

TOXEMIA OF PREGNANCY IN THE RABBIT*

I. CLINICAL MANIFESTATIONS AND PATHOLOGY†

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Toxic affections associated with pregnancy have been reported in several species of animals, notably the sheep (2) and guinea pig (3); it is also common knowledge among breeders that overfat animals of all kinds are apt to die during gestation or parturition, but the exact nature and pathogenesis of these disorders is not known. A highly fatal affection associated with pregnancy also occurs in the rabbit. This condition is of interest, not only as a disease of the rabbit, but also because it bears a close analogy to the toxemias of pregnancy in man and may provide a means for an experimental approach to problems connected with human toxemia.

Sporadic cases of this affection have been under observation in the laboratory breeding colony for several years. In the fall of 1935, the breeding colony was moved to Princeton, and with the resumption of breeding operations there was an unprecedented outbreak of fatal cases of toxemia. The present report is based on the study of material provided by this outbreak supplemented by data obtained from previous observations.

It should be made clear at the outset that, strictly speaking, the disease is not limited to pregnant rabbits, but occurs post partum and in pseudopregnant females. It is also evident that all cases of toxemia are not identical clinically or pathologically, but for the present no

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attempt will be made to classify cases except on the basis of apparent severity of the disease.

The object of the present paper is to report the results of clinical and pathological investigations, including the clinical history and symptomatology of the disease, blood chemistry and post mortem findings. Investigations bearing on the etiology of the disease will be presented in a subsequent paper.

Materials and Methods

The colony in which the disease occurs is maintained in active breeding service by this laboratory for the study of constitutional problems. The pedigrees and life histories of all animals are known, and the characteristics of the stock have been the subject of extensive investigation.

During the period under consideration the population was composed of fourteen pure breeds, including the Belgian, Beveren, Chinchilla, Dutch, English, Havana, Himalayan, Lilac, Polish, Rex, Sable and Silver Marten, Siamese Sable, French Silver and Tan breeds, and numerous hybrid lines. In November, 1935, the adult stock numbered 350 males and 650 females. Some of these animals were normal in all respects investigated; others showed minor abnormalities, and still others were heterozygous for lethal variations, but most of the population were known to be transmitters of some abnormality.

The colony is housed indoors in individual cages. The diet consists of hay, oats and a standard commercial ration which has been in use for several years. Frequent routine feedings are made and at all times the animals have access to a free supply of food and water. Previous to November, 1935, the colony was housed at The Rockefeller Institute in New York, but in the present situation in Princeton the only immediate changes in living conditions are larger quarters, more commodious cages and a new water supply.

The material for the present report is based on cases of toxemia which occurred between November, 1935, and December, 1936. During this period there were 72 fatal cases of the disorder. Diagnosis of non-fatal cases cannot always be made on a basis of symptomatology, and as facilities for blood analysis were limited and pathological examination of all suspected cases was impractical, the incidence could not be accurately determined. Clinical diagnosis was substantiated by chemical study of four cases that terminated in recovery, but the incidence of non-fatal cases was undoubtedly much greater, and the evidence at hand indicates that many instances of ante partum and post partum morbidity are mild cases of toxemia.

The facilities available did not allow constant examination of the colony, and because of the rapid course of the disease, the period of clinical observation was frequently limited to the terminal stages of the disease or affected animals were found dead. In a number of instances, however, the clinical course was followed from apparent health to complete prostration and a full history was obtained.

Complete autopsies were performed in all fatal cases and blood and various organs were cultured for bacteriological study. Pathological and bacteriological examinations were also made in two non-fatal instances of the disorder. Tissues for microscopic examination were fixed in Helly's and Petrunkevitch's (4) solutions and stained with hematoxylin and eosin. Pituitary glands were serially sectioned and contiguous sections stained with Mann's methylene blue and eosin or copper hematoxylin.

Blood studies included the determination of sugar, non-protein nitrogen, urea nitrogen, creatinine, uric acid, sodium chloride, calcium, inorganic phosphate, fat, cholesterol, acetone bodies, total serum proteins and albumin. The methods used in these determinations and the technical procedures followed were those recommended in a standard laboratory manual (5). No attempt was made to control the dietary status of animals at the time blood was obtained.

Chemical blood analyses have been made in twelve fatal and four non-fatal cases of toxemia. In addition, the blood of a large number of healthy females has been examined in order to determine normal values and the early or preclinical changes in toxemia. Thirty resting and six pregnant females of this series have continued in health to the present time and for comparative purposes are considered in subsequent paragraphs as a normal control group. Six other animals subsequently died of toxemia at periods ranging from 119 to 2 days after chemical blood examination, while five others showed abnormal values during apparent health, but to date have shown no clinical evidence of toxemia.

The clinical and pathological changes characteristic of the disorder suggested a toxic origin, and the possibility of food or water poisoning was examined both by feeding various constituents of the diet in excessive amounts to normal rabbits and by adding a metallic poison to the food.

Clinical Course

The symptomatology and clinical course of toxemia of pregnancy in the rabbit are variable. The disease may occur in a comparatively mild form followed by recovery, or pursue a rapid course to a fatal termination. The disorder in mild cases may escape clinical observation or be classified as a minor transitory disturbance, and its true nature not recognized until pathological examination following death from a subsequent attack or from unrelated causes shows healed lesions of toxemia. In typical cases, on the other hand, the disorder is obvious and presents a definite clinical picture.

Frequently, the disease sets in abruptly and from the beginning the manifestations are those of a sudden and severe intoxication. In other instances, the onset of severe manifestations is preceded by general signs of malaise which may be observed for 3 or 4 hours and gradually become more pronounced.

The signs of acidosis predominate the attack. Air hunger and dyspnea are apparent and acetone breath may be detected on close observation. Thirst is an early manifestation in some cases and may be extreme; occasional animals are found dead with their heads immersed in the water container. In other instances, however, the water intake is markedly diminished. Total suppression of urine is the rule in all cases, and in no instance has sufficient urine been found post mortem to permit a quantitative analysis. There is a loss of normal vigor and activity and the animals sit hunched in a corner of the cage with roughened coats and dull, lustreless eyes. They respond sluggishly to ordinary stimuli and the gait is slow and incoordinate when movement is forced. The ears are cold and the flow of blood in the marginal veins may be stopped with slight pressure. Convulsions occur in some cases and as a rule, are of the tonic type but may be clonic in character. Other animals remain lethargic, and generally, in all cases a comatose stage with relaxed sphincters, dilated pupils and widespread muscular asthenia precedes death. Cyanosis and marked respiratory distress with the appearance of a serosanguineous discharge from the nares are often terminal manifestations.

The period of obvious illness with conspicuous toxic signs in such cases is remarkably brief and rarely exceeds a few hours in duration. Frequently, the condition of a doe has been observed to change from apparent excellent health to complete prostration in the course of a half hour, and in only one animal under treatment has the clinical course lasted longer than 1 day. The condition of internal organs at autopsy indicates, however, that the course of disease may be of longer duration and that the disorder may be well advanced before clinical signs become apparent.

