at intervals, coordination disturbed, appeared very sick, but exhibited no other symptoms.

December 21: 9 a. m., found dead, stiff, and cold.

The total amount of caffein received by dog 24 was between 10.109 and 11.484 grams. As one of the capsules vomited was intact and the other broken open, the amount was probably about 10.75 grams. The fatal dose in this case was undoubtedly less than 185 mg per kilo—somewhere between 125 and 185 mg. Autopsy showed heart in diastole; posterior lobe of right lung deeply congested; liver engorged; gall cyst filled; spleen appeared normal; stomach well filled with semifluid mass; pyloric portion of stomach exhibited a severe inflammation of mucosa; mucosa of duodenum greatly inflamed and showed hemorrhagic areas and catarrhal exudate; remainder of small intestine also exhibited mild inflammation; kidneys deeply engorged, mesentery injected.

A comparison of the fatal doses of caffein in the experiments on high and low protein diet does not show much difference in the resistance to caffein, since 175 mg per kilo proved fatal to Nos. 30 and 32, while No. 29 died after receiving 150 mg per kilo, and No. 24 received 125 to 185 mg per kilo. Moreover, No. 28, which was changed from low to high protein diet, succumbed when given 125 mg per kilo. Observations made during the experimental period indicate, however, greater toxicity of caffein in the subjects on low protein diet. Dog 30 showed the effects of the drug when the dose was increased to 125 mg of caffein per kilo, while in No. 32, 150 mg per kilo were received without any manifestation of symptoms. Dog 31, which was likewise on a high protein diet, is evidently an exception, and its low resistance to caffein may be accounted for by the condition found at autopsy. In other dogs on low protein diet symptoms of intoxication appeared early in the experiment. In Nos. 29 and 24 it was observed as soon as the amount of caffein was increased to 75 mg per kilo. In dog 28 the first dose of caffein 50 mg per kilo was toxic. The symptoms of gastro-intestinal disturbance were especially marked after caffein on low protein diet. This may seem to contradict the results of experiments on dogs 11 and 20, in which larger doses of caffein failed to induce symptoms of intoxication. But it should be observed that the diet, which consisted almost exclusively of carbohydrates, was given only during the administration of caffein, while in the experiments of series B the subjects received a low protein diet for several weeks before the administration of caffein was begun, and it was continued through the entire caffein period. It will be remarked that the absence of cumulative action in the

experiments of the preceding series was also observed in dogs on high as well as on low protein diet. The appearance of symptoms after smaller doses of caffein in the latter experiments might suggest cumulative action, but since these symptoms disappeared on continued administration of the substance cumulation is clearly not indicated. The gastrointestinal lesions observed on post-mortem examination were, it will be recalled, also found in rabbits similarly treated. The explanation suggested probably applies also in the case of dogs.

SERIES C.

As already pointed out in the experiments on acute toxicity of caffein, young growing dogs are probably more resistant to caffein than adults. That this may also hold true in chronic caffein intoxication seemed indicated by the following experiments.

Dog 33. Black female puppy. Weight, 4 kilos. Had been continuously on a meat diet.

December 22: 2.30 p. m., received 0.69 gram of caffein (0.172 gram per kilo); 3.15 p. m., no symptoms except that feces were fetid.

December 23: 11.30 a. m., received 0.79 gram of caffein (0.197 gram per kilo); 1.30 p. m., no symptoms.

December 24: 11 a. m., received 0.87 gram of caffein (0.2009 gram per kilo); 4 p. m., no symptoms.

It will be observed that the only effect produced in dog 33 by feeding caffein was increased intestinal putrefaction, although 2.37 grams of caffein were given in three days. Additional data on the effects of the age of animals on the resistance to caffein seemed desirable. The following experiments were therefore carried out. Six puppies of the same litter were weaned when 7 to 8 weeks old and put on a milk diet. Three of them received this diet throughout the experimental period. Meat was substituted in the other three a few days before the administration of caffein was begun, and was continued until the end of the experiment. Caffein was given by mouth; the initial dose, which was administered for several days and then gradually increased, being 160 to 200 mg for each dog, except one, which received only 100 mg per kilo for several days and then an increased amount.

An intermission of a few days (during which no caffein was given) was allowed. This was done on account of some studies carried on at the same time on the effect of caffein on certain constituents of the urine.

PUP NO. 1.

