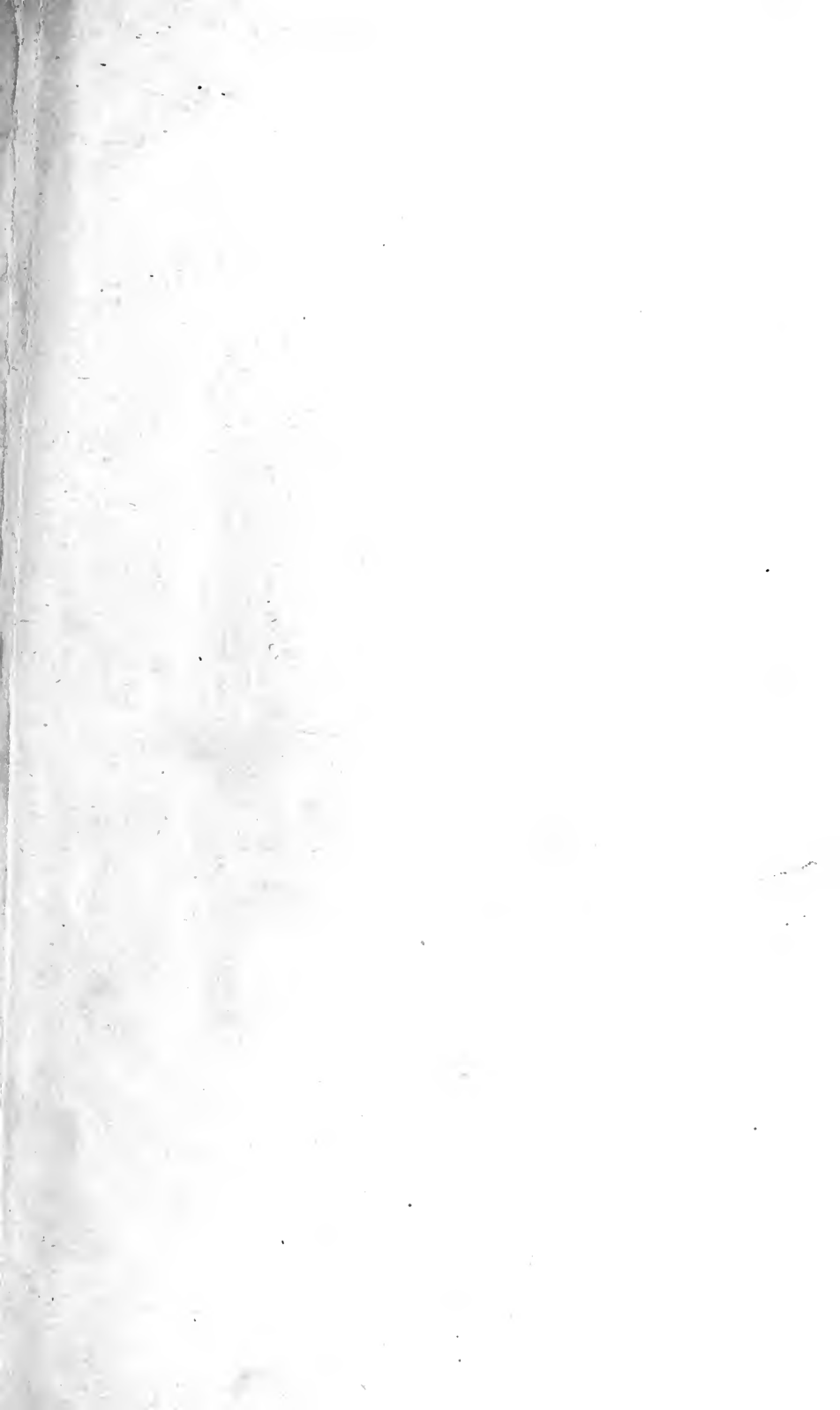


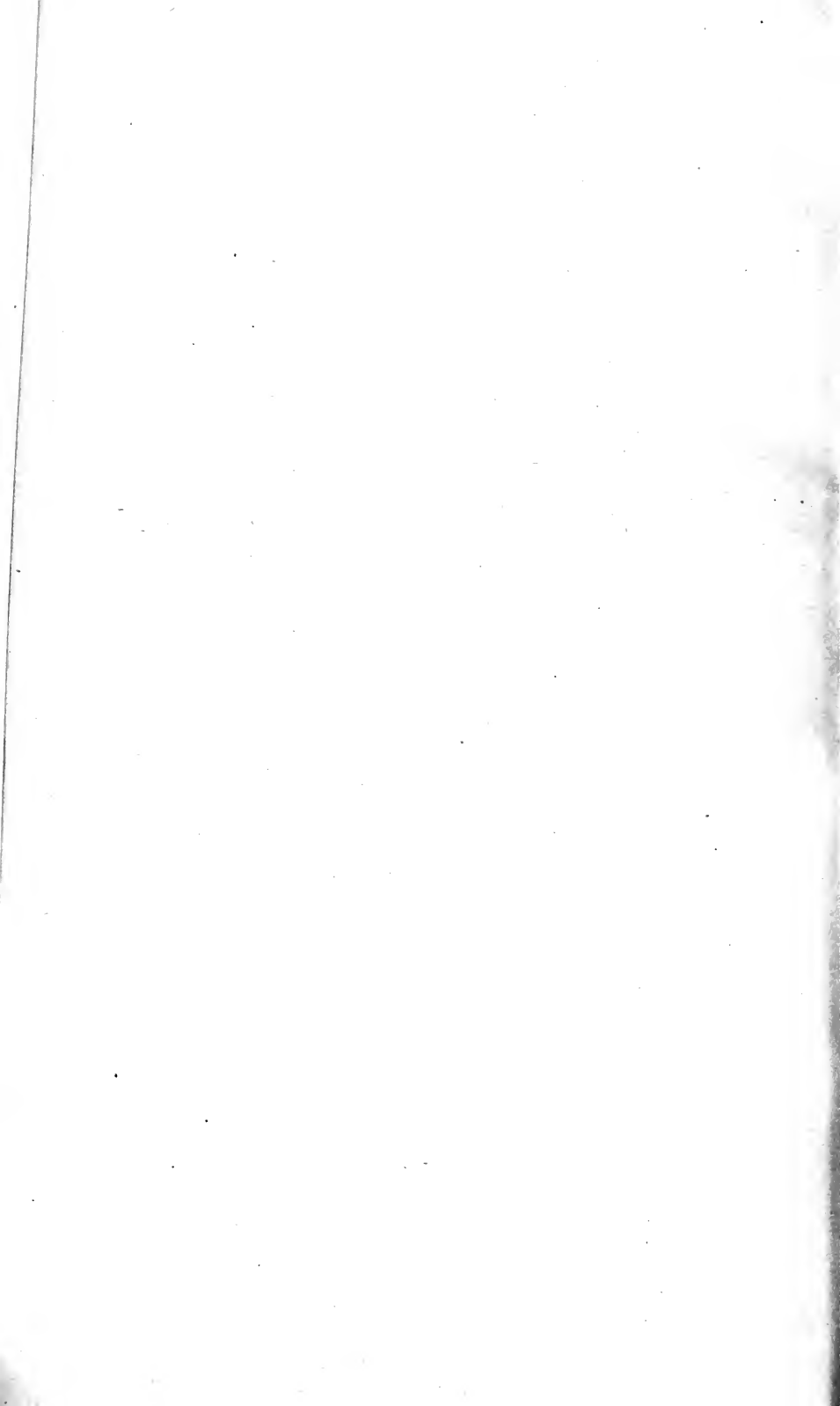
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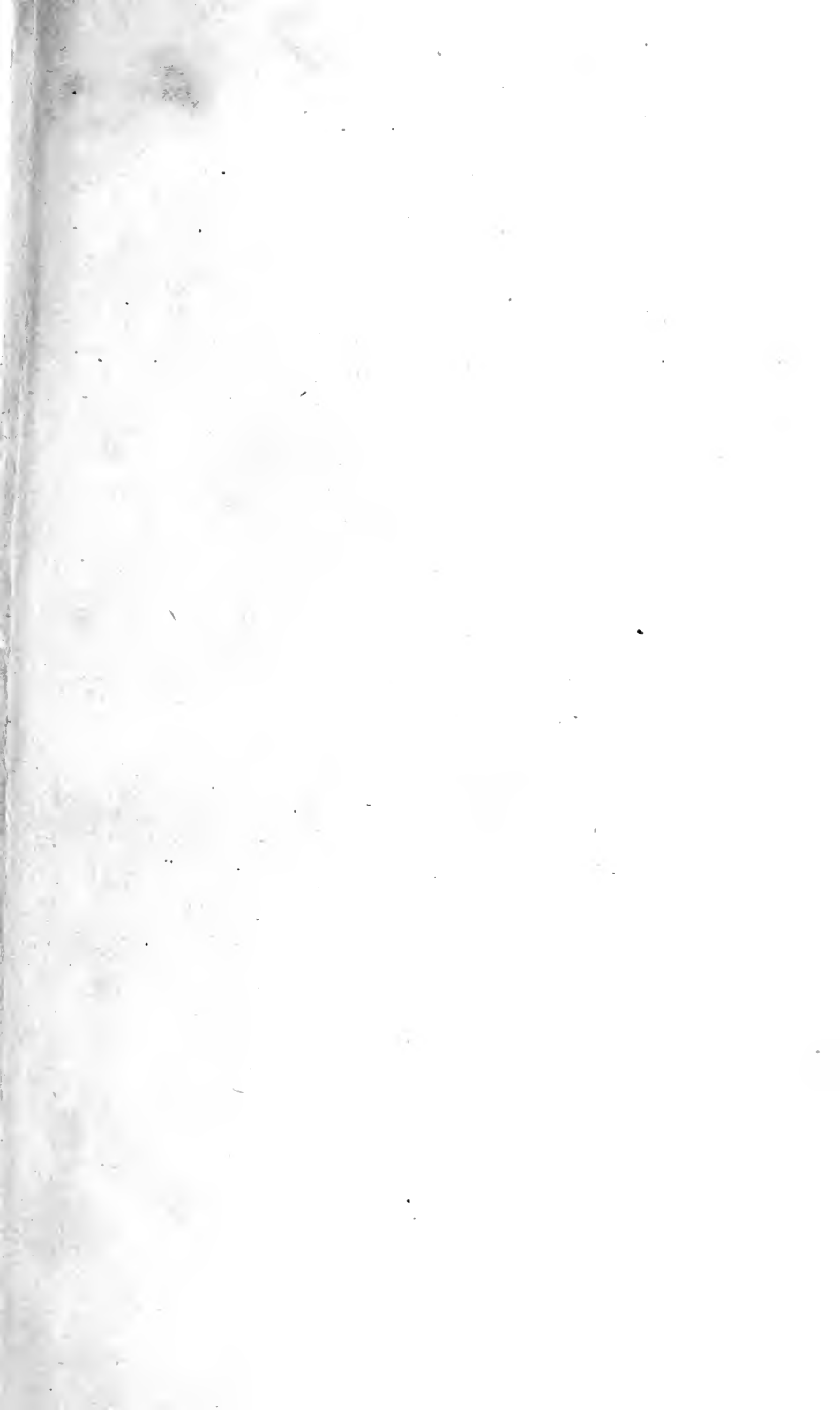


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U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF CHEMISTRY—BULLETIN No. 166.