In non-fatal cases the manifestations of toxemia are mild and usually not specific, and clinical diagnosis can rarely be made on the symptomatology, but rests rather on the occurrence of a protracted period of illness following abortion or desertion of a litter early in the puerperium. Occasionally, rapid respiration, dilated pupils, or a slight cyanotic tinge about the lips and nares may be observed, but generally, the manifestations are no more than those of slight disorder such as are present in a number of minor disturbances. Convulsions do not occur and acetone breath has not been detected. General morbidity with loss of appetite may persist for a week or more during which there is some loss of weight due largely to muscular wasting but without any noticeable depletion in fat depots. Fluid intake and urine excretion are markedly diminished. Recovery is gradual and leaves no outward sign of disability.

There is pathological and chemical evidence that mild cases may occur without symptoms, and while breeding records frequently show the past occurrence of abortion or desertion which may have been associated with the attack, in other instances there is no indication of reproductive abnormality in the history of the animal. Fatal asymptomatic cases also occur in which no outward sign suggests the condition

of the animal, and sudden death in the midst of nest building or other physiological activity is the first indication of disturbance.

In general, therefore, one may recognize three types of disease. First, a typical toxemia with characteristic manifestations which usually terminates in death within a few hours after the signs are first noted; second, a milder disorder which presents less characteristic signs but persists for a week or more and is followed by recovery; third, an asymptomatic affection which usually escapes recognition at the time but, in rare instances, may lead to sudden death.

Relation to Pregnancy

The disease is predominantly a disorder of pregnancy, but typical cases also occur post partum and in resting animals. Of the 72 fatal cases that occurred between November 1, 1935, and December 1, 1936, 43 or 59.3 per cent were in pregnant does, 15 or 20.8 per cent in post partum and 14 or 19.4 per cent in resting females.

There were 66 fatal cases in multiparous females, an incidence of 10.3 per cent, and only 6 cases, or an incidence of 2.6 per cent, in primiparae.

A more detailed account of the incidence in relation to pregnancy will be presented in a subsequent paper.

Blood Chemistry

The results obtained by chemical analysis of the blood of animals with toxemia were controlled in two ways: first, by comparison with values obtained in resting females of similar genetic origin and second, by a comparison with values obtained from pregnant females of the same genetic groups.

Resting Controls.—The values obtained for various constituents of the blood of thirty healthy resting females are presented in Table I. These results are divided into six groups, each of which is composed of animals of like genetic constitution but differing in some respect from those of other groups. While a consideration of the significance of group differences is beyond the scope of this paper, it may be mentioned that the mean values for these groups do show significant differences in one or more respects. In like manner, the values obtained for animals with toxemia are variable, but in all instances the

TABLE I
Values for Blood Constituents of Apparently Healthy Resting Female Rabbits

Group	Whole blood										Serum				Ratios		
	Sugar mg. per cent	Non-protein nitrogen mg. per cent	Urea nitrogen mg. per cent	Creatinine mg. per cent	Uric acid mg. per cent	Sodium chloride mg. per cent	Cholesterol mg. per cent	Fat mg. per cent	Calcium mg. per cent	Inorganic phosphate mg. per cent	Total protein gm. per cent	Albumin gm. per cent	Globulin gm. per cent	UN/NPN	P/Ca	A/G	
I	150	27.96	9.03	1.21		472			13.48	3.07	4.34	2.61	1.73	0.32	0.22	1.5	
	142.8	26.50	13.47	1.49		466			14.25	5.97	5.97	3.12	2.85	0.51		1.1	
	167.6	40.89	16.42	1.17		456			14.08	6.41	4.79	2.94	1.85	0.40	0.45	1.6	
II	145.6	32.47	12.78	1.51		442	116.8		13.13	6.84	5.62	3.58	2.04	0.39	0.52	1.7	
	152.2	37.72	13.63	1.47		419	107.2		15.19	7.68	6.03	4.04	1.99	0.36	0.50	1.9	
	148.6	41.64	14.33	1.52	0.94	478	95.2		13.81	6.46	5.86	3.81	2.05	0.34	0.46	1.8	
	145.6	38.10	13.72	1.53	0.81	495	105.4		14.11	5.93	6.34	4.14	2.20	0.36	0.42	1.8	
	152.2	35.43	13.15	1.53	0.78	479	95.2		12.99	6.12	5.28	3.07	2.21	0.37	0.47	1.4	
	136.3	32.13	17.43	1.51	0.64	469	105.3		13.38	5.51	5.16	3.13	2.03	0.54	0.41	1.5	
	134.6	30.60	10.56	1.67	0.63	434	102		13.91	4.78	5.02	3.21	1.81	0.34	0.34	1.7	
	150.5	39.45	16.06	1.81	1.00	453	106.9		13.32	4.00	5.34	3.35	1.99	0.40	0.30	1.7	
	142.8	34.86	13.89	1.68	0.80	442	106.4		13.77	4.59	6.21	3.25	2.96	0.39	0.33	1.1	
	145.7	35.70	14.07	1.53	0.75	449	108.6		14.74	4.11	5.92	3.28	2.64	0.39	0.27	1.2	
	III	126	36.9	16.13	1.40		445	123	842.7	15.48	3.75	5.44	3.46	1.98	0.43	0.24	1.7
		151.6	28.3	14.87	1.33		446	129.4	1183	13.72	3.84	5.25	3.03	2.22	0.52		1.4
153			11.37	1.51	0.67	485	112.8	968.2	13.72	5.71	5.39	3.09	2.30		0.41	1.3	
124		35.1	12.09	1.46	1.11	429	137.6	571.6	14.70	5.89	5.18	3.01	2.17	0.34	0.40	1.4	
130.3		40.5	12.50	1.44		475	132.2	517.9	13.58	6.37	5.53	3.43	2.10	0.30	0.46	1.6	
127		40.9	14.07	1.42		475	111.2	430.3	12.80	6.20	5.62	3.14	2.48	0.34	0.48	1.3	
167.6		37.5	15.51	1.52	0.68	458	124	502	13.58	7.38	5.00	3.16	1.84	0.41	0.54	1.7	
187.6	38.4	14.52	1.45	0.67	490	126.6	860.6	12.99	6.28	5.03	3.43	1.60	0.37	0.48	2.0		
138.8	35.4	13.23	1.52	0.55	442	115.8	403.3	14.35	5.86	5.40	3.50	1.90	0.37	0.40	1.8		
140.1	38.7	16.54	1.43	0.58	459	125	143.4	15.52	4.27	5.92	3.58	2.34	0.42	0.26	1.5		

deviation from normal as represented by control values is much greater than the difference between the various healthy groups.

Pregnant Controls.—With the view of determining the chemical changes that normally occur in pregnancy, the blood of six healthy females was studied and the results are shown in Table II. The determinations are not sufficiently numerous to allow a statistical comparison, but the results obtained indicate that the variations in blood chemistry accompanying pregnancy are very slight.

TABLE II
Values for Blood Constituents of Apparently Healthy Pregnant Rabbits

Group	Whole blood						Serum					Ratios		
	Sugar	Non-protein nitrogen	Urea nitrogen	Creatinine	Sodium chloride	Cholesterol	Calcium	Inorganic phosphate	Total protein	Albumin	Globulin	UN/NPN	P/Ca	A/G
	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	gm. per cent	gm. per cent	gm. per cent			
I	159.5	34.50	18.9	1.17	430		14.84	4.88	4.35	3.17	1.18	0.55	0.32	2.7
	118.1	41.64	15.3	1.14	470		15.35	6.08	5.07	2.92	2.15	0.37	0.39	1.4
IV	147	38.43	14.89	1.19	465	111.2	16.29					0.39		
	131.5	26.94	15.73	1.26	465		15.81		4.26	2.83	1.43	0.58		2.0
	125	30.18	15.84	1.15	462		16.39		5.85	3.40	2.45	0.53		1.4
VI	127	41.36	20.82	1.45	470		15.15	4.14	4.84	3.13	1.71	0.34	0.21	1.8

Toxemia

Chemical blood studies have been made both in fatal cases of toxemia and in cases with comparatively mild symptoms which terminated in recovery. Moreover, in a general survey of the colony, analyses were made on animals which later died of toxemia, and in addition abnormal values suggestive of toxemia were found in a number of apparently healthy rabbits.