Date.	Weight.	Food (milk).	Treatment (2 per cent caffein).	Symptoms.
	Grams.	*cc.*	*cc.*	
Apr. 21	1,450	300	10.0	No symptoms.
Apr. 22	1,520	300	10.0	Do,
Apr. 23	1,450	250	10.0	Do,
Apr. 24	1,375	250	10.0	Do,
Apr. 25	1,420	250	10.0	Do,
Apr. 26	1,390	250	None.	
Apr. 27	1,400	250	None.	
Apr. 28	1,405	250	None.	
Apr. 29	1,420	250	None.	Passed worms.
Apr. 30	1,430	250	None.	Do,
May 1	1,450	250	10.0	No symptoms.
May 2	1,515	250	15.0	Do,
May 3	1,475	250	15.0	Do,
May 4	1,495	250	15.0	Do,
May 5	1,515	250	22.0	Seems dull and whines.
May 6	1,535	250	20.0	Whines.
May 7	1,525	250	20.0	No symptoms.
May 8	1,530	250	20.0	Do,

May 9	1,500	250	23.0	Diarrhea; passed worms; tremor and rigidity of legs; whines.
May 10	1,490	250	None.	Completely recovered from the effects of 9th.
May 11	1,535	250	25.0	Can not balance itself; continually vomiting.
May 12	1,460	300	None.	Recovered from effects.
May 13	1,475	350	None.	In good condition.
May 14	1,545	250	None.	
May 15	1,550	250	None.	
May 16	1,555	250	None.	
May 17	1,560	250	25.0	Salivated in cage; stiffness of muscles.
May 18	1,450	250	None.	Weak and stiff; diarrhea.
May 19	1,500	250	None.	No symptoms.
May 20	1,565	250	None.	
May 21	1,545	250	None.	
May 22	(1)	250	None.	
May 23	1,595	250	27.0	Tremors; gait clumsy; incoordination of movements.
May 24	1,495	250	27.0	Diarrhea; vomited; weak and stiff; found dead 9 a. m. 25th.

1 Sunday.

Autopsy: Marked pulmonary congestion; liver very pale; heart wall injected; slight inflammation of stomach and intestines.

PUP NO. 2.

Date.	Weight.	Food (milk).	Treatment (2 per cent caffein).	Symptoms.
	Grams.	*cc.*	*cc.*	
Apr. 21	1,350	300	5.0	No symptoms.
Apr. 22	1,240	300	5.0	Do,
Apr. 23	1,250	200	7.5	Do,
Apr. 24	1,205	200	7.0	Do,
Apr. 25	1,220	200	7.0	Do,
Apr. 26	1,210	200	None.	
Apr. 27	1,210	200	None.	
Apr. 28	1,205	200	None.	
Apr. 29	1,200	200	None.	Passed worms.
Apr. 30	1,210	200	None.	
May 1	1,220	200	10.0	No symptoms.
May 2	1,220	200	10.0	Do,
May 3	1,235	200	10.0	Do,
May 4	1,235	200	10.0	Do,
May 5	1,235	200	17.0	Whines.
May 6	1,250	200	17.0	Do,
May 7	1,235	200	15.0	Diarrhea and worms.
May 8	1,250	200	15.0	Diarrhea.

May 9	1,165	200	18.0	Little or no symptoms.
May 10	1,235	200	None.	No symptoms.
May 11	1,300	200	20.0	Salivated in cage; refused to eat; draws up hind legs.
May 12	1,200	200	None.	Recovered.
May 13	1,215	200	None.	In good condition.
May 14	1,280	200	None.	
May 15	1,300	200	None.	
May 16	1,310	200	None.	
May 17	1,310	200	20.0	Salivated in cage; stiff; all symptoms.
May 18	1,250	200	None.	Weak and stiff.
May 19	1,245	200	None.	No symptoms.
May 20	1,310	200	None.	
May 21	1,325	200	None.	
May 22	1,325	200	None.	
May 23	1,325	200	22.0	Somewhat stiff.
May 24	1,315	200	22.0	Restless; scratches eyes; sick.

PUP NO. 3.