CARL L. ALSBERG, CHIEF OF BUREAU.

THE ELIMINATION AND TOXICITY OF
CAFFEIN IN NEPHRECTOMIZED
RABBITS.

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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF CHEMISTRY,
Washington, D. C., November 18, 1912.

SIR: I have the honor to submit for your approval a manuscript on the elimination and toxicity of caffein in nephrectomized rabbits, which is the third of a series of reports on caffein being made by the Pharmacological Laboratory of the Division of Drugs in connection with the enforcement of the food and drugs act. The first two numbers of the series are Bulletins 148 and 157. I recommend that this manuscript be published as Bulletin No. 166 of the Bureau of Chemistry.

Respectfully,

R. E. DOOLITTLE,
Acting Chief.

Hon. JAMES WILSON,
Secretary of Agriculture.

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THE ELIMINATION AND TOXICITY OF CAFFEIN IN NEPHRECTOMIZED RABBITS.

ELIMINATION OF CAFFEIN IN NEPHRECTOMIZED RABBITS.

INTRODUCTION.

That the elimination of foreign substances from the healthy body is accomplished chiefly by the kidney is a fact well established by numerous investigations and has been found to be true not only for compounds differing widely in chemical composition but also for those differing in physiological action. The urinary tract is not the sole channel, however, for ridding the body of substances not normally present in the tissues. The stomach and intestines, as well as the digestive glands, likewise perform the function of excretion, in some cases supplementing the activity of the kidney, and in others serving as the sole path for the removal of substances which might find lodgment in the tissues either as a result of accident or disease or when introduced artificially for experimental purposes. Although much information has been secured on elimination into the gastrointestinal canal in health, elimination by this channel in various renal affections, when accompanied by deficient excretion of solids or when the kidney is entirely removed, has not as yet been made the subject of extensive investigation.

REVIEW OF THE LITERATURE.

Statements that impairment of the renal function or removal of the kidney may be accompanied by a compensatory increase in the elimination of some substances by the gastrointestinal canal have appeared from time to time in the literature, but have never been supported by data sufficient to render them of scientific value, although clinicians have utilized such information for therapeutic purposes. Bing⁶ observed a greater output of chlorids into the stomach in a few cases of chronic nephritis in which the kidney became impermeable to these salts. Similar observations regarding chlorids were made by Javal,¹¹ according to whose analysis from 3 to 4 grams of sodium chlorid were found in the feces of patients suffering from Bright's disease during an attack of diarrhea, while in the healthy subject only from 0.1 to 0.2 gram was obtained in the feces of 24 hours. Widal and Javal,²⁰ who also analyzed the vomitus in cases of chronic

* The superior figures refer to the bibliography at the end.

Bright's disease, stated that the amount of chlorin recovered exceeded the total quantity ingested and eliminated in the urine, thus showing undoubted compensatory elimination by the gastric epithelium. They also observed that some of their patients vomited a fluid containing chlorids when milk formed the exclusive diet.

That other organs may behave in a manner similar to the stomach and intestines appears from the following studies. Zezschwitz³⁰ examined the saliva of healthy people for urea, and found only traces in some and no evidence of its presence in others. In cases of nephritis, however, especially in chronic cases, he found appreciable quantities of urea in the saliva. Powers²⁴ maintained many years ago, on the basis of observations he made, that the elimination of total nitrogen and urea by the skin was increased in cases of chronic nephritis.

The presence of a mechanism by which vicarious action or compensatory elimination is brought about in the body has also received support from experiments on animals. Claude Bernard⁵ was probably the first to bring forward evidence of increased elimination, if not of true vicarious action, by the gut, for he found that urea is eliminated by the intestinal epithelium in dogs after the removal of both kidneys from the circulation. He also demonstrated the presence of potassium ferrocyanid in the saliva of dogs from which the kidneys were removed or in which the ureters were tied. This substance when injected into the circulation is not eliminated in the saliva of normal dogs. Corroborative evidence of vicarious elimination was furnished more recently by Achard and Loper.¹ After the administration of potassium ferrocyanid to dogs whose kidneys were removed they detected this substance in the lachrymal secretions, where in the case of normal dogs it is not found.

Renewed interest in this subject has been shown within the last few years. McCallum¹⁵ endeavored to show that the intestine supplements the eliminative function of the kidney. After infusing large quantities of sodium chlorid into rabbits whose kidneys were excluded from the circulation, he found large quantities of sugar in the intestines and stomach. In one experiment in which the kidneys were left intact the amount of glucose found in the small intestine after the infusion of sodium chlorid was much less than that in the animals in which the kidneys were deprived of their function. On the basis of this evidence McCallum concluded that the intestine assists the kidney in the function of elimination.

This conclusion was disputed recently by Kleiner,¹² who carried out a series of experiments on nephrectomized rabbits and on a large number of controls which received solutions of dextrose intravenously. He concluded that double nephrectomy increased the gastrointestinal elimination of dextrose, but the increase was too small to

be regarded as of any value in furnishing evidence of the existence of a compensatory mechanism in the stomach and intestines. Evidence that the gastrointestinal canal is a channel of elimination in pathological conditions was furnished recently by Grigaut and Richet⁸ in a series of experiments on dogs which had been starved for 24 hours and operated upon under anæsthesia. After the subcutaneous injection of sodium chlorid, urea, and glucose, these substances were found in the intestines, and the first two also in the stomach. The very recent work of Roger and Garnier²⁵ lends support to the contention that a compensatory mechanism of elimination is present in the intestines when the kidneys are removed. In experiments carried out on rabbits having both kidneys removed, 200 cc of fluid were found in the upper part of the small intestine after the infusion of large quantities of Locke's solution. With similar treatment of rabbits with kidneys intact the amount of fluid did not exceed 40 cc.

From what has already been stated, it is clear that vicarious action or compensatory elimination by the stomach and intestines undoubtedly takes place when the kidney is abnormal or absent. Evidence is accumulating, however, to prove that this is not universally the case; in all probability such a mechanism does exist for some substances but apparently fails to develop for others. It is interesting to recall here the views of Bouchard⁷ on this subject, as he long ago denied the existence of vicarious elimination by the gastrointestinal canal or by the skin in conditions of renal insufficiency, for he believed that an increased quantity of fluid which may be secreted by these organs under such conditions does not necessarily mean augmentation of the amounts of the solid substances dissolved. He therefore maintained that these organs do not supplement the functions of the kidney. The experiments of Meltzer and Lucas¹⁷ with magnesium sulphate showed that this substance is much more toxic for nephrectomized rabbits, the toxicity under these conditions being increased 50 per cent. The absence of vicarious elimination is also indicated in experiments with subminimum doses of this salt. Animals that received small doses at intervals of several hours succumbed when the sum was equal to the maximum toxic or fatal dose. Studies on the elimination of radium by Salant and Meyer²⁶ have likewise shown that new paths for the elimination of this substance are not developed in the rabbit after the removal of both kidneys.

In a recent communication on the elimination of nitrogen and sodium chlorid by the skin in cases of chronic nephritis, Loofs¹⁴ stated that the quantities present in this condition were the same as in healthy subjects. There is, perhaps, a difference in the mechanism for the elimination of various substances, organic and inorganic, in disease. The following investigation shows, however, that such differences exist also in health, as has long been established for a number

of alkaloids, but only within recent years for inorganic substances. In this connection it is of interest to recall the work of Mendel^{19,20,21} and his collaborators, who have found by experiments on animals that the channel of elimination varies for different inorganic salts, magnesium and calcium salts being eliminated entirely by the kidney. According to Hanford,⁹ cæsium is eliminated by the kidney and bowel. Although Mendel and Thacher²² found strontium in the urine, it is chiefly eliminated by the bowel, as it was found in greater quantities in the feces after parenteral introduction of its salts. After subcutaneous injection barium is likewise eliminated by the-bowel, and only traces of it appear in the urine. Rubidium, on the other hand, seems to be excreted chiefly in the urine and to a much smaller extent by the bowel.

Although it is known that alkaloids may be eliminated by the walls of the stomach and intestine in health, very few of them have been investigated in regard to their excretion into the intestines or stomach in abnormal conditions. As pointed out in a previous investigation²⁷ by this laboratory, studies on the elimination of caffeine were made by a number of investigators, but for the reasons set forth its reinvestigation was undertaken. It was found that this substance is eliminated by the stomach and intestine as well as by the kidney, but the relative amounts found in the contents of the gastrointestinal canal, especially in the contents of the intestine, are greater with a diet of oats than with one of carrots. Since this difference in the rate of elimination may be accounted for by the increased activity of the kidney on a diet of carrots, the desirability of testing the eliminative capacity of the gastrointestinal canal of the rabbit for caffeine presented itself as affording an opportunity to test the existence of a compensatory mechanism for the elimination of caffeine after the removal of both kidneys.

EXPERIMENTS.

The experiments were carried out on well-nourished rabbits of medium size, some only 4 or 5 months old, but all apparently in good condition at the time of the experiment. All the operations were performed under ether anæsthesia. Except in one series in which the abdominal route was chosen, the kidneys were removed by lumbar incision. The renal pedicle was securely ligated, the kidney excised and removed, and the wound then closed. With few exceptions the loss of blood was slight. The animals stood the operations very well; they could walk shortly afterwards, and their general condition was very good. The duration of life varied considerably, some living less than 24 hours, but most of them surviving the operation from 2 to 3 days. In some exceptional cases the duration of life was much longer, for one rabbit lived 5 days and another 7

days after double nephrectomy. The feces were in almost all cases well formed and hard, but in a few animals they were soft. Diarrhea after double nephrectomy was seldom observed. Caffein was administered by subcutaneous injection. The plan of the experiment, as well as the method for the determination of caffein, was the same as that given in Bureau of Chemistry Bulletin 157. No important deviations were made, except that in most experiments the contents of the various portions of the gastrointestinal canal of one rabbit were examined instead of combining those of several subjects.

ELIMINATION OF CAFFEIN DURING THE FIRST 22 HOURS (SERIES I, II, AND III).

SERIES I: *Rabbits 730 and 731.*—Gray; weight, 2,370 and 2,010 grams, respectively; diet, oats, for several months.

January 12: Nephrectomized at 5 p. m.

January 13: Both subjects were alive and seemingly well, not having shown any symptoms in the interim. They were chloroformed 22 hours after double nephrectomy. The combined bile amounted to about 10 cc, and did not contain any caffein. The combined stomach contents weighed about 150 grams and contained 1.16 per cent of the caffein administered. The feces and intestinal contents of rabbit No. 730, which was a pregnant female almost ready to deliver, contained 8.50 per cent of the caffein administered. Of the quantity of caffein injected, 1.23 per cent was recovered from the uterus and its contents, making a total of 10.89 per cent caffein recovered from all sources following the subcutaneous injection of caffein (100 mg per kilo) 22 hours previously.