Fatal Cases of Toxemia.—Chemical blood values determined in twelve fatal cases of toxemia are shown in Table III. A notable feature of the disorder is the extreme difficulty of obtaining blood from the marginal ear vein, and a number of animals died during heart

TABLE III
Values for Blood Constituents in Advanced Stages of Fatal Toxemia of Pregnancy

Rabbit No.	Whole blood										Plasma					Serum					Ratios		
	Sugar	Non-protein nitrogen	Urea nitrogen	Creatinine	Uric acid	Sodium chloride	Cholesterol	Fat	Acetone	Calcium	Inorganic phosphate	Total protein	Albumin	Globulin	UN NPN	P/Ca	A/G						
	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent								
1	35.4	60.8	29.1	4.34											0.47		0.69						
2	89.3										7.40	3.04	4.36				0.91						
3	95.5	65.7	54.8	4.58	460		1022		13.92	9.37	5.90	2.82	3.08	0.83	0.67		0.74						
4							592	++		7.80	4.27	1.82	2.45	0.47			1.14						
5	47.1	64.1	30.2	4.76		125					5.38	2.87	2.51										
6	167.6	107.1	54.9	2.98		106.4	1757	++	9.97	8.84	3.20	1.27	1.93	0.51	0.88		0.65						
7	155.2	64.3	37.2	4.19		282.4	3656	++	9.70	13.27	4.08	2.03	2.05	0.57	1.36		0.99						
8	146.4	62.5	27.6	5.59		93.6	931	+		14.42	3.90	2.20	1.70	0.44			1.29						
9	309.2	140.1	107.1	8.3	1.23	306	3586	++	8.82	11.71	6.13	3.09		0.76	1.32		1.0						
10	425.2	191	159.5	12.12		395			11.40	8.89	5.54	2.22	3.32	0.83	0.78		0.67						
11	304.4	175.8	136.3	12.67		366					5.12	1.57	3.55	0.77			0.44						
12	312.4	53.9	37.5	5.09		160	1972	+	12.83	12.01	3.96	1.81	2.15	0.69	0.93		0.84						

puncture. All other animals died within a few hours of bleeding and the values, therefore, represent the terminal stages of the disease.

Values for various constituents of the blood showed marked changes in the disorder, and while they varied in different animals, the direction of alteration in all constituents with the exception of sugar was constant.

On a basis of blood sugar determinations, the fatal cases of toxemia were divisible into three groups, one of which showed a marked hypoglycemia, another a marked hyperglycemia, and a third approximately normal values. The non-protein nitrogen, urea nitrogen and creatinine values were markedly elevated in all groups and were greatest in the hyperglycemic group. This increase, however, was not a result of blood concentration, as is shown by the disproportionate rise of these and other blood constituents in relation to sugar. The urea partition of the non-protein nitrogen was greater than normal in all animals and in two instances reached the high figure of 83 per cent. Uric acid was also increased but, unfortunately, its value was determined in only one instance.

The sodium chloride value was elevated in one of the two animals in which it was determined. Calcium was decreased and inorganic phosphate increased to such a degree that in all cases the P/Ca ratio approached or was greater than unity. Fat and cholesterol were not uniformly altered, but in general the values were greater than those obtained in healthy animals and in some instances the increase was pronounced. The qualitative acetone test was positive in all cases in which it was made. Serum proteins were slightly increased in one or two instances but generally were somewhat lowered. The albumin and globulin fractions were markedly altered, and in the majority of cases their ratio was reversed.

Preclinical Values.—The course of toxemia in fatal cases is so rapid that consecutive blood studies during the period of clinical illness have so far not been possible. A number of animals, however, that were bled as part of the normal control group subsequently died of toxemia and their blood values give some indication of the preclinical phase of the disorder. The determinations are listed in Table IV, and it should be emphasized that at the time they were made, the animals showed no signs or symptoms of ill health and to all appearance were in excellent condition.

The animal listed first in this series was examined 119 days before the onset of toxic signs and the only abnormalities encountered were a high sugar and low non-protein nitrogen and urea nitrogen values. Likewise the blood of the second animal obtained 75 days before death showed only a slight deviation from the normal manifest in low serum protein value. However, reexamination of this animal 7 days before death disclosed an entirely different blood picture with an increased non-protein nitrogen, low urea nitrogen, increased serum proteins and inversion of the albumin-globulin ratio.

The third animal in the table was examined 36 days before the occurrence of toxemia, and except for low sugar, sodium chloride and creatinine values, the

TABLE IV
Values for Blood Constituents at Various Times Prior to the Onset of Toxemia of Pregnancy

Rabbit No.	Time before death days	Condition at bleeding	Whole blood					Serum					Ratios			
			Sugar	Non-protein nitrogen	Urea nitrogen	Creatinine	Sodium chloride	Cholesterol	Calcium	Inorganic phosphate	Total protein	Albumin	Globulin	UN/NPN	P/Ca	A/G
			mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	gm. per cent	gm. per cent	gm. per cent		
1	119	R	192.9	22.95	12.7	1.42	445	120	15.77	4.68	5.51	3.58	1.93	0.55	0.29	0.19
2	75	R	145.7	33.57	15.19	1.10	430		15.13	3.6	4.44	3.42	1.02	0.45	0.23	3.3
	7	P	140.1	40.89	8.04	1.69		291.4			5.52	2.49	3.03	0.19		0.8
3	36	R	75	36.36	15.4	0.87	267		14.93		5.49	3.37	2.12	0.42		1.5
4	6	P	129.3	39.1	26.6	1.64	473		13.34	6.16				0.67	0.46	
5	5	P	205.4	31.47	11.8	1.61		85	11.88	3.34	3.86	1.92	1.94	0.37	0.28	0.9
6	2	P	131.2	32.37	20.45	1.68	460	100.4	12.54	5.26	4.14	2.86	1.28	0.63	0.41	2.2

R = resting. P = pregnant.

blood was entirely within the limits of normal. On the other hand, the blood of the next animal, analyzed 6 days before death, showed definite abnormalities with nitrogen retention and a high urea partition.

The fifth animal was examined 5 days before the occurrence of illness and showed high sugar, low serum protein values, and inversion of the albumin-globulin ratio.

The blood of the final animal of this series was analyzed 2 days before death and the suggestive findings were increased urea nitrogen and creatinine with slightly lowered calcium and increased inorganic phosphate values.

It should be noted that the four series of determinations showing most marked abnormalities were made during the gestation period

that terminated in death, while the other determinations were made at various intervals before the fatal pregnancy. The findings in the pregnant group show clearly that the disease may be well advanced before clinical symptoms become apparent. Evidence of a disordered metabolism may be found in the chemical blood picture for as long as a week before the condition is reflected in the animal's behavior, but the degree of variation from normal gives no positive indication of the severity of the condition or the proximity of death.