Apr. 21	1,215	300	None.	
Apr. 22	1,220	300	None.	
Apr. 23	1,220	200	None.	
Apr. 24	1,200	200	None.	
Apr. 25	1,205	200	None.	
Apr. 26	1,195	200	None.	
Apr. 27	1,200	200	(1)	
Apr. 28	1,215	200	None.	
Apr. 29	1,220	200	None.	
Apr. 30	1,200	200	None.	
May 1	1,225	200	10.0	No symptoms.
May 2	1,230	200	10.0	Do,
May 3	1,235	200	10.0	Coughs and whines.
May 4	1,245	200	10.0	Passed worms.
May 5	1,270	200	17.0	Eyes appear dim and is continually scratching them.
May 6	1,260	200	17.0	Appears restless and draws up hind legs when walking.
May 7	1,240	200	15.0	Eyes dim; passed worms; diarrhea.
May 8	1,265	200	15.0	Coughing continually; very restless.
May 9	1,240	200	18.0	12 noon; salivated in cage; passed worms; diarrhea; foaming at mouth; can not balance himself; rigidity and tremor of hind legs. 2.15, found dead.

[1] Urine squeezed from bladder.

Autopsy: Severe pulmonary congestion; catarrhal gastritis; mild enteritis with small hemorrhagic areas on mucosa.

PUP NO. 4.

Date.	Weight.	Food.	Treatment (2 per cent caffein).	Symptoms.
	Grams.	*Milk cc.*	*cc.*	
Apr. 28	1,670	300		
Apr. 29	1,670	300		
Apr. 30	1,670	300		
May 1	1,690	300		
May 2	1,690	300		
May 3		300		
May 4	1,720	300		
May 5	1,735	300		
		Meat (grams).		
May 6	1,760	60		
May 7	1,745	80		
May 8	1,710	180		
May 9	1,750	180		
May 10	1,750	180		
May 11	1,755	180	10.0	No symptoms.
May 12	1,730	180	None.	
May 13	1,785	180	None.	
May 14	1,835	115	10.0	Do,
May 15	1,820	115	10.0	Do,
May 16	1,835	115	10.0	Passed worms.
May 17	1,860	115	10.0	Feces soft and black.
May 18	1,855	115	15.0	Stiff; loss of appetite.
May 19	1,770	115	15.0	Loss of appetite.
May 20	1,755	115	15.0	Do.
May 21	1,780	115	17.0	Restless.
May 22		115	17.0	Feces soft and black.
May 23	1,785	115	17.0	Loss of appetite.
May 24	1,795	115		Loss of appetite; threw up worms.
May 25	1,630	115	20.0	Loss of appetite; worms; cough; diarrhea.
May 26	1,600	115	23.0	Weak; no appetite; diarrhea; cough.
May 27				Found dead, 9 a. m.

Autopsy.—Lung uniformly congested; liver deeply congested; heart muscle pale with hemorrhagic areas; kidneys pale with hemorrhagic spots on surface and in cortex; slight catarrhal inflammation of stomach and the small intestines.

PUP NO. 5.

Date.	Weight.	Food.	Treatment (2 per cent caffein).	Symptoms.
	Grams.	*Milk cc.*	*cc.*	
Apr. 28	1,745	300		
Apr. 29	1,745	300		

Apr. 30	1,750	300		
May 1	1,765	300		
May 2	1,765	300		
May 3		300		
May 4	1,490	300		
May 5	1,805	300		
		Meat (*grams*).		
May 6	1,815	60		
May 7	1,825	80		
May 8	1,770	180		
May 9	1,795	180		
May 10	1,805	180		
May 11	1,800	180	10.0	No symptoms.
May 12	1,720	180	None.	
May 13	1,815	180	None.	
May 14	1,845	115	10.0	Do,
May 15	1,830	115	10.0	Do,
May 16	1,815	115	10.0	Loss of weight; no other symptoms.
May 17	1,830	115	15.0	No symptoms.
May 18	1,835	115	15.0	Stiffness.
May 19	1,825	115	15.0	No symptoms.
May 20	1,850	115	15.0	A little stiff.
May 21	1,835	115	17.0	No symptoms.
May 22		115	17.0	
May 23	1,820	115	17.0	Do.
May 24	1,835	115	20.0	Do.
May 25	1,840	115	20.0	Feces soft and black.
May 26	1,820	115	23.0	
May 27	1,840	115	25.0	A little stiff.
May 28	1,830	115	25.0	
May 29		115	None.	
May 30		115	None.	
May 31	1,770	115	None.	
June 1	1,765	115	25.0	Diarrhea; stiff in hind legs.
June 2	1,750	115	27.5	Diarrhea and worms.
June 3	1,635		27.5	Paralyzed; vomited; died at 3 p. m.