The elimination of caffein in this experiment indicates a marked compensation of the excretory function of the gut, since the amount was several times as large as in normal rabbits fed on carrots and nearly twice the amount of that in rabbits which received oats. This may be seen on comparing the above data with those in the following experiment taken from Bulletin No. 157, page 17.

Caffein recovered from normal rabbits killed 24 hours after injecting 150 mg per kilo.

Data.	Rabbits on carrot diet, 720 mg caffein injected.		Rabbits on oat diet, 660 mg caffein injected.	
	Mg.	Per cent.	Mg.	Per cent.
In urine.....	54	7.5	31.3	4.75
Intestinal contents.....	12	1.7	19.3	3.56
Stomach contents.....	10	1.4	11.2	1.70
Feces.....	2	.3	1.2	.20
Total.....	78	10.9	63.0	10.21

The difference in the elimination by the gut between the normal and nephrectomized rabbits is therefore marked. While in the former the maximum amount of caffein found was only 3.5 per cent, the amount present in the gut of the nephrectomized rabbit was 8.5 per cent, or nearly two and one-half times as much as in the normal rabbits. The quantity of caffein in the stomach was practically the same in nephrectomized as in normal rabbits. This seems to

be exceptional, as inspection of the protocols of the next two series shows that much greater amounts of caffeine were eliminated also by this channel.

SERIES II AND III.—*Nephrectomized rabbits which were chloroformed 22 hours after subcutaneous injection of caffeine. Diet, oats.*

Series.	Rabbit No.	Weight.	Food consumed.	Water.	Symptoms.	Caffeine recovered in composite sample.		
						Intestines.	Stomach.	Total.
		<i>Grams.</i>		<i>cc.</i>		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Series II: 100 mg caffeine per kilo.....	732	1,675	None....	100	None....
	733	1,920	...do....	15	...do....	5.35	3.53	8.88
	734	1,930	...do....	60	...do....
Series III: 150 mg caffeine per kilo.....	736	2,005	...do....	35	...do....
	735	2,020	...do....	85	...do....	4.40	3.30	7.70
	737	1,685	...do....	90	...do....

The increased rate of elimination of caffeine by the intestines was also shown in Series II and III. In both series of experiments elimination was more marked by the stomach than was found to be the case in Series I, or in normal rabbits, the amount of caffeine recovered in the gastric contents being 3.5 and 3.3 per cent of the amounts administered, which is fully twice the quantity recovered in Series I, or in rabbits with kidneys intact. The elimination of caffeine by the intestinal epithelium was considerably less than in Series I, but was more marked than in the normal rabbits just referred to. In the next series of experiments the rate of elimination was found to be appreciably greater in the stomach and was also much more marked in the intestines.

ELIMINATION OF CAFFEIN IN FROM 7 TO 28 HOURS (SERIES IV).

Rabbit 738.—Gray; weight, 1,725 grams.

February 1: 10 a. m., double nephrectomy; 3.20 p. m., 17 cc of 2 per cent caffeine injected subcutaneously; 4.30 p. m., hypersensitive; 10 p. m., found dead.

Rabbit 739.—Gray; weight, 1,643 grams.

February 2: 10.30 a. m., double nephrectomy; 3.20 p. m., 16.5 cc of 2 per cent caffeine injected subcutaneously.

February 3: 2.30 p. m., chloroformed, contents of stomach and intestine removed and examined for caffeine.

Rabbit 741.—Gray; weight, 1,685 grams.

February 2: 3.30 p. m., double nephrectomy; 3.45 p. m., 17 cc of 2 per cent caffeine injected subcutaneously.

February 3: 4 p. m., chloroformed and contents of stomach and intestine removed.

Half an hour after receiving caffeine all the rabbits of this series showed symptoms of caffeine intoxication, such as increased reflexes, photophobia, and restlessness. Of these three subjects rabbit No. 738 was found dead 12 hours after the nephrectomy and 7 hours after the injection of caffeine. Rabbit No. 738 was run independently for a determination of the caffeine recovered.

SERIES IV.—*Three nephrectomized rabbits which received 200 mg caffein per kilo.*

Rabbit No.	Duration of life.	Weight.	Food consumed.	Water.	Caffein recovered.		
					Intestines.	Stomach.	Total.
738	Hours. 12	Grams. 1,725	None....	cc. 50	Per cent. 10.43	Per cent. 5.43	Per cent. 15.91
739	28	1,643	...do.....	90	} 7.45	4.33	11.78
741	24.5	1,685	...do.....	90			

The relative and absolute amounts of caffein eliminated in this experiment were much greater than in those already considered. Especially noteworthy is the rate of elimination in rabbit No. 738, which excreted in seven hours nearly one-sixth of the caffein injected. This, it will be observed, is greater than the relative amounts excreted by the kidney and gastrointestinal canal of the rabbits previously examined in this laboratory (see Bulletin 157) or of those of the present investigation. The difference in the doses employed might have been responsible for the greater output of caffein, but, as will be seen later, this was not the case. Abnormality or accident might also be thought of as factors in this connection. Before deciding, however, that such was the case the necessity of obtaining additional data on the rate of elimination of caffein at various intervals after its administration in nephrectomized rabbits presented itself, and the experiments of the following series were undertaken with this object in view.

ELIMINATION OF CAFFEIN IN FROM 1½ TO 3 HOURS (SERIES V).

The kidneys were in all cases removed by the abdominal route under ether anæsthesia. The operations were practically bloodless. The recovery from the operation seemed to be very slow, the rabbits remaining in a greatly weakened condition up to the end. After the administration of caffein the rabbits became hypersensitive.

SERIES V.—*Caffein elimination in nephrectomized rabbits after subcutaneous injection of 150 mg per kilo.*

Rabbit No.	Weight.	Symptoms.	Mode of death.	Duration of life.	Caffein recovered.			
					Intestines.	Stomach.	Total.	
742	Grams. 1,835	Paralysis within 2 hours.	Chloroformed...	Hours. 3.00	Per cent. 9.00	Per cent. 3.69	Per cent. 12.69	
743	1,645	...do.....	Natural.....	2.75	11.80	1.90	13.70	
744	1,760	Paralysis within 1 hour.	...do.....	1.50	7.70	1.60	9.30	
Average.....						9.50	2.39	11.89

It will be noticed that the rate of elimination was especially marked in the intestines, the amounts of caffein varying considerably, but it was not quite in proportion to the time which elapsed between the administration of the caffein and the death of the animal. This is far in excess of the relative amounts recovered from the urine of rabbits at a corresponding period of time after the injection of caffein. As stated previously (Bulletin 157), from 3 to 4 per cent of the caffein introduced was found in the urine three and three and a half hours after the administration of the drug.

As the striking results obtained in Series V and in rabbit No. 738 might have been due to abnormal conditions, the rabbits in all of these cases having lived only a few hours after nephrectomy, it seemed desirable to repeat the experiments on other rabbits, employing the usual route for the removal of the kidney. Caffein was injected subcutaneously shortly after the kidneys were removed.

ELIMINATION OF CAFFEIN IN 4 HOURS (SERIES VI).

Double nephrectomy was performed by lumbar incision under ether anæsthesia. The average time of the operations, which were practically bloodless, was 15 minutes. The characteristic caffein symptoms, such as increased sensitiveness, avoidance of light, and extreme restlessness, were marked in each of the subjects, although to a somewhat less degree in rabbit No. 757. The general condition of the rabbits remained favorable up to the end, there being no manifestation of weakness.

SERIES VI.—*Caffein elimination in nephrectomized rabbits after subcutaneous injection of 200 mg per kilo.*

Rabbit No.	Weight.	Symptoms	Mode of death.	Duration of life.	Caffein recovered.		
					Intestines.	Stomach.	Total.
	<i>Grams.</i>			<i>Hours.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
757	1, 775	Extremely sensitive	Chloroformed . . .	4	6.65	3.49	10.12
758	1, 920	do	do	4	6.20	5.40	11.60
759	1, 825	do	do	4	8.32	4.38	12.70
Average					7.05	4.42	11.47

The results obtained in this series were practically the same as those in Series IV and V, the absolute and relative amounts of caffein eliminated being quite large. The elimination by the intestinal epithelium in the experiments of this series is interesting, as a decided difference in the rate of elimination was observed in rabbit No. 759, which amounted to about 1 to 2.5 per cent more than in the other two rabbits.

The experiments of Series VII were made to test the suggestion that elimination is probably more rapid during the first few hours

after injection, and that caffein is reabsorbed later from some part of the gastrointestinal canal.

ELIMINATION OF CAFFEIN IN 6 HOURS (SERIES VII).

Three rabbits were nephrectomized by lumbar incision, the operation requiring on an average 20 minutes. The animals were then injected with caffein subcutaneously, receiving 200 mg per kilo, and were chloroformed at the end of six hours. Rabbit No. 778 became strongly hypersensitive shortly after injection and remained in that condition to the end. Rabbit No. 779 behaved similarly, though the degree of hypersensitiveness was less. Rabbit No. 780 showed the same symptoms and in addition had a short convulsion.

SERIES VII.—*Caffein recovered in nephrectomized rabbits which had received 200 mg per kilo.*

Rabbit No.	Weight.	Caffein recovered.		
		Intestines and feces.	Stomach.	Total.
	<i>Grams.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
778	1,320	6.44	3.41	9.85
779	1,210	5.23	5.42	10.65
780	1,328	6.57	3.02	9.59
Average....		6.08	3.95	10.03

Although the difference in the rate of elimination as compared with that in Series IV and V is not very great, it is of sufficient importance to merit attention. Representing the average of three experiments, it is not to be regarded as accidental. Moreover, it may be noticed that the amounts recovered were especially diminished in the intestines. It is quite possible, therefore, that the progressive diminution in the amounts of caffein present in the stomach and intestines was due to reabsorption. To test the validity of this hypothesis experiments were performed in which the elimination of caffein was studied after longer intervals of time. This was accomplished by collecting and examining the feces in each case until the rabbit died. The stomach contents as well as the contents of the intestines were then examined separately for caffein. The results are shown in detail in the next series of experiments.

ELIMINATION OF CAFFEIN IN 21 HOURS, 5 AND 7 DAYS (SERIES VIII).

To explain the absence of caffein in the stomach and intestines in rabbits Nos. 775 and 777 and the recovery of small quantities only in the feces collected since its administration until the death of the animals, it must be assumed that it is either reabsorbed into the circulation or decomposed in the lumen of the gastrointestinal canal. As the latter hypothesis is highly improbable, since caffein is known

to resist putrefaction, its absorption from some part of the gastrointestinal tract is the only logical conclusion.