Blood Chemistry and Reproductive History.—The blood values found in resting animals at considerable periods of time before the clinical

TABLE V
Irregular Values for Blood Constituents of Apparently Healthy Female Rabbits

Rabbit No.	Condition at bleeding	Whole blood					Serum					Ratios		
		Sugar	Non-protein nitrogen	Urea nitrogen	Creatinine	Sodium chloride	Calcium	Inorganic phosphate	Total protein	Albumin	Globulin	UN NPN	P/Ca	A/G
		mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	gm. per cent	gm. per cent	gm. per cent			
1	P	147.8	28.65	8.8	1.33	460	14.16	3.6	5.29	3.93	1.36	0.30	0.25	2.8
2	P	114.5	35.81	10.52	0.96	460	13.61	5.09	4.79	2.75	2.04	0.29	0.37	1.3
3	R	182.8	29.4	11.02	1.5	440	11.59	4.42	4.94	2.53	2.41	0.38	0.38	1.05
4	R	145.7	40.24	22.05	1.61	410	16.98	3.90	7.25	3.57	3.68	0.54	0.23	0.9
5	R	94.3	28.65	11.02	1.94	460	9.31	1.80	3.09	1.65	1.44	0.38	0.19	1.1

R = resting. P = pregnant.

occurrence of the disorder are not markedly abnormal but show definite alteration in one or more particulars. Moreover, the presence of a functional disturbance is reflected in their immediate breeding history. The first two animals in this series were mated shortly after the listed blood values had been determined, and while no abnormalities were noted during gestation, the resulting litter in one instance was born dead and in the other was deserted shortly after birth. Subsequently both were remated and died of toxemia during the ensuing pregnancy. The determinations on the third animal were made just previous to a fertile mating and the gestation period terminated in fatal toxemia.

There have been other instances, however, in which comparable

abnormal values were obtained, but despite numerous pregnancies the animals have continued in apparent health to the present time. These values are listed in Table V. The blood for analysis in the first two instances was taken during the last week of gestation and the remaining determinations were made on resting animals.

Cases of Toxemia with Comparatively Mild Symptoms.—Table VI contains the results of chemical analysis of the blood of four animals obtained during the course of a disturbance which resembled fatal toxemia, both clinically and pathologically, but terminated in recovery rather than in death. With the exception of the absence of acetone bodies, the blood changes are of the same general order but less marked than those noted in fatal toxemia.

In the first instance the manifestations were mild and would have escaped casual observation. The blood showed nitrogen retention with lowered calcium, inorganic phosphate and total serum proteins. $\frac{UN}{NPN}$ was increased but P/Ca and A/G were within normal limits. The signs in the second case were more severe and were accompanied by a slight hyperglycemia, increased non-protein nitrogen with normal urea and a high inorganic phosphate value. It should be noted in this connection that alteration of P/Ca is more closely related to the severity of clinical signs than are changes in other ratios, and an increase as great as that seen in the present instance is usually noted only in fatal cases.

The first analysis of the blood of the third animal in the table was made approximately 10 months before the occurrence of the disorder and showed no irregularity. The second determinations were made while toxic symptoms were marked, but the values were only slightly different from those obtained during health, the only definite alteration being a decrease in serum proteins. Sugar, non-protein nitrogen, urea nitrogen, creatinine and sodium chloride showed small increases. In the final analysis the values for sugar and sodium chloride showed a decided rise. Serum proteins remained low and inorganic phosphate showed a slight increase. Calcium and creatinine were unchanged and all other values were lowered.

It is of considerable interest that the last blood analysis was made at a time when clinical signs were least pronounced and recovery appeared imminent, but the results obtained indicated a metabolic disorder of increasing severity rather than recovery. This animal was killed for pathological study shortly after the last analysis and while a comparison of the values obtained in this animal with those of fatal cases of toxemia would suggest a metabolic disorder of considerably less severity, the lesions were hardly distinguishable from those of fatal cases.

Analysis of the blood of the last animal of this series was begun a few days after the appearance of toxic signs and the last determinations were made shortly

TABLE VI
Values for Blood Constituents in Non-Fatal Cases of Toxemia

Rabbit No.	Date	Whole blood							Plasma					Serum					Ratios		
		Sugar mg. per cent	Non-protein nitrogen mg. per cent	Urea nitrogen mg. per cent	Creatinine mg. per cent	Uric acid mg. per cent	Sodium chloride mg. per cent	Cholesterol mg. per cent	Fat mg. per cent	Acetone	Calcium mg. per cent	Inorganic phosphate mg. per cent	Total protein gm. per cent	Albumin gm. per cent	Globulin gm. per cent	UN NPN	P/Ca	A/G			
1	1936	147.5	50.55	30.81	1.71	0.89	449			11.34	3.00	3.44	2.21	1.23	0.60	0.26	1.8				
		171.4	46.38	15.4	1.43	0.78	469			12.54	8.83	5.66	3.12	2.54	0.33	0.70	1.2				
3	Jan. 17	153	35.3	13.23	1.26		470			12.99	4.8	5.15	3.14	2.01	0.37	0.36	1.5				
	Nov. 6	156.2	36.9	14.07	1.64	0.79	528	113.2	574	12.41	4.78	3.14	1.92	1.22	0.38	0.38	1.5				
	Nov. 9	180.8	30.39	12.36	1.68	0.77	554	90.8	644	12.41	5.20	3.38	1.87	1.51	0.40	0.41	1.2				
4	Oct. 26	185.2	48.9	20.82	1.74	0.79	498	208.2		13.26	5.75	6.72	3.29	3.43	0.42	0.43	0.96				
	Nov. 9	129.2	72.5	35.73	3.44	0.72	459	217.4	430	11.83	4.5	5.82	3.28	2.54	0.49	0.38	1.3				
	Nov. 16	139.6	87.86	41.20	3.20	0.74	485	240		12.47	6.54	5.82	2.24	3.58	0.46	0.52	0.6				

before death from pneumonia and pulmonary abscess. The first values show an inversion of the albumin-globulin ratio and a rise of all blood constituents with the exception of calcium. In subsequent analyses, the values for sugar, calcium, sodium chloride and total serum proteins tended toward decrease while other constituents continued to increase, and the ratios $\frac{UN}{NPN}$, P/Ca and A/G became increasingly abnormal.

The blood chemistry of this animal was probably influenced by the coexisting infection, and the values obtained are less indicative of the metabolic changes in toxemia than those of the preceding instances.

Pathology

The fatal and milder clinical types of toxemia are generally not distinguishable pathologically and show no consistent difference either in the nature or extent of lesions. The disease is divisible into different pathological types based on alteration of the liver, but these types are apparently not associated with constant clinical or chemical findings. The most striking lesions are found in the liver and kidney as in human toxemia, but changes of interest and of possible pathogenetic importance occur in other organs, and for this reason the gross and microscopic anatomy will be described in some detail.

Gross Morbid Anatomy.—At autopsy all animals that died of the disorder were well nourished and the majority were overfat. The increase in fat generally occurred in normal depots, being greatest in the retroperitoneal region, but in occasional animals an abnormal distribution of fatty pads in the region of the shoulder girdle was noted. Animals that were killed after a prolonged period of illness during which loss of appetite was a pronounced symptom showed considerable muscular wasting without, however, any appreciable diminution in fat stores.