PUP NO. 6.

Date.	Weight.	Food.	Treatment (2 per cent caffein).	Symptoms.
	Grams.	*Milk cc.*	*cc.*	
Apr.		300		

28				
Apr. 29	1,280	300		
Apr. 30	1,290	300		
May 1	1,315	300		
May 2	1,330	300		
May 3		300		
May 4	1,360	300		
May 5	1,365	300		
		Meat (grams).		
May 6	1,395	60		
May 7	1,365	80		
May 8	1,340	180		
May 9	1,380	180		
May 10	1,400	180		
May 11	1,425	180	14.5	No symptoms.
May 12	1,470	180	None.	
May 13	1,485	180	None.	
May 14	1,510	115	14.5	Do,
May 15	1,500	115	14.5	Do,
May 16	1,485	115	14.5	Passed worms.
May 17	1,480	115	14.5	
May 18	1,485	115	19.5	Feces soft and black; almost diarrhea.
May 19	1,495	115	19.5	
May 20	1,500	115	19.5	Scratches her eyes and chases her tail.
May 21	1,500	115	17.0	
May 22		115	17.0	
May 23	1,470	115	17.0	
May 24	1,465	115	20.0	
May 25	1,450	115	20.0	Feces soft and black.
May 26	1,450	115	23.0	Diarrhea and worms.
May 27	1,355	115	23.0	Refused to eat all food.
May 28	1,270	115	23.0	Threw up worms, stiff, and has skin over both eyes.
May 29				Found dead.

Highest amount of caffein given, 362 mg per kilo. No autopsy.

Examination of the results obtained in the experiments of series C shows that young and growing dogs tolerate large amounts of caffein. In four subjects of this series, Nos. 1, 2, 3, and 6, no effect was observed when moderately large amounts (160 to 200 mg per kilo of caffein) were fed. Symptoms were noticed only when these amounts of caffein were increased from 50 to 60 per cent. The other two dogs, Nos. 4 and 5, of this series were less resistant, however, to caffein, as 0.16 gram of the drug per kilo induced well-marked symptoms. Since these were fed meat, while Nos. 1, 2, and 3 received milk, the difference in toxicity may be due to the diet employed, but No. 6, which likewise received a meat diet, failed to show the effects of caffein when 200 mg per kilo were fed. On the other hand, it should be noticed that No. 1 died after receiving 360 mg per kilo, No. 2 survived a dose of 334 mg, while No. 3 died after a dose of 322 mg per kilo of caffein. The

fatal doses for Nos. 4, 5, and 6 were 287, 335, and 300 mg per kilo, respectively. Although the differences are too small to justify any definite conclusion regarding the effect of a milk diet or of a meat diet on the toxicity of caffein, the results nevertheless suggest a reasonable possibility that caffein is more toxic to young dogs when on an exclusively meat diet than when fed milk. It is perfectly evident, however, that the resistance to caffein in either case is very great, almost twice that of adult subjects. As shown in series A and B, 125 to 175 mg per kilo proved fatal to all but two animals in these experiments, while symptoms of toxicity appeared after much smaller doses. In other respects the behavior of young dogs toward caffein was the same as that of the adult. In neither case was cumulation nor tolerance observed under the conditions of these experiments. The findings at autopsy were likewise similar, as gastro-enteritis was the chief lesion observed on macroscopic examination. It might be mentioned, however, in this connection, that the symptoms of caffein intoxication in young dogs often presented marked differences from those observed in those of more advanced age. The resemblance of the effects of caffein in young puppies and in rabbits was very striking. In both, the tonic with clonic convulsions were observed after a sufficient quantity of caffein was administered. In the dogs which were fully grown a large dose of caffein was usually followed by tonic convulsions and almost instantaneous death.

Moderately large amounts of caffein fed daily to puppies for several days—in some cases as long as 10 days—induced mild symptoms only. No cumulative effect was observed in any of the experiments of series C. There seems to be tolerance of certain functions toward caffein, but no general tolerance of the body could be obtained in these experiments. Caffein is apparently less toxic for adult dogs on high than on low protein diet. In young and growing dogs caffein is somewhat less toxic when milk, rather than meat, forms the exclusive diet. Some pathological conditions apparently increase the toxicity of caffein also in dogs. The symptoms of caffein intoxication observed in young dogs are in some respects different from those in full grown and older animals, and resemble those noticed in rabbits.