SERIES VIII.—*Recovery of caffeine in rabbits nephrectomized by lumbar incision and 5 or 6 hours later injected subcutaneously with 150 mg per kilo.*

Rabbit No.	Weight.	Symptoms.	Mode of death.	Duration of life.	Caffein recovered.		
					Intestines.	Feces.	Stomach.
775	<i>Grams.</i> 1,505	Diarrhea and pneumonia developed toward end of experiment.	Natural.....	5 days...	<i>Per cent.</i> None.	<i>Per cent.</i> 1.4	None.
776	1,380	Frequent and severe convulsions at end.do.....	21 hours.	7.25	None.
777	1,165do.....	About 7 days.	None.	1.9	None.

Although the existence of compensatory elimination by the stomach and intestines in conditions of renal insufficiency is frequently assumed by clinicians, this is still a matter of dispute among investigators. The results of the experiments recorded in the present investigation are therefore of interest as bearing upon an important problem in physiology, which may also be of value in human and veterinary therapeutics. As shown in Bulletin 157, from 3.5 to 5 per cent of the caffeine administered is eliminated into the lumen of the gastrointestinal canal of normal rabbits at the end of from 22 to 24 hours and the same was found true in guinea pigs. After the removal of the kidney the amounts of caffeine recovered from the contents of the stomach and intestines of rabbits varied between 7.7 and 11.78 per cent of the quantity administered, which is therefore about twice as much as is eliminated in normal animals by this channel, the increase being especially marked in the intestines, as may be seen by referring to the protocols. Examination of the contents of the gastrointestinal canal from one and one-half to four hours after the administration of caffeine revealed the presence of amounts proportionally much larger, thus showing an increased rate of elimination by this channel at this period. This was also studied in normal rabbits in the experiments of Series IX.

ELIMINATION OF CAFFEIN IN RABBITS IN WHICH URINE WAS SCANTY OR ABSENT
(SERIES IX).

Rabbit 839.—Weight, 1,370 grams; laparotomized.

July 18, 1912: The common bile duct was ligated and 48 hours later the subject was given 150 mg per kilo caffeine subcutaneously. Four hours later the subject was chloroformed, and the following determinations made: Caffein recovered in urine, 5.15 per cent; in intestines, 8.75 per cent; in stomach, 2.50 per cent; total, 16.40 per cent.

Rabbit 834.—In early pregnancy; laparotomized; weight, 1,650 grams.

July 18, 1912: 11.30, ligature around the upper part of the pylorus and the small intestine, below the common bile duct, the bile duct also ligated, ethyl chlorid used,

considerable bleeding; 1.30 p. m., received subcutaneously 150 mg caffein per kilo; 2.30 p. m., strongly hypersensitive, restless, and avoiding the light; 5 p. m., died in tetanic convulsions; caffein found in intestinal tract, 4.32 per cent; in stomach, 5 per cent; total caffein recovered, 10.77 per cent; duration of life three and one-half hours; 15 cc urine; no caffein.

Rabbit 835.—In late pregnancy; weight, 2,220 grams.

July 18, 1912: 11.45, laparotomized, as rabbit No. 834, except the bile duct was not ligated; 1.30 p. m., received subcutaneously 150 mg caffein per kilo; 2.15 p. m., hypersensitive and restless; 3.45 p. m., clonic convulsion; died; duration of life two and one-fourth hours; caffein in intestines, 4.32 per cent; in stomach, 3.1 per cent; uterus and contents, 8.1 per cent; total caffein, 15.72 per cent; no urine secreted, for none found in bladder.

Normal rabbits.—July 18, 1912: Caffein elimination, four hours after subcutaneous injection of 150 mg caffein per kilo.

Caffein recovered from normal rabbits.

Rabbit No.	Weight.	Symptoms.	Mode of death.	Duration of life.	Caffein recovered.			
					Intestines.	Stomach.	Urine.	Total.
840	Grams. 1,715	Markedly increased reflexes.	Chloroformed.	Hours. 4	Per cent. 8.92	Per cent. 3.20	Per cent. 1.00	Per cent. 13.12
841	1,445do.....do.....	4	10.70	3.40	1.00	15.10
842	1,850do.....do.....	4	12.70	2.20	None.	14.90
Average.....					14.37			

In Series IX elimination into the gastrointestinal canal varied between 12.1 and 14.9 per cent, which is higher than in the case of nephrectomized rabbits. It will be noticed, however, that with one exception the amount recovered from the urine was insignificant, while in one case no caffein at all could be detected. It is significant that in rabbit No. 839, in the gastrointestinal canal of which the smallest amount was found, the quantity of caffein in the urine was higher than in the other rabbits, thus pointing to a decided tendency to vicarious elimination. The experiments with rabbits Nos. 834 and 835 apparently contradict this statement, but the vastly increased toxicity of caffein in these two cases is probably responsible for the decreased elimination of caffein into the gastrointestinal canal. Additional experiments were therefore undertaken to ascertain the relation between elimination in the urine and into the gastrointestinal canal.

ELIMINATION OF CAFFEIN IN RABBITS IN WHICH URINE WAS ABUNDANT (SERIES X).

• Caffein was given to a number of rabbits. Only those were used in which diuresis was marked.

Rabbit 861.—Pregnant; diet, oats and cabbage; water consumed, 100 cc.

September 7, 1912: 11.30 a. m., received 17 cc of 2 per cent caffein injected subcutaneously, or 150 mg per kilo; 3.50 p. m., bladder emptied; total urine, 150 cc. Per cent caffein recovered in urine, 6.41; stomach, 2.93; intestines, 5.42; total, 14.76.

Rabbit 864.—Diet, oats and cabbage; water consumed, 100 cc.

September 7, 1912: 12.00 m., received 14.5 cc of 2 per cent caffein injected subcutaneously, or 150 mg per kilo; 4.05 p. m., bladder emptied; rabbit chloroformed; total urine, 110 cc. Per cent caffein recovered in urine, 4.34; stomach, 4.83; intestines, 6.98; total, 16.15.

Rabbit 858.—September 7, 1912: 11.45 a. m., received 16 cc of 2 per cent caffein, or 150 mg per kilo; bladder empty; 4 p. m., bladder emptied and the rabbit chloroformed; total urine, 60 cc. Per cent caffein recovered in urine, 3.69; uterus, 3.00; stomach, 2.72; intestines, 6.9; total, 16.25.

Rabbit 859.—Diet, oats and cabbage; water consumed, 100 cc for three days.

September 7, 1912: 11.20, bladder emptied, received 12.25 cc of 2 per cent caffein, injected subcutaneously, drank 65 cc of water; 3.40, bladder emptied, animal chloroformed; urine, 100 cc. Per cent caffein recovered in urine, 3.72; stomach, 4.08; intestines, 8.53; total, 16.33.

In the above experiments the amount of caffein recovered from the contents of the stomach and intestines was from 8.35 to 12.61 per cent, while in Series IX the amount varied between 12.1 and 14.9 per cent, thus pointing to a decided increase in the eliminative function of the gastrointestinal canal when the kidney is unable to eliminate this substance. This is also shown when the kidneys were entirely removed, in Series VI, as well as in the experiments with rabbits Nos. 742 and 743 (p. 13).

RESULTS OF EXPERIMENTS.

Elimination of caffein in normal rabbits.

Rabbit No.	Caffein recovered.				Time.
	Urine.	Intestine.	Stomach.	Stomach and intestines.	
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Hours.</i>
839	5.15	8.75	2.5	11.25	4
840	1.00	8.92	3.2	12.12	4
841	1.00	10.7	3.4	14.1	4
842	-----	12.7	2.2	14.9	4
¹ 834	-----	4.32	5.00	9.32	3.5
² 835	-----	4.32	3.1	7.42	2.25
861	6.41	5.42	2.93	8.35	4
864	4.32	6.98	4.83	11.81	4
858	3.63	6.9	2.72	9.62	4
859	3.72	8.53	4.08	12.61	4

¹ 15 cc urine; no caffein.

² No urine.

Elimination of caffein in nephrectomized rabbits.

Series.	Intestine.	Stomach.	Total.	Time.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Hours.</i>
I	8.5	1.16	9.66	22
II	5.35	3.53	8.8	22
III	4.4	3.30	7.7	22
IV	7.45	4.33	11.78	24-28
IV-738	10.43	5.48	15.91	7
V-742	9.7	3.69	12.69	3
V-743	11.8	1.9	13.7	2.75
V-744	7.7	1.6	9.3	1.5
VI	7.05	4.42	11.47	4
VII	6.08	3.95	10.03	6

It may be argued that the difference in the amounts of caffeine found in the gastrointestinal canal under those conditions may be due to a decreased rate of absorption in nephrectomized rabbits or when renal function is inhibited, but it has been shown by several investigators that this is not likely to be the case. Indeed, experimental evidence points the other way. It was shown by Meltzer and Salant¹² and later by Loeb and Fleischer¹³ that the rate of absorption from the peritoneal cavity is usually faster after the removal of the kidneys.

The elimination of caffeine in nephrectomized rabbits takes place largely in the intestines, but appreciable amounts have also been found in the gastric contents, the amounts recovered from the stomach varying between 3.3 and 5.28 per cent, while in the intestines 4.47 to 10.48 per cent of the amounts injected were separated from the intestinal contents 22 hours after the administration of caffeine. The total amounts of caffeine eliminated 22 hours after its administration were 8.88 per cent, 7.70 per cent, and 11.78 per cent. By comparing these results with the elimination of caffeine by the kidney, stomach, and intestines of normal rabbits we find that the difference is very small. Thus, the total amounts on a diet of oats and carrots 24 hours after injection were 10.21 and 10.9 per cent of the amount of caffeine given, or an average of 10.55 per cent, while the average for the three series of nephrectomized rabbits was 9.45 per cent. There is therefore little difference in the amounts eliminated when the kidneys are intact or when they are removed, owing to the stimulation to greater activity of the epithelium of the gastrointestinal canal, especially that of the intestines. In this connection the fact may also be mentioned that the presence of caffeine in the intestines is due entirely to excretion by the intestinal epithelium. This is proved by the following experiments in which the small intestine was ligated at its upper end:

Rabbit 810.—Weight, 1,820 grams.

May 18, 1912: 11.20 a. m., laparotomy, small intestine tied near the pyloric opening; two ligatures, one above and one below the common bile duct; 12 m., received 360 mg caffeine, or 200 mg per kilo, showed the usual symptoms of caffeine intoxication; 3.30 p. m., chloroformed, about 40 cc of urine found in the bladder, but no caffeine present, the stomach contents contained 1.15 per cent and the intestinal contents 7.7 per cent, or a total of 8.85 per cent caffeine.

Rabbit 811.—Weight, 1,600 grams.

May 18, 1912: 11.40 a. m., same operation as in No. 810, except that there was one ligature below the bile duct and the bile duct itself was ligated; 12 m., received 200 mg caffeine per kilo, or a total of 320 mg, showed strongly the usual symptoms of caffeine intoxication; 1.45 p. m., found dead, about 30 cc urine were found in the bladder, but there was no caffeine present, the stomach contents contained 2.22 per cent and the intestinal contents 4.81 per cent, or a total of 7.03 per cent, caffeine.