In the majority of cases, whether in pseudopregnant or pregnant animals, and irrespective of the duration of pregnancy, the mammary glands were engorged and actively secreting. The feti in pregnant does were usually dead and on dissection frequently showed liver changes comparable with those observed in the mother. In the majority of instances the placentae were intact and appeared healthy, but in a few cases degenerating placentae without feti were found free in the uterine cavity. This, however, is not an unusual finding in animals killed during gestation and may be due to accidental dislocation or expression of lethal hereditary factors. The ovaries in all cases, in pregnant, pseudopregnant and parturient animals, contained large corpora lutea.

The stomach usually contained food, and the only lesion of the gastrointestinal tract proper was the occasional occurrence of petechial hemorrhages on the surface of the large intestine. Small areas of fat necroses were frequently found in the

mesenteric fat and in some instances there was extensive necrosis of the pancreas and neighboring adipose tissue.

The liver varied in weight from 55 to 210 gm. in animals of different size but averaged 5.5 per cent of the net body weight. The gross appearance was also variable. Frequently, it was uniformly yellow in color or yellow with irregular pinkish red blotches scattered over its surface. Surface markings were absent and all lobular differentiation lost. In such cases the consistency was that of soap and the cut surface imparted a peculiar greasy sensation to the fingers. Rarely, the organ was of a dull greyish brown color with scattered small pale subcapsular areas and fair preservation of lobular markings. The gall bladder was usually filled with dark green bile and showed no abnormality, but occasionally its walls were thickened and its contents granular.

The kidneys were also large and pale and depressed scarred areas were common. In many instances they showed no marked abnormality in appearance, but in others the alteration was striking. The capsule was stretched and stripped easily, revealing a bulging cortex, yellow in color with irregular pink blotches. Section showed an absence of cortical markings with frequent hemorrhagic areas. The urinary bladder was empty in most instances, and in no case of toxemia was sufficient urine found at autopsy to allow a quantitative analysis.

The spleen was always small, averaging less than a gram in weight, and little blood could be expressed from its cut surface.

The pleural and pericardial cavities usually contained an excess of fluid which in many instances was blood-stained and gelatinous in consistency. The lungs were congested and edematous. The heart muscle was flabby and pale and the ventricles were dilated with large amounts of chicken fat clot. Frequently, the right auricle and the vena cavae were devoid of blood and filled with a white milky fluid which on microscopic examination was found to be made up largely of fat globules.

The adrenals were small and their combined weight averaged 0.468 gm. or 0.022 per cent of the net body weight. They were pale yellow in color and extremely soft in consistency. The cortex was wide and frequently contained small adenomata.

The thyroid and parathyroid glands were extremely small and pale. They could be differentiated from the surrounding tissues only with the aid of a magnifying lens, and as a clean-cut separation was not possible, no weights were made.

The hypophysis was always greatly enlarged and averaged 0.044 gm. or 0.002 per cent of the net body weight. In the majority of cases no gross abnormality was noted, but in three instances the posterior half of the gland was found replaced by a large cyst containing colorless fluid.

The brain was wet and edematous, but no other gross abnormality was observed.

Microscopic Examination.—The liver alteration in the great majority of cases consisted of widespread fatty infiltration and degeneration, but in other instances fatty changes were not marked and focal areas of necrosis were the predominating lesion.

Fatty changes were extreme in many cases and involved the entire lobule so that sections resembled adipose tissue. The fat droplets generally tended to be of large size toward the center of the lobule and frequently occupied the entire cell. Nuclei were pushed to the cell margin and were observed in all stages of degeneration. Occasional cells remained intact in this area, but the protoplasm was granular and the nuclei pyknotic. At the periphery of the lobule, the fat droplets were small and were distributed evenly throughout all the cells with a resulting honeycombed appearance. Nuclei were not displaced and degenerative changes were not marked (Fig. 1). Sinusoids throughout the organ were narrowed or obliterated by the encroachment of swollen cells. Occasionally, all cells contained fat droplets exclusively of the large or small type, but the distribution described above was usual (Fig. 2). Special fixation and staining confirmed the presence of fat and showed the complete absence of glycogen. Focal hemorrhages and small discrete areas of necrosis without hemorrhage were occasionally found, but more frequently large irregular areas and sometimes entire lobes were necrotic with thrombosed vessels (Fig. 3).

In other cases fatty degeneration was limited to the periphery of the lobule while the central portion was necrotic or contained patches of necrosis in its peripheral zone (Fig. 4). It should be pointed out in this connection that following the extensive portal fatty change, the periphery of the central zone functioned as the periphery of the lobule and the necrotic areas were, therefore, similar in distribution to those frequently found in eclampsia in man.

In still other instances fatty changes were not widespread, but were limited to focal areas in which all cells were involved irrespective of lobular relationships. In such cases focal necrotic areas were common, widespread throughout the organ and tended toward a peripheral or mid-zonal distribution (Figs. 5 and 6). These areas were sharply circumscribed and adjacent cells showed no alteration. There was no cellular exudation and hemorrhage was rare. The vessels and sinusoids were dilated with blood cells and occasional hyalin thrombi were found. Fibrinous thrombi, however, as often reported in human eclampsia were not observed.

Frequently, both in fatal cases and in animals killed for pathological examination during protracted post partum illness, fibroblastic proliferation into the necrotic areas was noted, implying an older lesion than the clinical history indicated. Moreover, focal and disseminated areas of mature connective tissue were a common finding and may be interpreted as the terminal stage of such a healing process (Fig. 7). If this interpretation is correct, recurrence of toxemia is not uncommon, and as similar areas of fibrosis are found in multiparous does dying of other causes with no previous history of toxemia, it supplies further evidence of the occurrence of asymptomatic cases. The fibrous lesions referred to are readily distinguished from hepatic coccidiosis which is rarely seen in our colony.

Examination of the livers of feti removed from fatal cases showed degenerative and necrotic lesions comparable with those found in the mother.

There was apparently no correlation between the extent of hepatic and renal damage. Kidney lesions were almost exclusively retrogressive in character and

varied from fatty tubular changes to complete cortical necrosis. Active inflammatory lesions were extremely rare.

In many cases the outstanding picture was that of tubular fatty degeneration (Fig. 8). Glomeruli were also affected but to a much less extent. Small areas of hemorrhage were common and rarely hyalin thrombi in arterioles and thrombosed glomeruli were noted. Infrequent inflammatory changes were of the nature of intercapillary glomerulitis with increase of endothelial cells and narrowing of the capillary lumen.

In other instances, cortical damage was extreme with degeneration and necrosis of all elements and advanced dissolution of the entire zone (Fig. 9). The microscopic picture closely resembled that seen in symmetrical cortical necrosis in man and only hazy outlines of swollen glomeruli and granular vacuolated tubular cells with faint pyknotic nuclei remained discernible. Interlobular arteries were thrombosed and in occasional better preserved areas thrombosed glomeruli were recognizable. Medullary structures were intact, but intertubular hyalin changes were common.

Old lesions, including wedge-shaped fibrotic areas, diffuse interstitial fibrosis, scars from focal areas of tubular degeneration with round cell infiltration, together with degenerated and fibrous glomeruli were frequently encountered. The relationship of lesions of this order to past or repeated attacks of toxemia is difficult to evaluate in the present instances, inasmuch as the possibility of parasitic infestation could not be eliminated. Comparison of the lesions found in animals known to have had repeated attacks of toxemia with those encountered in the general population, however, warrants the conclusion that such lesions may be induced by toxemia.