DISCUSSION OF RESULTS.

It was pointed out at some length in the introduction that the toxicity of some drugs may not be the same for all forms of life. This observation was also made by some investigators who experimented with caffein on different species of animals. Thus Maurel[55] stated that caffein is twice as toxic for the frog as for the rabbit when administered by mouth. Fröhner's[26] experiments, on the other hand, made on domestic animals, failed to show great differences in the toxicity of caffein. According to this observer, horses seem to be more susceptible than cattle, goats, and swine, the minimum toxic dose being the same for all of these, while the resistance of the dog to caffein is about midway between that of the horse and the other animals mentioned. It may be remarked, however, that Fröhner made only 13 experiments. That these data are inadequate for the formation of any conclusions as to the toxicity of caffein is evident since the most striking effect of caffein observed in the work herein reported was the comparatively wide range of variation in the resistance of individuals of the same species to this drug. This was found to be the case even when the conditions of experimentation were approximately uniform, and was observed whatever the mode of administration of the drug employed. The toxicity for different individuals also varied in acute as well as in chronic intoxication. It is for this reason that the number of tests employed were often quite large, for no conclusions of any value could be drawn without averaging the results of a sufficiently large number of experiments. Furthermore, it is to be borne in mind that the action of a drug may differ according to the mode of its introduction into the body and that different species of animals may vary in this regard. This is especially true of some substances when given by mouth, the range in toxicity for certain species of animals being much greater when thus administered than when injected subcutaneously or intravenously.

Maurel's[56] investigations are of interest in this connection, as his work embraces a systematic study of the toxicity of a large number of substances in the rabbit, pigeon, and frog when given by mouth, subcutaneously, intravenously, or when injected into the muscles. According to this investigator the range of variation of the toxicity of a substance is widest when given by mouth. Potassium sulphocyanid, for example, is about 2.5 times as toxic for the frog as for the rabbit when given by mouth. Quinin hydrobromid is three times as toxic for the frog as for the pigeon, while for the rabbit it is twice as toxic as for the pigeon. When given by hypodermic injection the toxic dose per kilo weight is practically the same for all three species. The difference of resistance according to the mode of administration is even more marked for spartein sulphate. When given by mouth the toxicity for the rabbit is six times as great as for the frog, but when injected subcutaneously the toxic dose is about the same for the rabbit and for the frog. The relation of the mode of administration to toxicity is further shown in the following substances: For the rabbit the minimum fatal dose per kilo of quinin hydrobromid is 1.5 grams administered by mouth, 0.5 gram when injected subcutaneously, and 0.07 gram by the intravenous path, while strychnin sulphate is twice as toxic administered intravenously as subcutaneously, and six times as toxic as when administered by mouth. The mode of introduction, however, does not always affect the toxicity of a substance. This is made evident by the action of strychnin on frogs in which, according to Maurel[56], the toxic dose is the same whether given by mouth or injected into the subcutaneous tissues. This appears to hold true also for other animals as demonstrated by the experiments of Hatcher[35] on the cat, in which he observed that strychnin is as readily absorbed from a full stomach as from the subcutaneous tissues. These findings are extremely interesting, especially in view of Maurel's[57] work on the subject, according to which he finds that a substance is much less toxic when given by mouth than when administered by hypodermic injection or intravenously. That this generalization does not admit, however, of universal application is made evident by the work of various experimenters. Claude Bernard[10] observed that curara is as poisonous for the pigeon when given by mouth as when injected subcutaneously, while Zalesky[86] found that samandarin is more toxic for frogs when introduced into the stomach than by injection into the lymph sacs. Our experiments with caffein likewise show that Maurel's generalization does not always hold good, since it was found in experiments with gray rabbits that the minimum fatal dose is but moderately greater by mouth than by the subcutaneous path.

Equally interesting is the observation of the writer, that in the guinea pig the difference in the toxicity between the subcutaneous and intraperitoneal injections is very slight, while in the cat the toxicity of caffein is the same whether given by mouth or injected into the subcutaneous tissues, and is markedly less when injected into the peritoneal cavity. The experiments on dogs show considerable variation of effective dose when given by mouth, but the interesting observation was made that the toxic dose by mouth may be smaller in some cases than the average dose by subcutaneous injection. If the resistance to caffein by subcutaneous injection of the different species of animals experimented upon in the present research be compared, it will be noticed that the gray rabbit or Belgian hare, which is more resistant than the other varieties employed, stands more caffein in proportion to the weight of the body than the other animals.