It will be observed that the intestine contained about the same amount of caffeine as was found in the experiments in which communication between stomach and intestines was open. The absence of caffeine in the urine is interesting, as it suggests impermeability of

the kidney as a result of anæsthesia, for it was found (Bulletin 157) that the elimination of caffein in rabbits normally begins 15 to 40 minutes after its subcutaneous injection. It is interesting to note that the bile contained very small quantities, only milligram quantities being found in the normal rabbit as well as in the rabbit without kidneys.

The kidneys and intestines, and to a much smaller extent the stomach, are therefore the chief organs for the elimination of caffein. It is important to appreciate, however, that the rôle which the gastrointestinal canal plays in this connection is quite different from that of the kidney. Although, like the latter, it relieves the circulation and probably other organs in the body of a considerable amount of caffein, this action is really not excretory in nature, for the caffein is stored only temporarily in the lumen of the intestines or stomach only to be returned gradually to the circulation, as very small amounts of caffein were removed in the feces. The function of the digestive tract is in this case more nearly akin to that which the liver possesses for numerous substances. Hence even the removal of the kidney fails to transform the stomach and intestines into true organs for the excretion of caffein; the results obtained in the present investigation indicate that after double nephrectomy there is merely an increase in the activity of the mechanism which existed in the normal rabbit. It is evident, therefore, that a formation *de novo* of a mechanism for the elimination of foreign substances does not take place under these conditions. Observations on the bile show that even if a mechanism exists in the normal subject but is poorly developed, it apparently remains unchanged after nephrectomy. The conflicting views held by investigators as regards the development of vicarious elimination by the wall of the gastrointestinal canal are perhaps due to lack of appreciation that vicarious action may consist in the stimulation of a previously existing function to greater activity.

TOXICITY OF CAFFEIN IN NEPHRECTOMIZED RABBITS.

INTRODUCTION.

In the experiments of the preceding section it was noticed that moderately large doses of caffein failed to produce a fatal result. Indeed, in some rabbits there was no manifestation of symptoms after the administration of 150 mg of caffein per kilo, while in Series IV rabbits which received 200 mg per kilo lived 24 to 28 hours before they were killed. It is interesting to recall that in a previous study (Bureau of Chemistry Bul. 148) 200 mg per kilo proved toxic, although not necessarily fatal in most cases for normal rabbits. Retarded absorption might be given as a cause for the greater resistance to caffein, but the appearance of symptoms of caffein intoxi-

cation in these experiments soon after its injection precludes such a possibility. The toxicity of caffein may be expected to be greater in the rabbit after the removal of both kidneys, since the kidney is the chief organ for the elimination of caffein. Increased excretion into the gastrointestinal canal is probably of not much importance, however, in modifying the toxicity of caffein, for it has been shown that reabsorption takes place.

Moreover, there is reason to believe, from previous studies by various investigators, that the action of a substance on a normal animal furnishes no guide to its toxicity when the renal function is impaired or altogether abolished. It may be recalled in this connection that similar results were obtained in experiments with strychnin on rabbits by Meltzer and Salant¹⁸ and by Meltzer and Langmann,¹⁶ who corroborated these findings on guinea pigs. The reaction of animals after removal of the kidney is not the same, however, toward all substances. Claude Bernard⁴ showed long ago that curare becomes poisonous when given by mouth to dogs in which the ureters are tied or the kidneys removed. Since it has been established that this substance may ordinarily be taken by mouth without injurious effects, the findings of Claude Bernard, corroborated by Herrman, are of interest as showing undoubted accumulation in the body of a poison which is normally eliminated by the kidney. This is shown even more strikingly by the experiments of Meltzer and Lucas,¹⁷ who brought forward evidence of diminished resistance to magnesium sulphate in rabbits deprived of both kidneys. These investigators have also found that cumulation of this substance takes place after removal of the kidneys, which is not the case in the normal subject. On the contrary, the experimental data obtained in the first part of this investigation strongly suggest decreased toxicity of caffein after the removal of the kidney. Additional evidence, however, was considered necessary to determine accurately the resistance of the rabbit to caffein in this condition. For this purpose several methods of inquiry were employed, the following being particularly valuable and suggestive:

The effect of repeated nontoxic doses administered at long intervals was tried to test whether or not the action of caffein is cumulative after the removal of both kidneys. Since it was found that 200 mg per kilo are surely toxic, the injection of several subminimum doses, the total of which is equal to the toxic dose, should produce symptoms if cumulation takes place. If, on the other hand, there is some mechanism by which caffein is destroyed more easily or neutralized in nephrectomized rabbits, the administration of several doses of this size, if given at proper intervals, may be expected to be without any effect. Moderate amounts of caffein were given at fairly long intervals in the series following to test whether cumulation takes place.

EXPERIMENTS.

CUMULATION WITH MODERATELY LARGE DOSES (SERIES I).

Rabbit 110.—Gray; weight, 1,400 grams.

March 17: 11.30 a. m., both kidneys removed by lumbar route; 2 p. m., 7 cc of 2 per cent aqueous caffein solution injected subcutaneously in tissues of back.

March 18: 9.30 a. m., 7 cc of 2 per cent aqueous caffein solution injected subcutaneously in tissues of back; 1.45 p. m., no symptoms; 1.50 p. m., 7 cc of 2 per cent aqueous caffein solution injected subcutaneously in tissues of back; 4.30 p. m., rabbit became paralyzed; this was followed by convulsion, marked opisthotonos, and death.

At first sight the result obtained in rabbit No. 110 seems to point to the absence of cumulation, as two doses of 100 mg caffein per kilo administered at an interval of about 20 hours may be given without producing symptoms of toxicity. The fate of the first dose, aside from the portion that found its way into the gastrointestinal canal, which, as was shown above, is about 8 or 10 per cent of the amount injected, admits of two possibilities. Either it was destroyed or it was neutralized partly or entirely in the body by substances formed after removal of the kidney. The effect of the third injection would indicate, however, that some of the first dose was still in the body in an active condition, since, as was observed in this investigation, 0.2 gram per kilo is not fatal within three hours. That cumulation, though to a small degree, does apparently take place is further indicated in rabbit No. 229.

The effect of the second injection of caffein in this rabbit makes this evident, since a single dose of this size is well borne by nephrectomized rabbits, as is illustrated in rabbit No. 232, which died about 18 hours after receiving 150 mg caffein per kilo. Experiments on rabbit No. 229 show the same after a single dose. It may be recalled that such a dose given on two successive days likewise proved fatal to normal rabbits.

Rabbit 229.—Black and white male; weight, 2,140 grams.

November 1: 3 p. m., both kidneys removed by dorsal route under ether anæsthesia and wound closed; practically no loss of blood, perhaps 1 cc; 3.05 p. m., rabbit recovered from operation and was running around in the room; 3.30 p. m., 15 cc of 2 per cent caffein (140 mg per kilo) injected subcutaneously; under observation until 5 p. m.

November 2: 9 a. m., condition of rabbit excellent; feces in cage hard; no evidence of diarrhea; 9.30 a. m., 15 cc of 2 per cent caffein injected subcutaneously; 12 m., rabbit restless; this continued until 1.30 p. m., when paralysis was observed; 1.45 p. m., convulsion and death.

Rabbit 232.—Gray male; weight, 2,010 grams.

November 2: 11 a. m., double nephrectomy; 1.45 p. m., 15 cc of 2 per cent caffein injected subcutaneously in the back; operation uneventful; loss of blood slight.

November 3: 9 a. m., found dead; body still quite warm.

CUMULATION WITH SMALLER DOSES (SERIES II).

The questions of cumulation and neutralization were tested again in experiments in which smaller doses were repeated at proper intervals, but only one dose of medium size was administered. By

this method it was thought the total quantity introduced could be increased without producing fatal consequences. Series II and III show the result of this treatment.

Rabbit 797.—Gray male; weight, 1,740 grams.

May 3: 2 p. m., double nephrectomy; 3.30 p. m., 9 cc of 2 per cent caffein injected subcutaneously; 5.30 p. m., somewhat hypersensitive.

May 4: 8.25 a. m., reflexes increased, condition otherwise good; 11.10 a. m., 11 cc of 2 per cent caffein injected subcutaneously; 12.30 p. m., increased reflexes observed.

May 5: 10 a. m., condition good, no symptoms; 11 a. m., 17 cc of 1 per cent caffein

May 6: 9 a. m., condition good, no symptoms; 11 a. m., hypersensitive; 2.15 p. m., died.

Rabbit 798.—Gray female; weight, 1,205 grams.

May 3: 2.25 p. m., double nephrectomy; 3.35 p. m., 6 cc of 2 per cent caffein injected subcutaneously; 5.30 p. m., slightly sensitive.

May 4: 8.30 a. m., no symptoms; 11.13 a. m., 8 cc of 2 per cent caffein injected subcutaneously; 12.30 p. m., reflexes increased.

May 5: 10 a. m., condition good, no symptoms; 11 a. m., 12 cc of 1 per cent caffein injected subcutaneously.

May 6: 9 a. m., found dead; cold and stiff.

Total caffein injected, 0.33 gram per kilo.

Rabbit 799.—Gray female; weight, 1,165 grams.

May 3: 3 p. m., double nephrectomy, attended by considerable loss of blood; 4.35 p. m., 6 cc of 2 per cent caffein injected subcutaneously; 5.30 p. m., no symptoms.

May 4: 9 a. m., no symptoms; weight, 1,080 grams; 10.30 a. m., 8 cc of 2 per cent caffein injected subcutaneously; 12.30 p. m., reflexes increased markedly; 1 p. m., found dead and stiff, body warm.

Although some of the rabbits of this series became hypersensitive, thus showing symptoms of caffein poisoning after the first dose, there was no evidence of cumulation under the conditions employed in the series, since all of them survived the total amount, which is a fatal dose for the normal rabbit. Rabbit No. 797 was in good condition 22 hours after it received the third dose of caffein, thus making a total of 0.57 gram or about 327 mg per kilo, figuring on the basis of the initial weight, which would make at least 350 mg per kilo if the loss of weight from day to day is taken into consideration. The same holds true for rabbit No. 798. The case of rabbit No. 799 is probably due to hemorrhage and may be regarded as exceptional, which is made highly probable by the behavior of rabbits Nos. 803, 804, and 805 in the following series of experiments:

CUMULATION WITH SMALL DOSES (SERIES III).

Rabbit 804.—White female; weight, 1,755 grams.

May 9: 3.25 p. m., double nephrectomy; 4.32 p. m., 9 cc of 2 per cent caffein injected subcutaneously; 5.30 p. m., no symptoms.

May 10: 12 m., 13 cc of 2 per cent caffein injected subcutaneously; 4.30 p. m., no symptoms.