The heart muscle showed intense and widespread fatty changes (Fig. 10). The Malpighian corpuscles of the spleen were small and germinal centers were absent. The pulp was pale staining, washed out in appearance and contained numerous refractile fat globules. In addition to the necrotic lesions previously mentioned, the pancreas showed occasional areas of fibrosis; islands of Langerhans were unusually large and numerous, but hyalin changes were rarely observed.

The fascicular zone of the adrenals was very wide and frequently occupied the entire width of the cortex (Fig. 11). There was an abundance of lipoid throughout the zone; fatty degeneration with necrosis was marked at the inner boundary of the cortex and occasionally was so marked that a fatty necrotic layer entirely separated the cortex from the medulla (Fig. 12). Small hemorrhages were frequently found and cortical adenomata were common.

The thyroid was inactive, follicles were large, lined by low cuboidal epithelium and filled with pale staining colloid.

The hypophysis was studied by means of serial sections in many instances. A good differentiation between granular cells was obtained with Mann's methylene blue and eosin, and contiguous sections were stained with copper hematoxylin to differentiate the cells of the pars intermedia from the basophils of the anterior lobe.

A variety of changes were found in the anterior lobe. Cell counts were not made, but an increase in the number of acidophils was apparent and there was a marked tendency toward grouping of cells with similar staining qualities in different portions of the gland giving the appearance of multiple adenomata. Degranularization of chromophils was a constant feature and was most marked in the vicinity of blood vessels but varied in extent in different sections and in different glands. There were frequently large colloid accumulations in the portion of the lobe adjacent to the pars intermedia. Cells in the vicinity of such accumulations showed degenerative changes and were sometimes necrotic.

The pars intermedia was considerably enlarged in its central portion and the sheath of intermedia surrounding the pars posterior was increased in width. Colloid cysts were numerous and often contained pale eosinophilic concretions resembling the horny pearls of a squamous cell carcinoma. Invasion of the pars posterior with intermedia cells was common, and frequently strands and columns of these cells were found deep in the anterior lobe (Fig. 13). Acidophils were occasionally observed in the intermediate zone, and in one instance a localized area of acidophilic cells resembling an adenoma was found (Fig. 14).

The posterior lobe contained numerous Herring bodies and occasional small dark staining colloid cysts. In instances in which large cysts were found at autopsy, microscopic examination showed them to be limited to the posterior lobe with walls made up of compressed nervous tissue.

The pars tuberalis was hypertrophied and its blood vessels were packed with colloid material to the exclusion of blood cells.

Bacteriological Examination

Blood cultures were made in the terminal stages of the disease in many instances and were uniformly negative. Blood and organ cultures made directly after death have occasionally shown the presence of a few bacteria which were regarded as contaminants or post mortem invaders.

Toxic Factors

The similarity of certain of the pathological changes to those described in poisoning with toxic weeds such as snakeroot (6) suggested the possibility of a contamination in the hay supply of the colony. The hay was, therefore, thoroughly examined, but no toxic weed could be identified. Moreover, a collection of weeds found in the hay was fed exclusively to a number of animals without ill effect.

The water supply of the colony was derived from deep wells and showed no abnormality other than a high mineral content. However, the possibility of the presence of some poison was investigated

by adding scale from the distilling apparatus to the feed of a group of animals. Large amounts of scale were administered in this manner with no resulting illness.

The work on frencing in tobacco plants in the Laboratory of Plant Pathology suggested the possibility of thallium in the water in amounts too small for chemical detection (7), and as the lesions of thallium poisoning are not unlike some of those found in toxemia, a number of animals of the most susceptible group were given small amounts of thallium acetate in their drinking water. Each animal received 3 mg. of thallium acetate daily and the experiment was continued until each had received 50 mg. No ill effects other than a slight loosening of the hair resulted from this treatment and there were no manifestations in any way suggestive of toxemia of pregnancy. Thallium acetate is a cumulative poison and the lethal dose must, therefore, be more than 50 mg. It may be assumed then that had the disease been due to thallium poisoning, the first animal to die would have ingested at least that amount. As each animal drinks approximately 150 cc. of water a day and the first death occurred about 1 month after removal to Princeton, this would mean a concentration of thallium in the water of 0.01 per cent, an easily detectable amount. Tests of the water, however, have all been negative.

DISCUSSION

An accurate and complete interpretation of the problems arising from a study of this disorder is not possible at this stage of the investigation, but many of the findings are suggestive and are worthy of consideration, not only as they affect a distinct disease entity in the rabbit, but also because the disorder apparently bears a relationship to the toxemias of pregnancy in man. The extent of this relationship and evidence pointing to the endogenous origin of the disease in the rabbit will be discussed in subsequent paragraphs, but consideration of etiological factors proper will be deferred to a later paper.

The toxemias of pregnancy in man have been classified on a symptomatic basis and range from slight disturbances to eclampsia, but clear-cut clinical differentiation is not always possible and no characteristic pathological changes distinguish the various types. The disease in the rabbit also presents similar clinical types. The acute

rapidly fatal disorder is analogous to eclampsia, while the less acute non-fatal disturbance is, in many respects, comparable to pre-eclamptic toxemia, and in both species the absence of acidosis and convulsions characterizes the less severe disease. The asymptomatic disorder associated with abortion or desertion and the milder toxemic attacks of short duration may be compared to pernicious vomiting of pregnancy, although the characteristic clinical feature is absent due possibly to the presence of a strong cardiac sphincter in the rabbit. Other asymptomatic cases evidenced only by the finding of healed hepatic (or renal) lesions may correspond to the type designated as presumable toxemia in man.

There is considerable controversy regarding the blood chemical findings in eclampsia; the majority opinion is that the sugar may be normal but is often increased and rarely decreased. The non-protein nitrogen, urea nitrogen and creatinine values are not elevated except in cases associated with kidney damage, acetone bodies are increased, uric acid and inorganic phosphate are elevated, calcium is approximately normal, fats and lipoids are not increased more than in normal pregnancy, total proteins are slightly elevated and the albumin-globulin ratio is decreased. The alteration in pre-eclamptic toxemias is less definite and consists mainly in a slight increase of uric acid and a decrease in the $\frac{UN}{NPN}$ ratio.

These findings are in general agreement with those obtained in the rabbit. The blood sugar values varied between hyperglycemia, hypoglycemia and normal levels in animals of different genetic constitution, but the character of the finding was comparatively constant for animals of a given group. In other respects the alteration, although more marked, was in the same direction as noted in man. In non-fatal cases the changes were less pronounced and evidence of acidosis was not found.

The abnormal values found in a number of animals during apparently normal gestation are of particular interest and may be interpreted as evidence of the occurrence of asymptomatic attacks of the disorder. Similar abnormal values were also obtained in healthy resting animals, and it is not improbable that despite the absence of clinical symptoms, these animals also suffered previous attacks of

toxemia. The persistence of an abnormal blood picture well into the period of clinical recovery has been noted in one instance, and it may be that abnormal values persist for a considerable period of time following even an asymptomatic attack and that their presence does not interfere with ordinary behavior or pregnancy.