Although the minimum fatal dose was found to be somewhat larger for the guinea pig than for the gray rabbit when caffein was injected intraperitoneally, it was on the contrary smaller by other paths of introduction, and approximated quite closely the minimum fatal dose for rabbits of the other varieties. Cats as well as dogs were found to be distinctly less resistant to caffein than the herbivora.

There are a number of factors far more important than zoological differences which influence the toxicity of caffein. Some of these are age, season, and pathologic conditions. As these factors

have already been dwelt upon in their appropriate places, further discussion might seem unnecessary, but owing to their importance in determining the action of a drug, emphasis is desirable. Especially is this the case with pathological conditions in relation to toxicity. While no positive proof of diminished resistance to caffein in pathological conditions was obtained by subjecting the suggestion to experimental test, it was observed in these experiments on rabbits that death occurred in some individuals after small doses which are usually not even toxic. The findings at autopsy indicate the presence of pathological conditions. The same was observed in some experiments on cats and dogs. It is extremely probable, therefore, that disease modifies the reaction of the organism to caffein as well as to other drugs.[78]

That the resistance to drugs may vary according to the age of the subject has been maintained by a number of pharmacologists. According to Guinard,[30] young dogs, rabbits, and guinea pigs are very susceptible to morphin, resembling children in this regard.[E] The minimum fatal dose for these animals is about one-third less than for the adult. This is not true, however, for the young of other species. Cats under 15 days of age tolerate twice the toxic dose of morphin for the adult cat. Young beeves and goats are likewise more resistant to this alkaloid than adults. On the other hand, according to Livon,[54] young guinea pigs are more sensitive to alkaloids than adults. The toxicity of caffein, as shown in the present investigation, was found to be less in the young than in the adult. In dogs the young subjects are in some instances almost twice as resistant as adults. The difference was found to be less in cats and rabbits than in dogs, but it was quite marked.

The effect of season on the toxicity of drugs has been discussed in the section on the experiments on guinea pigs, which were more resistant to caffein in the fall than in February and March. The effect of season seems to vary with the animal, but it may also differ with the substance employed. In Noe's[65] studies on this subject cantharidin was found to be more toxic for the hedgehog in November than in July. The effect of season was different for morphin, as it was observed that the resistance of the hedgehog was greater at the end of the summer than earlier in the season.

The relation of diet to toxicity of drugs has been studied by Hunt.[39] His experiments indicate that this is an important factor in the resistance to acetonitril. The studies here reported on the effect of diet on toxicity of caffein in rabbits were confined to experiments with oats and carrots and do not show any modification of the resistance to caffein. The question of diet in chronic intoxication in dogs, however, suggests that in these animals diet may affect the toxicity of caffein, although the data on this subject are far from satisfactory. There is nevertheless sufficient evidence to suggest that a high protein diet for the adult dog tends to greater resistance of the animal to caffein and similarly the growing dog tolerates larger quantities of caffein on a milk diet than on a diet of meat.

This brings us to a consideration of the behavior of caffein in chronic intoxication. Although in both rabbits and dogs absence of cumulation was evident, in other respects decided differences in the resistance to caffein were observed. While the rabbit tolerates more than twice the single dose of caffein per kilo for the dog, the result is quite different in repeated dosage of the drug, the rabbit succumbing to continued administration of much smaller doses of the drug than the dog. This is probably due to lesions of the gastro-intestinal canal caused by caffein which occasions loss of appetite much more readily in the rabbit than in the dog. The abundant energy reserve in the dog makes it possible for this animal to stand inanition much longer than the rabbit and other herbivora. The difference in the behavior of the rabbit and dog toward caffein is interesting as showing complete reversal of resistance in acute and chronic intoxication. From the statement in the introduction it is evident that the size of the single toxic or lethal dose of a substance is in no wise an index of the active degree of its toxicity. The experiments with caffein here reported furnish additional evidence that this is true, at least for the rabbit.

GENERAL SUMMARY AND CONCLUSIONS.