May 11: 9 a. m., reflexes increased; weight, 1,630 grams; 2 p. m., reflexes increased; no other symptoms; 8.5 cc of 2 per cent caffein injected subcutaneously; 4.30 p. m., still alive.

May 12: 11.30 a. m., found dead.

Rabbit 803.—White male; weight, 1,825 grams.

May 9: 3 p. m., double nephrectomy; 4.30 p. m., 10 cc of 2 per cent caffein injected subcutaneously.

May 10: 9 a. m., condition good; no symptoms; 10.30 a. m., 2 cc of blood drawn from ear; 12 m., 15 cc of 2 per cent caffein injected subcutaneously; 5 p. m., no symptoms, but reflexes slightly increased.

May 11: 9 a. m., condition good, but slight increase of reflexes present; 2.10 p. m., weight, 1,755 grams; 9 cc of 2 per cent caffein injected subcutaneously.

May 12: 4.30 p. m., rabbit alive, reflexes increased, but no other symptoms, standing in cage, general condition good.

May 13: 9 a. m., found dead.

Rabbit 805.—White female; weight, 2,241 grams.

May 9: 3.50 p. m., double nephrectomy; 4.33 p. m., 11 cc of 2 per cent caffein injected subcutaneously; 5.30 p. m., no symptoms.

May 10: Condition good, no symptoms; 10.30 a. m., 2 cc of blood drawn from ear vein; 12 m., 15 cc (?) of 2 per cent caffein injected subcutaneously; 5.30 p. m., no symptoms.

May 11: 9 a. m., found dead; warm; abdomen swollen; rabbit apparently had diarrhea.

The experiment on rabbit No. 803 fully corroborates the results obtained in the previous series, the rabbit having survived a total amount of 0.68 gram of caffein, or approximately 0.4 gram per kilo. Although the history of rabbit No. 805 might seem to suggest cumulative effect, closer analysis shows that this is improbable. After the first dose no effect was observed nor were any symptoms present at any time for about 5½ hours after the administration of the second dose of caffein. Death in this case was therefore not due to caffein, but was in all probability the result of the operation. Rabbit No. 804 survived a total dose of 250 mg per kilo and lived at least 2½ hours after an additional injection of 100 mg caffein per kilo was made. Since a dose of 250 mg per kilo was found to be fatal in most cases when injected subcutaneously into normal rabbits, there can be no doubt of the absence of cumulation of the drug in this case also. This may be accounted for by the increased rate of elimination into the stomach and intestines which takes place when the kidneys are removed by increased oxidation, or it may be due to the formation of substances antagonistic to caffein. That the increased excretion of caffein through the walls of the stomach and intestines is not the cause of the absence of cumulation is evident, for, as pointed out before, reabsorption takes place.

It is hardly conceivable that oxidation in the tissues should be affected. According to Von Noorden²⁸ purin metabolism is not influenced in chronic renal diseases, while protein catabolism seems to be decreased. The formation of substances antagonistic to caffein is therefore highly probable. No evidence of their presence, however, could be obtained by the methods thus far employed in the present investigation. The following experiments were carried out to test this suggestion:

• For special purposes.

TEST FOR THE PRESENCE OF SUBSTANCES ANTAGONISTIC TO CAFFEIN (SERIES IV AND V).

Rabbits Nos. 800 and 801 received 0.3 gram per kilo a short time after the removal of both kidneys; the same dose was repeated on rabbits Nos. 815 and 816 about 24 hours after nephrectomy. The results are as follows:

SERIES IV, GROUP A.—*Caffein administered within a few hours after operation.*

Rabbit 801.—Gray female; weight, 1,385 grams.

May 6: 11.30 a. m., double nephrectomy; 3.52 p. m., condition good; 21 cc of 2 per cent caffein injected subcutaneously; 3.58 p. m., mild convulsion reported; 5 p. m., convulsion and death.

Rabbit 800.—Gray male; weight, 1,620 grams.

May 6: 11.50 a. m., double nephrectomy; 3.50 p. m., 25 cc of 2 per cent caffein injected subcutaneously (in several places); 4 p. m., rabbit looked sick, twitching of muscles of face and neck, anterior extremities paralyzed, respiration deeper and less frequent than usual and somewhat difficult; 4.30 p. m., died; no convulsions had been observed, although under constant observation.

SERIES IV, GROUP B.—*Caffein administered on the day after operation.*

Rabbit 815.—Belgian hare, male; weight, 1,755 grams.

May 21: 3.15 p. m., double nephrectomy by lumbar incision, loss of blood slight, condition after operation good.

May 22: 10 a. m., paralysis of anterior extremities; 10.20 a. m., temperature 101.2° F., weight 1,635 grams; 10.55 a. m., 25 cc of 2 per cent caffein injected subcutaneously; 11.30 a. m., slight increase of reflexes, no other symptoms; 12.20 p. m., reflexes markedly increased, breathing also abnormally increased; 1.30 p. m., rabbit lying on its side, struggled occasionally, in a comatose condition, dyspnoea quite marked; 1.35 p. m., died.

Rabbit 816.—Belgian hare, male; weight, 1,765 grams.

May 21: 3.35 p. m., double nephrectomy, loss of blood slight, condition good after operation.

May 22: 10.15 a. m., temperature 101° F., weight 1,745 grams; 10.50 a. m., 25 cc of 2 per cent caffein injected subcutaneously; 11.30 a. m., reflexes markedly increased; 12.20 p. m., symptoms of caffein poisoning pronounced, rabbit very hypersensitive, responded with a tremor when touched, respiration increased in rate and depth; 1.30 p. m., rabbit hypersensitive, attitude normal; 2.15 p. m., paralysis of extremities, dyspnoea, increased sensitiveness; 5 p. m., condition unchanged.

May 3: 9 a. m., found dead; cold and stiff.

Analysis of the results obtained in these two groups of experiments tends to show a somewhat decreased toxicity of caffein when given some time after the removal of the kidneys. Although rabbits Nos. 815 and 816 did not survive the effect of a large dose of caffein, it is nevertheless obvious that the resistance was greater than in rabbits Nos. 800 and 801, both of which died soon after caffein was injected. They seemed to be overwhelmed with the effect of the drug. In rabbit No. 800 paralysis set in without the development of convulsions.

Thus only a depressing effect followed the injection of a dose which in the normal rabbit is usually fatal, with the production of convul-

sions. These results do not support, therefore, the view expressed above, that substances antagonistic to caffeine may form after nephrectomy, for the rapid death of rabbits Nos. 800 and 801 shows a decidedly increased toxicity of caffeine when injected after the lapse of a few hours following double nephrectomy. This may be due to more rapid absorption at this time as has been shown for substances other than caffeine. Meltzer and Salant¹⁸ observed increased absorption of salt solution from the peritoneal cavity of nephrectomized rabbits. This was also found by Loeb and Fleischer under similar conditions. Such being the case, the diminished resistance to caffeine may be expected to continue, and indeed become more marked the longer the interval between double nephrectomy and the administration of caffeine, since, as was shown by Archard and Loper, the osmotic pressure of the blood increases steadily after removal of the kidneys. That the toxicity, however, is less under these conditions is shown in rabbits Nos. 815 and 816. The greater resistance as compared with rabbits Nos. 800 and 801 suggests, on the contrary, the presence of substances which are capable of counteracting the effect of caffeine. Additional support is afforded by the following experiments:

SERIES V,* GROUP A.—*Caffein administered shortly after operation, under ether anæsthesia.*

Rabbit 790.—Gray female; weight, 1,810 grams.

April 26: 11.55 p. m., double nephrectomy by lumbar incision; 2.30 p. m., 18.5 cc of 2 per cent warm caffeine solution injected subcutaneously in the back; 3.15 p. m., reflexes increased moderately; 5.15 p. m., reflexes much more marked.

April 27: 11 a. m., paresis of extremities and impaired coordination, dyspnoea, spasms; died at 11.20 a. m. Lived 21 hours after injection of caffeine.

Rabbit 788.—Gray female; weight, 1,520 grams.

April 26: 11.30 a. m., double nephrectomy by lumbar incision; 2.30 p. m., 15.5 cc of 2 per cent warm caffeine solution injected subcutaneously in the back; 3.15 and 5.15 p. m., reflexes as in the other two rabbits of this series.

April 27: 11 a. m., reflexes increased, moderate degree of incoordination of posterior extremities, respiration deeper and slower.

April 28: 2.45 p. m., rabbit alive, standing in cage, looked somewhat sick, depressed, breathing more slowly than normal; 4.30 p. m., still alive.

April 29: found dead; apparently died in convulsions. Lived more than 2 days.

Rabbit 789.—Gray female; weight, 1,630 grams.

April 26: 11.30 p. m., bilateral nephrectomy performed by lumbar incision, loss of blood slight (perhaps 1 cc); 2.30 p. m., 16.5 cc of 2 per cent warm solution of caffeine injected subcutaneously in the back; 3.15 and 5.15 p. m., reflexes as in rabbit No. 790.

April 27: 8.45 a. m., rabbit found dead.

Lived less than 18 hours.

SERIES V, GROUP B.—*Caffein administered on day after operation.*

Rabbit 820.—White and yellow female; weight, 1,885 grams.

May 22: 1 p. m., double nephrectomy.

* 200 mg. caffeine per kilo.

May 23: 9 a. m., condition good; 10 a. m., weight 1,845 grams, 19 cc of 2 per cent caffein injected subcutaneously; 11 a. m., reflexes increased, very sensitive; 5.15 p. m., rabbit standing in cage, reflexes increased, condition otherwise good; observed frequently all day, no paralysis or convulsions occurred.

May 24: 8.15 a. m., rabbit standing in cage, still hypersensitive, condition otherwise good; 4.30 p. m., rabbit still alive, standing in cage, reflexes increased, but no convulsions noticed; observations made several times during the day.

May 25: 8.50 a. m., rabbit alive, in good condition, still hypersensitive, but somewhat less so than day before; died about 11 a. m.

Lived 49 hours after caffein injection, or 70 hours after double nephrectomy.

Rabbit 819.—Yellow male.

May 22: 11 a. m., weight, 1,765 grams; 1.30 p. m., double nephrectomy.

May 23: 9 a. m., condition good; 10 a. m., weight 1,725 grams, 17.25 cc of 2 per cent caffein solution injected subcutaneously; 11 a. m., hypersensitive, reflexes very much increased, observed at frequent intervals all day; 5.15 p. m., condition about the same as at 11 a. m., rabbit standing in the cage, no paralysis.

May 24: 8.15 a. m., rabbit found dead; stiff and cold.

Lived more than 18 hours after caffein injection and between 28 and 46 hours after double nephrectomy.

Rabbit 821.—Black male; weight, 1,850 grams.

May 22: 2.10 p. m., double nephrectomy.

May 23: 9 a. m., condition good; 10 a. m., weight 1,815 grams, 18.5 cc of 2 per cent caffein injected subcutaneously; 11 a. m., reflexes exaggerated, rabbit hypersensitive, observed at frequent intervals, no paralysis or convulsions; 5.15 p. m., still very sensitive, condition as at 11 a. m.