It is generally stated that the principal organs affected in eclampsia are the liver, kidneys, heart and brain. The most distinctive hepatic lesion is focal necrosis, which may be peripheral in distribution but is frequently widespread throughout the lobule. In other instances necrotic lesions are absent and areas of hemorrhage or widespread fatty degeneration are found. Renal lesions are for the most part degenerative in character and acute inflammatory changes are rare. The heart often shows fatty degeneration and the brain edema or congestion.

Similar pathological changes are found in fatal toxemia of pregnancy in the rabbit, but in addition there are marked endocrine disturbances consisting of fatty degeneration and necrosis of the adrenals, hypoplasia of the thyroid and hyperplasia of the hypophysis with pronounced alteration of the pars intermedia and its secretory products.

The disorder in the rabbit is thus in many respects analogous with toxemia of pregnancy in man. The clinical and pathological manifestations in the two species are similar, and the only differences in the disease picture are found in the greater extent of lesions in the rabbit; these variations may arise from generic differences.

The clinical manifestations and many of the pathological changes of the disorder are suggestive of an intoxication, but investigation gave no indication of a toxic substance of extraneous origin and there was no evidence that the disease was of contagious or infectious origin. Moreover, the occurrence of typical cases of toxemia in pseudopregnant and post partum animals eliminates the possibility of a toxic factor arising from the products of conception. There is, however, considerable evidence that the disorder is of endogenous origin and is associated with a disturbance of physiological functions concerned in reproductive processes. Evidence bearing on this aspect of the problem will be presented in a subsequent paper.

SUMMARY

The clinical manifestations and pathology of a disorder associated with pregnancy in the rabbit have been described. The disorder bears a close analogy to toxemia of pregnancy in man and offers an experimental approach to the problems associated with that condition. The evidence at hand indicates that the disorder is of endogenous origin and arises from a disturbance of functions concerned in reproductive processes.

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EXPLANATION OF PLATES

PLATE 24

FIG. 1. Section of liver showing marked fatty changes. The fat droplets tend to be of large size toward the center of the lobule and of small size toward the periphery. Hematoxylin and eosin. $\times 66$.

FIG. 2. Section of liver also showing marked fatty changes but with all fat droplets of small size. Hematoxylin and eosin. $\times 66$.

FIG. 3. Section of liver showing thrombosis of a large vessel and a large wedge-shaped area of necrosis. Hematoxylin and eosin. $\times 66$.

FIG. 4. Section of liver showing fatty changes in the periphery of the lobule with necrosis in the mid-zonal and central areas. Hematoxylin and eosin. $\times 92$.

FIG. 5. Section of liver showing focal areas of necrosis in the peripheral and mid-zonal areas of the lobule. Hematoxylin and eosin. $\times 66$.

PLATE 25

FIG. 6. Section of liver showing sharply defined areas of necrosis in the periphery of the lobule with proliferation of fibroblasts into the necrotic areas. Hematoxylin and eosin. $\times 110$.

FIG. 7. Section of liver showing fatty changes with diffuse cirrhosis. This section was taken from a fatal case of toxemia and the animal was known to have had previous attacks of the disorder. Hematoxylin and eosin. $\times 66$.

FIG. 8. Section of kidney showing fatty degeneration of tubular epithelium. Scharlach red. $\times 235$.

FIG. 9. Section of kidney showing degeneration and necrosis of all cortical elements. Hematoxylin and eosin. $\times 110$.

FIG. 10. Section of myocardium showing marked fatty degeneration of muscle fibers. Scharlach red. $\times 235$.

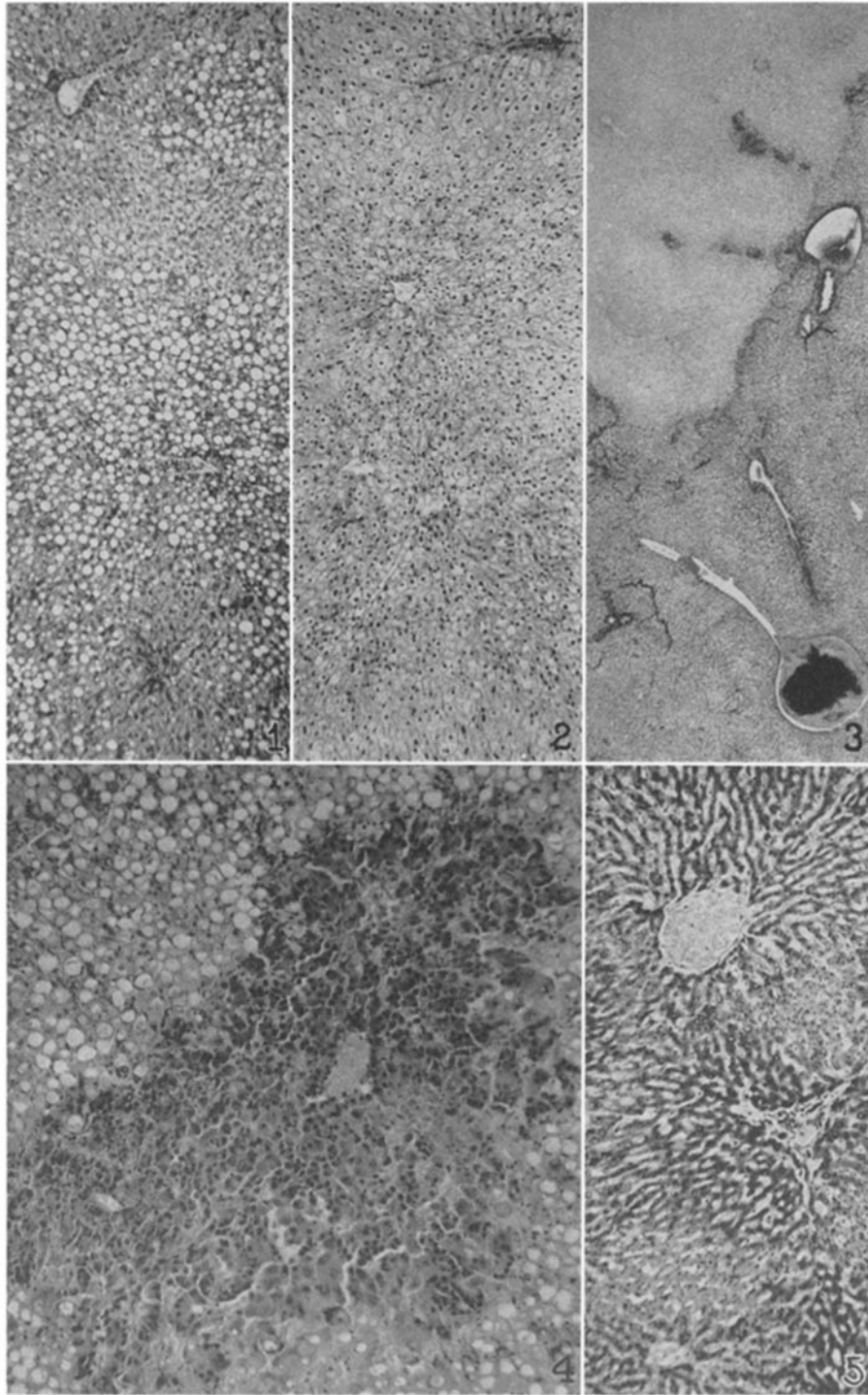
FIG. 11. Section of adrenal showing narrow glomerular zone with an abundance of lipid in the cells of the zona fasciculata. Hematoxylin and eosin. $\times 110$.