The toxicity of caffein in the rabbit varies with the mode of its administration, being least when given by mouth and greatest by intravenous administration. The toxicity is from 15 to 20 per cent greater by subcutaneous injections than by mouth, but is about half of that when injected into the peritoneal cavity. No difference was observed in the toxicity of caffein whether administered into gluteal or into the lumbar muscles. When introduced by this route the toxicity was found to be less by one-third than when it is injected into the peritoneal cavity, but is about 30 per cent more toxic than the subcutaneous injections. White or black rabbits were found to be less resistant to caffein than gray rabbits.

The resistance of the guinea pig to caffein, as of the rabbit, is greatest when given by mouth. The minimum fatal dose is less by intraperitoneal injections, but greater than by subcutaneous injections, thus differing from the rabbit in this regard. The adult cat is less resistant than the guinea pig or rabbit to caffein. The minimum lethal dose by mouth is the same as by subcutaneous, and is less than by intraperitoneal, injection. The minimum fatal dose for dogs was found to be the same by mouth as by subcutaneous injection and is almost the same as for the cat. The toxicity of caffein varies in the guinea pig according to season of the year.

Age is likewise a factor in the toxicity of caffein, young animals being more resistant than the full-grown and older animals; this was shown in experiments on rabbits, cats, and dogs. The symptoms of caffein poisoning also were different in puppies and in full-grown dogs. Different diets, such as carrots and oats, did not influence the resistance of rabbits and guinea pigs to caffein. Low protein diet tends to decrease resistance to caffein in dogs. Young growing dogs are less resistant to caffein on a meat than on a milk diet. Caffein is not cumulative in the rabbit or dog, even if administered for a considerable length of time. Some degree of tolerance may be induced in the rabbit under certain conditions, but not in dogs under the conditions of the experiments made in this investigation. The possibility, however, that dogs may acquire tolerance for caffein is not excluded. Although the rabbit tolerates a much larger single dose of caffein than the dog, it was found, in experiments on chronic intoxication that the rabbit is less resistant to caffein than the dog. The toxicity of caffein is probably increased under pathological conditions, since comparatively smaller doses were fatal to rabbits, cats, and dogs, when marked lesions not due to caffein were found at autopsy. Glycosuria was observed in rabbits, guinea pigs, and cats when caffein was given in sufficient amounts.

TABLE 18.—*Acute caffein intoxication: Table showing average minimum toxic and minimum fatal doses for adult animals.*

Table headings:

SC: Subcutaneously.
BM: By mouth.
IP: Intraperitoneally.
IM: Intramuscular.
IV: Intravenous.

Animal.	Effect of dose.	Dose per kilo (grams)				
		SC.	BM.	IP.	IM.	IV.
Rabbit, gray	{Toxic	0.15	0.325	0.100-0.125	0.13-0.15	0.05
	{Fatal	.30	.350	.150	.20	0.10-.16
Rabbit, white or black	{Toxic					
	{Fatal	.20	.290			
Guinea pig	{Toxic	0.15-.16	.150	.200		
	{Fatal	.20-.24	0.280-.300	.240-.250		
Cat	{Toxic	.12-.14	.125	.125-.150		
	{Fatal	.15	.150	.180-.200		
Dog	{Toxic		.100-.120			
	{Fatal	.15-.16	.140-.150			

NOTE.—The doses given in this table are approximate.

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FOOTNOTES:

[A] The small figures refer to the bibliography at the end of this bulletin.

[B] Survived first dose.

[C] Time of injection inadvertently omitted, but was probably not slower than in the other cases of this series.

[D] Cat probably old; had been in the laboratory for several weeks before the experiment. Gained in weight 175 grams.

[E] A case of accidental poisoning reported recently by Wichura (Münich. Med. Woch., 1911, No. 30, p. 1618) throws some doubt on the accepted view that the susceptibility of young children to morphin is greater than that of adults. Wichura also found that the therapeutic doses of codein preparations ordinarily recommended for children in pleuritic cough are not effective in this condition.

Transcriber's Notes

Obvious typographical errors have been corrected, but variations in spelling, punctuation and hyphenation have been retained.

Rabbit 396. The date August 15 has been corrected to August 19.

The data for Rabbit 123 is presented as printed in the original, although it is likely that the Caffein per kilo. values should be preceded by a decimal point (or that the heading should be Mg.).

Standard footnotes are identied alphabetically. Numeric references within the text refer to the bibliography.

The headings of Table 18 have been abbreviated for clarity on small screens.

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