May 24: 8.15 a. m., rabbit found dead; stiff and cold.

The results of these six experiments show that absorption of caffein was quite rapid, symptoms of caffein intoxication appearing within from 45 to 60 minutes after its injection, but they were much more marked in the rabbits of group B (819, 820, and 821) than in those of group A (788, 789, and 790). This suggests a diminished tolerance for the drug with the lapse of time after nephrectomy, but the duration of life in group B after the administration of caffein does not indicate such a condition. It will be noticed that one rabbit in each group lived 2 days after receiving caffein, while two other members of each group died in the night following the administration of the drug. Moreover, the result of anæsthesia in rabbits Nos. 788, 789, and 790 should not be left out of account. It is conceivable that at the time caffein was injected into these rabbits from two to three hours after the operation, enough ether remained in the tissues to antagonize, to some extent at least, the stimulating effect of caffein. This seems to contradict the results of the experiments with large doses of caffein in rabbits 800 and 801. It is significant, however, that symptoms of excitement were absent in one rabbit and were not especially marked in the other, thus pointing to the antagonistic effect of ether. Symptoms of nervous disturbance may therefore be suppressed when larger amounts of caffein are given shortly after the removal of the kidney before the effects of anæsthesia have passed off. The increased toxicity of caffein under these

conditions—that is, when administered a few hours after nephrectomy—is in all probability due to its effect on the heart. Ether and caffeine, when given in large amounts, are thus antagonistic where the nervous system is concerned and synergistic with respect to cardiac action.

Again, it should be observed that the duration of life after the injection of 0.2 gram caffeine per kilo was the same whether caffeine was administered from two to three hours after removal of the kidneys or whether it was administered the next day. The increased osmotic pressure at this time should make absorption more rapid and therefore the toxicity greater, while the antagonistic effect of ether when caffeine is given after the operation should diminish the toxicity, but the fact that such was not the case justifies the conclusion that some mechanism which neutralizes the toxicity of caffeine was present. Moreover, some experiments with smaller doses show that the resistance to caffeine is greater after removal of the kidneys. It was shown in Bulletin 148 that for some rabbits 100 mg of caffeine per kilo proved fatal by subcutaneous injection. Such a dose, however, failed in the great majority of instances to produce any symptoms in nephrectomized rabbits. This may be seen by referring to the protocols of experiments on rabbits Nos. 797, 798, 799, 803 and 804, (p. 23), and is also shown in the following experiments:

FURTHER TESTS, WITH MEDIUM DOSES, FOR THE TOXICITY OF CAFFEIN (SERIES VI).

Rabbit 794.—Gray female; weight, 1,440 grams.

May 1: 12.23 p. m., double nephrectomy, an overdose of ether caused suspension of respiration, rabbit was revived by artificial respiration and operation was continued, loss of blood about 1 cc; 3.38 p. m., 8 cc of 2 per cent caffeine injected subcutaneously; 5.30 p. m., reflexes increased, hypersensitive, avoided light.

May 2: 9 a. m., reflexes increased, but less than day before, still avoided light, otherwise condition good, feces normal; 1 p. m., rabbit found dead, still warm and rigor mortis present.

Lived about 21 hours after the injection of caffeine.

Rabbit 795.—Gray female; weight, 1,375 grams.

May 1: 11.52 a. m., double nephrectomy, ether narcosis, operation about 18 minutes, uneventful, estimated loss of blood about 1 cc; 3.35 p. m., condition good, 8 cc of 2 per cent caffeine injected subcutaneously; 5.30 p. m., condition good, no symptoms.

May 2: 9 a. m., condition of rabbit good, feces soft; 10 p. m., rabbit looked comatose, paralyzed.

May 3: 9 a. m., found dead.

Lived more than 30 hours after injection of caffeine.

Rabbit 796.—Gray female; weight, 1,460 grams.

May 1: 12.50 p. m., ether narcosis, double nephrectomy, loss of blood slight, about 1 cc, operation uneventful; 3.35 p. m., condition good, 8 cc of 2 per cent caffeine injected subcutaneously; 5.30 p. m., rabbit paralyzed, feces soft.

May 2: 9.30 a. m., paralysis more marked than day before, feces soft; 2.25 p. m., was bled to death.

Lived 23 hours after injection of caffeine.

Since the duration of life in this series of experiments was not less than 20 hours, the amounts of caffein injected, about 110 mg per kilo, can not be regarded as fatal. That absorption was good is indicated by the symptoms manifested by rabbit No. 794, as the effects of caffein could be noticed in less than 2 hours after its administration. The condition of rabbit No. 796 was probably accidental and not due to caffein nor to removal of the kidney, as paralysis occurs sometimes spontaneously. On the other hand, those symptoms had never been observed so soon after nephrectomy or after small doses of caffein. Moreover, since no symptoms of excitement were present, this dose of caffein can not be regarded as toxic.

EFFECT OF OPERATION ON TOXICITY (SERIES VII).

As a further test of the resistance of the rabbit to caffein under this condition, it seemed advisable to control the operative procedure involved in the removal of the kidney, for it is possible that the effect of exposing the kidney and the anæsthesia may modify the toxicity of caffein independently of the removal of the organ. This was carried out in the following experiments on 6 rabbits, Nos. 812, 813, 814, 824, 825, and 826. One, No. 813 (165 mg per kilo), showed symptoms of severe intoxication in one and one-half hours after it received caffein and died during the night; No. 812 (200 mg per kilo) lived one and one-half hours; No. 824 (146 mg per kilo) was found paralyzed the next day; No. 814 (150 mg per kilo) showed mild symptoms of caffein intoxication and survived. The other two showed no effect of caffein. The operation, aside from the removal of the kidney, therefore increased the toxicity of caffein. This is shown in the following protocols to the experiment:

Rabbit 812.—White female; weight, 1,950 grams.

May 20: 3.05 p. m., kidney exposed by lumbar incision but not removed; 4.15 p. m., condition good, 15 cc of 2 per cent caffein injected subcutaneously; 5.45 p. m., rabbit dead, stiff and warm.

Rabbit 813.—Black male; weight, 1,785 grams.

May 20: 2.45 p. m., kidney exposed by lumbar incision but not removed, wound closed; 4.20 p. m., 15 cc of 2 per cent caffein injected subcutaneously; 5.45 p. m., reflexes very much increased and rabbit avoided light; symptoms of severe caffein intoxication present.

May 21: 9 a. m., found dead.

Rabbit 814.—Black male; weight, 2,080 grams.

May 20: 3.35 p. m., kidneys exposed by lumbar incision and wound closed, but kidney not removed; 4.17 p. m., 16 cc of 2 per cent caffein subcutaneously injected; 5.45 p. m., reflexes increased, but not very marked.

May 21: 9 a. m., alive, in good condition.

May 22: 11 a. m., weight, 1,935 grams, appetite good.

May 23: Alive, condition good.

Rabbit 825.—Belgian hare, female; weight, 1,290 grams.

May 28: 3.15 p. m., ether anæsthesia, kidneys exposed but not removed, wound closed; 4.43 p. m., received 8 cc of 2 per cent caffein (124 mg per kilo) injected subcutaneously.

May 29: 9 a. m., weight 1,250 grams, condition good.

Under observation until June 10, when weight was 1,345 grams and condition good.

Rabbit 826.—White female; weight, 1,760 grams.

May 28: 3.30 p. m., kidneys exposed by lumbar incision under ether anæsthesia, but not nephrectomized; 4.42 p. m., 12 cc of 2 per cent caffein (136 mg per kilo) injected subcutaneously.

Under observation until June 16, when found dead. Rabbit lost 300 grams weight until June 1, after which it began to gain weight slowly.

Rabbit 824.—Belgian hare, female; weight, 1,710 grams.

May 28: 3.50 p. m., kidneys exposed by lumbar incision under ether anæsthesia; 4.40 p. m., 12.5 cc of 2 per cent caffein (146 mg per kilo) injected subcutaneously.

May 29: paralyzed.

RÉSUMÉ AND DISCUSSION.

A careful study of the preceding experiments shows that in the majority of cases a single dose of from 100 to 150 mg caffein per kilo may be given to a nephrectomized rabbit without producing marked symptoms. Reference to Bulletin 148 shows that 150 mg of caffein per kilo were usually toxic, while smaller doses were without effect except in such cases as were associated with some abnormality. These, as may be seen, proved fatal. Doses of 200 mg caffein per kilo were always toxic for the normal rabbit and proved fatal in some but not in all cases. Nephrectomized rabbits likewise manifested symptoms of toxicity when such amounts were administered. The duration of life after injection varied between 18 and 48 hours (approximately). A dose of 200 mg per kilo can not be regarded, therefore, as surely fatal for nephrectomized rabbits, but it is invariably toxic. The tolerance for the single dose of this alkaloid is probably a little greater than in the normal rabbit. Furthermore a dose of 150 mg caffein per kilo may produce death when repeated within 24 hours, while smaller doses may be given daily without causing death in nephrectomized rabbits. This is shown in Series III (p. 23), in which the total amount injected, 0.3 to 0.4 gram caffein per kilo, was well borne by rabbits deprived of both kidneys. Larger doses, 0.3 gram caffein per kilo, were also given at a single injection in nephrectomized rabbits. This proved to be rapidly fatal when administered from two to three hours after nephrectomy, while such a dose given about 24 hours after nephrectomy was distinctly less toxic, as the animals lived several hours after receiving caffein.

The evidence presented in this investigation points strongly, therefore, to the formation of substances that counteract the effect of caffein when both kidneys have been removed. By the control experiments showing greater toxicity as a result of merely exposing the kidney this contention is greatly reinforced.

CONCLUSIONS.

The data presented in the present investigation justify the following conclusions:

1. The removal of both kidneys in the rabbit stimulates to a marked degree the elimination of caffeine by the stomach, and to a greater degree the elimination by the intestine.

2. The amounts of caffeine found several hours after injection compared with the amounts recovered about 24 hours after injection indicate that absorption goes on simultaneously with excretion. That reabsorption takes place is shown by the absence of caffeine from the gastrointestinal canal and by the presence of very small quantities of it in the feces of nephrectomized rabbits which lived 5 to 7 days after the injection.

3. Elimination is most active during the first few hours after injection of caffeine, being more rapid by the intestine than by the kidney.