PLATE 26

FIG. 12. Section of adrenal showing a fatty necrotic zone separating the cortex from the medulla. Hematoxylin and eosin. $\times 20$.

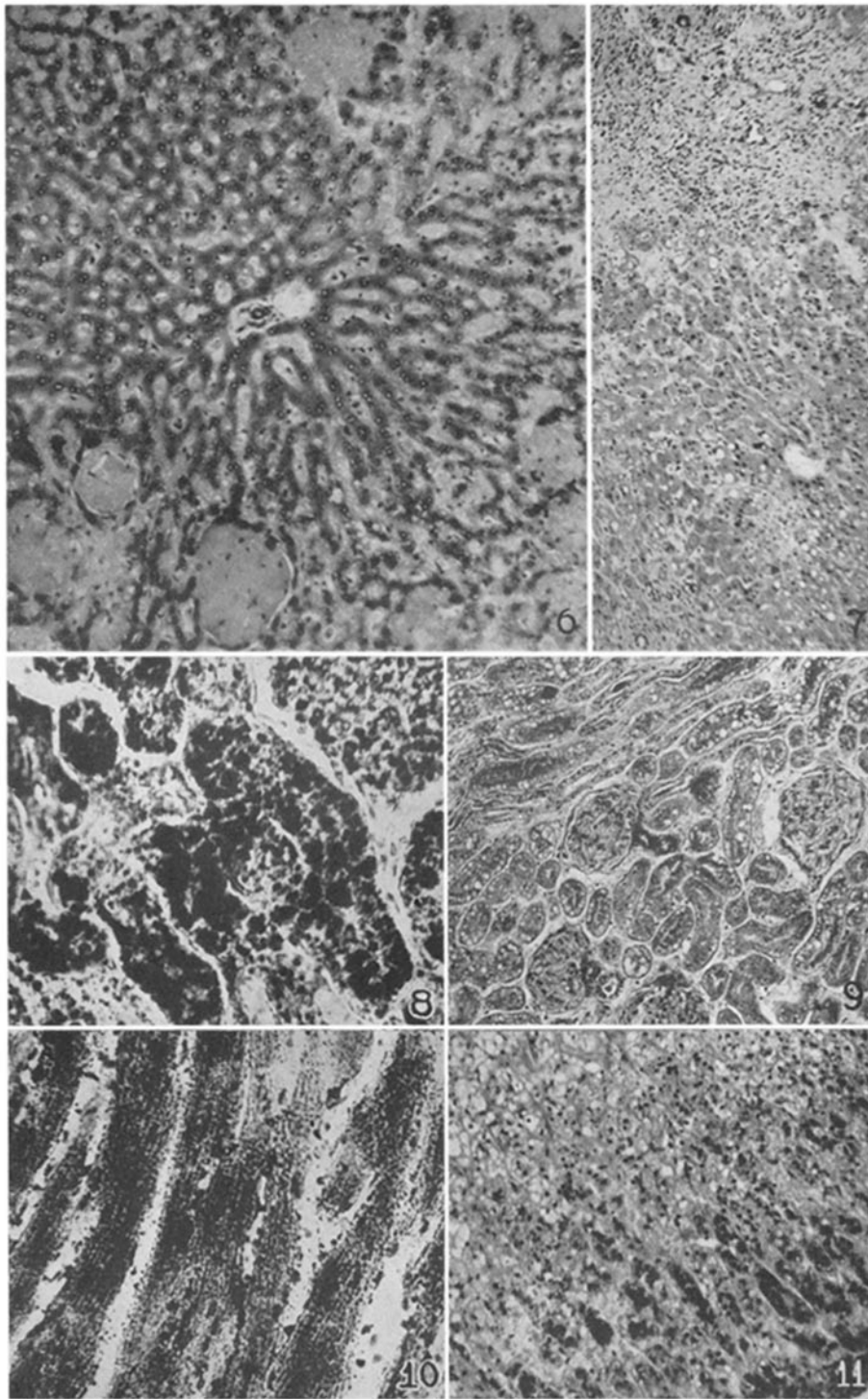
FIG. 13. Section of hypophysis showing invasion of the posterior lobe by cells of the intermediate lobe. Mann's methylene blue and eosin. $\times 41$.

FIG. 14. Section of hypophysis showing a localized area of acidophilic cells resembling an adenoma in the pars intermedia. Mann's methylene blue and eosin. $\times 100$.



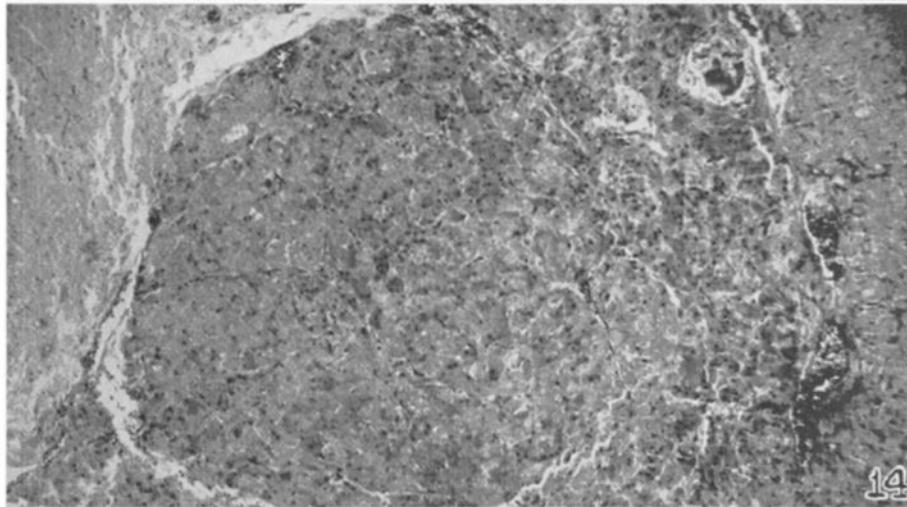
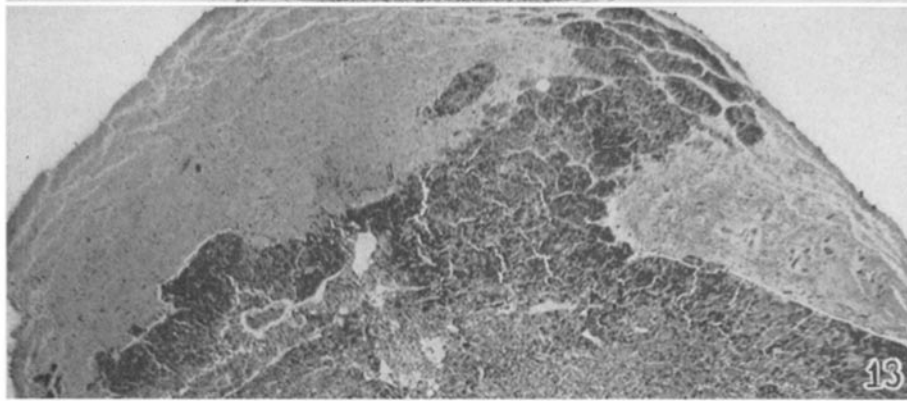
Photographed by J. A. Carlile

(Greene: Toxemia of pregnancy in rabbit. I)



Photographed by J. A. Carlile

(Greene: Toxemia of pregnancy in rabbit. 1)



Photographed by J. A. Carlile

(Greene: Toxemia of pregnancy in rabbit. I)