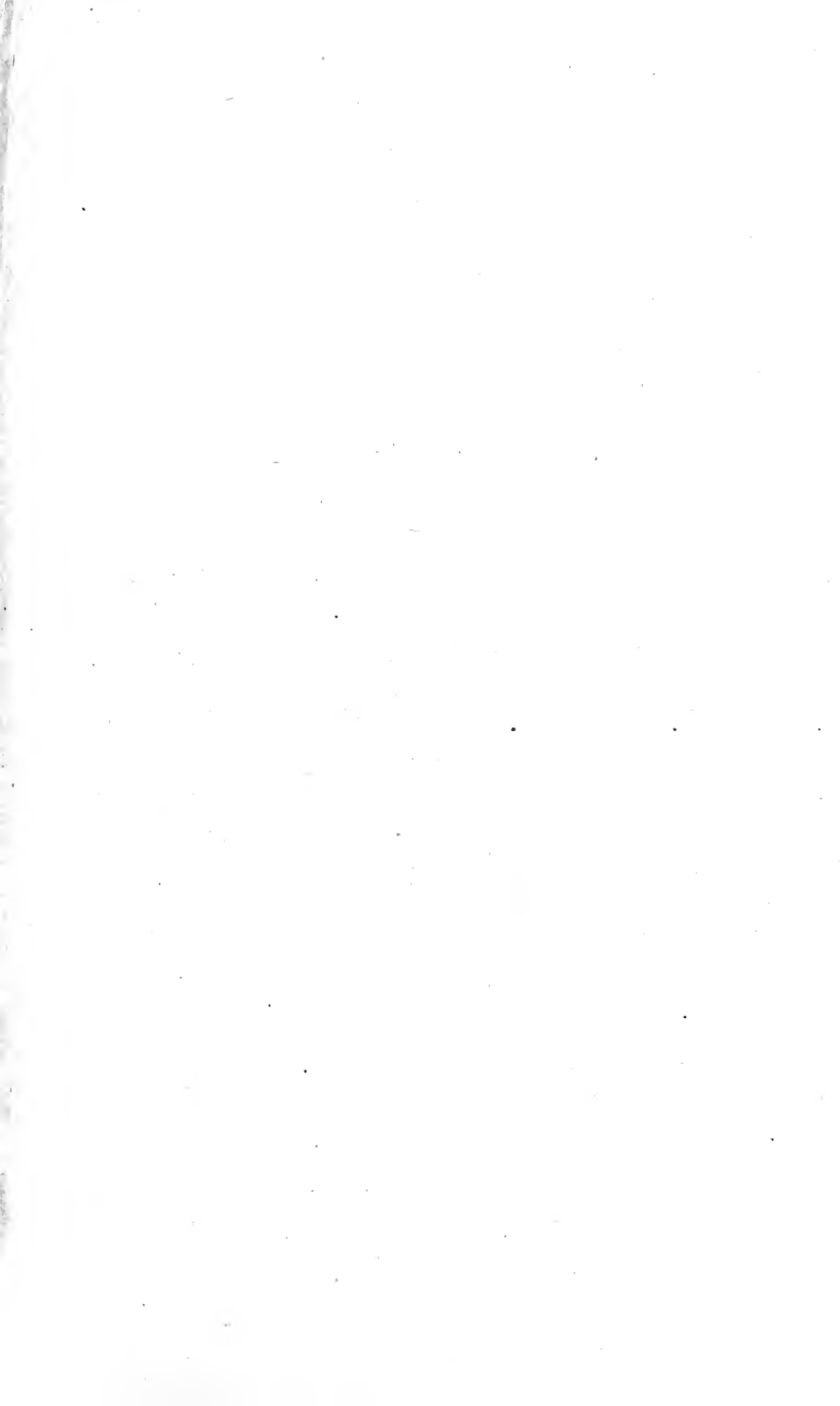
4. The undiminished resistance to the single dose and tolerance for large amounts of caffeine when subminimum doses are injected at sufficiently long intervals points to the formation of substances antagonistic to caffeine after the removal of both kidneys.

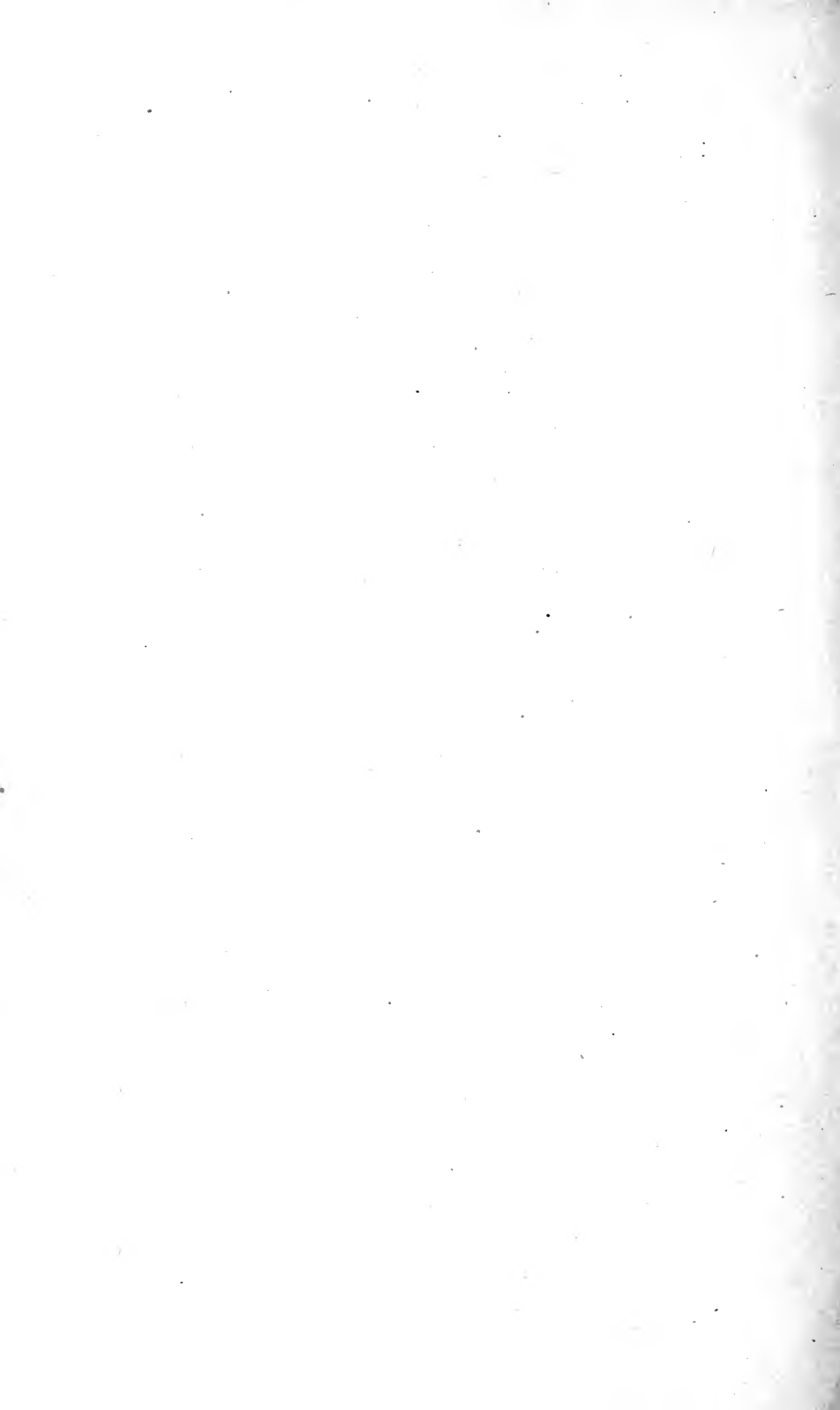
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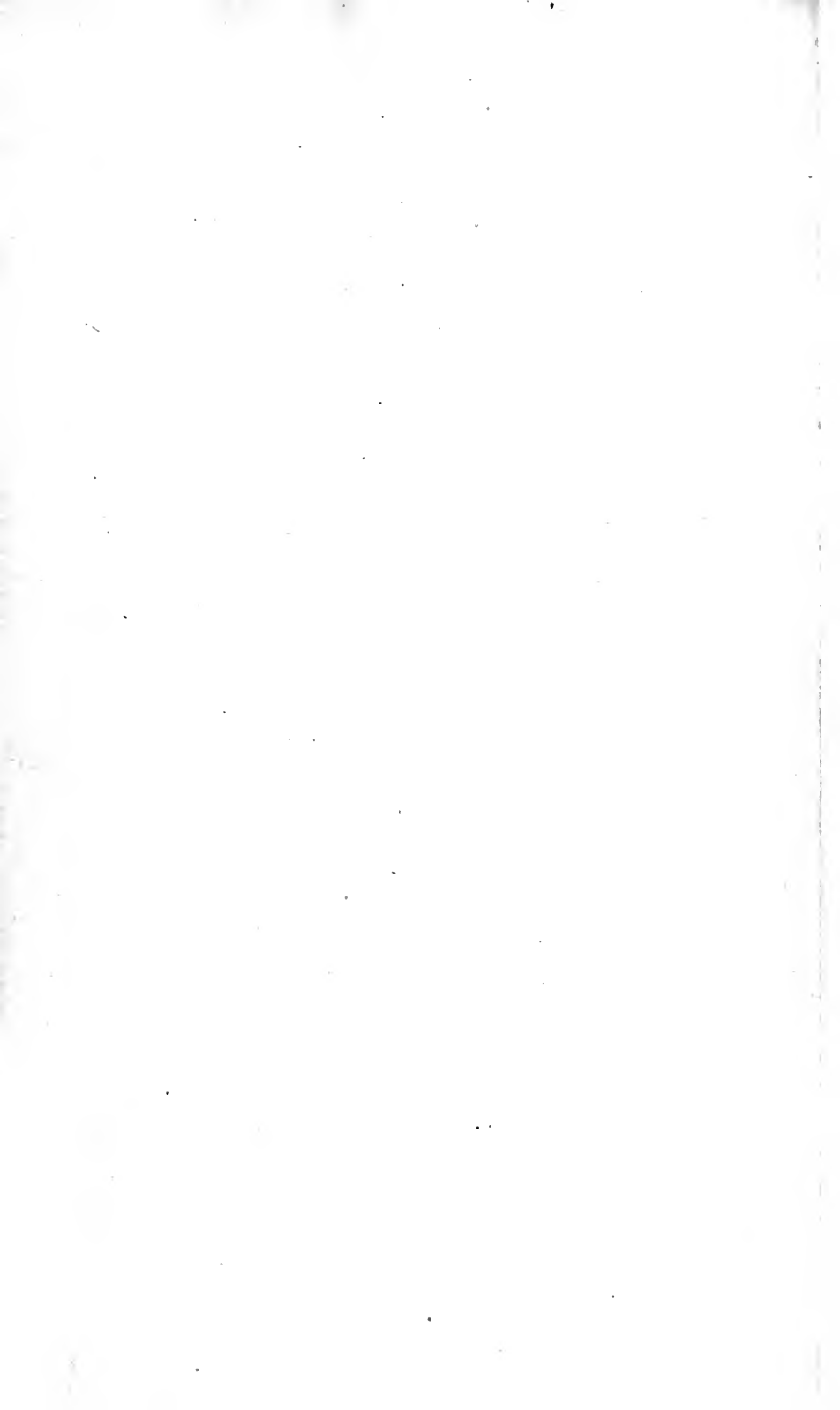
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