no atheromata and no reports of finding atheromata in alloxan diabetic rabbits have appeared in the literature.

In discussion we would like to mention one observation concerning the food intake of these animals. It was noted soon after the beginning of the experiment that, contrary to previous experiences with regular diets in which diabetic animals consumed much more food than normal rabbits, these diabetic rabbits did not eat the oily diet nearly so enthusiastically as did the controls. A greater amount of cholesterol had to be added per unit weight of ration to insure that each rabbit received at least 0.3 g daily. Facilities did not permit us to determine the exact daily consumption of each rabbit. The control animals, therefore, may have received somewhat more cholesterol per day than did the diabetic animals, but it is certain that they did not receive more than twice as much. It is our opinion that the difference in intake was not sufficiently great to account for the very great difference in time of onset of atheromata.

Whatever the mechanism, and despite the above mentioned criticisms, we feel that these findings clearly indicate that under the conditions of this experiment alloxan diabetes retards in time and degree the development of cholesterol atheromatosis in the rabbit, and that it represents a conclusive demonstration that hypercholesterolemia of itself does not lead to the formation of atheromata.

Summary. Alloxan diabetic rabbits, when fed a cholesterol enriched diet, developed blood cholesterol levels much higher than did normal rabbits fed the same diet. Atheromata appeared consistently in controls (and also in alloxan injected, nondiabetic rabbits) after 41 days on this diet, but only to a slight degree in one diabetic rabbit fed the same diet for 98 days. There was no qualitative difference in the character of the lesions in the 3 groups.

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Studies on the Pathogenesis of Experimental Necrotizing Arteritis.* (17336)

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Previous studies from this laboratory[1-3] have shown that acute necrotizing arteritis can be produced with regularity in dogs by feeding a specified high fat diet for a period of 8 weeks or longer then sacrificing them through renal damage. Three factors, time, diet and renal damage, seem to be essential in the production of these lesions.

Methods have been published in detail previously.[2] Briefly these consist of feeding healthy adult dogs a high fat diet for a period of 8 weeks or longer, then sacrificing them through renal damage. The active "dietary factor" is found in cod liver oil but is apparently not unique to cod liver oil. The oil may be added to a kennel diet of unselected table scraps or to a "standard diet", consisting of calves liver (raw wet weight), 32 parts; cane sugar, 25 parts; corn starch, 25 parts; butter, 12 parts and cod liver oil, 6 parts, with equal results. Renal damage has been produced by any one of 3 ways: 1) uranium nitrate subcutaneously; 2) mercuric chloride intravenously; and 3) bilateral nephrectomy; all equally effective.

In all experiments to date, renal damage has been essential. To determine whether or not damage to tissues and organs other than the kidney would precipitate the arterial le-

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1 Holman, R. L., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 307.

2 Holman, R. L., and Swanton, M. C., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 87.

3 Holman, R. L., *So. Med. J.*, 1949, **42**, 108.

TABLE I.
Studies on the Pathogenesis of Experimental Necrotizing Arteritis.

Group	Dog No.	Period on standard diet before injury, wks	Type of injury	Total dose	Survival interval, days	Terminal NPN	Arterial lesions
Group A	47-41	10	Chloroform anesthesia	28 hr	20	50	0
Chloroform	47-46	9	"	10 hr	6	68	0
Injury	47-50	9	"	13 hr	9	50	0
to	47-74	13	"	12 hr	7	5*	0
liver							
Group B	47-73	12	Turpentine	32 cc	18	37*	0
Suppuration	47-64	13	Peritonitis		21	12*	0
	48-94	14	Cellulitis		3	58*	0
Group C	49-34	9	Bilateral ureteral ligature		6	255*†	0
Bilateral ureteral ligature	49-35	9	"		6	168*‡	0

* Blood urea nitrogen instead of non-protein nitrogen.
† Terminal blood serum phosphorus 21 mg per 100 ml.
‡ Terminal blood serum phosphorus 13 mg per 100 ml and terminal blood carbon dioxide combining power 38 volumes %.

sions in properly fed dogs, the following two series of experiments were undertaken:

1) *Chloroform injury to the liver.* Four properly fed dogs (Table I, Group A) were subjected to light chloroform anesthesia for periods totalling 10-28 hours in 6-20 days. Marked liver injury was indicated by the appearance of jaundice and was confirmed by histological study of the liver. None of these 4 dogs showed gross or microscopic lesions in the arterial system.

2) Suppuration (Table I, Group B). One dog (47-13) was given repeated subcutaneous injections of turpentine for a total dose of 32 cc in 18 days. Two of the sterile abscesses broke down and each discharged about 100 cc of sanguinopurulent material. The total white blood cell count rose progressively to 45,500. At the time of autopsy there were 12 subcutaneous abscesses estimated to contain a total of 400 cc of similar purulent material but no arterial lesions.

The experimental induction of intestinal obstruction was attempted in two other dogs but neither operation accomplished its intended purpose. Instead, in one dog (47-64) the ligature (umbilical tape about 1 foot above the ileocecal valve) cut through the bowel and the dog died with generalized peritonitis on the 21st day. In the second attempt (Dog 48-94) the small intestine about 2 feet below the ligament of Treitz, was divided between ligatures and the closed proximal end was sutured into the subcutaneum of the abdominal wall. This proximal stump "blew out" and the dog died on the 3rd day with massive fecal contamination of the subcutaneum (associated with cellulitis and fat necrosis) that extended from the hypogastrium to below the inguinal ligament. Despite the massive necrosis and suppuration no arterial lesions were found in either of these two dogs.

Since severe renal damage has been a prerequisite for the arterial lesions the following experiments were undertaken in an attempt to define more accurately what phase or part of "renal insufficiency" was responsible for the subsequent anatomical changes in the arterial system.

Bilateral ureteral ligations. Under nembutal anaesthesia both ureters of 2 properly fed dogs (Table I, Group C) were doubly ligated and divided between ligatures at a level midway between the uretero-pelvic and ureto-vesical junctions. Each dog stopped eating on the 2nd post-operative day and each showed a progressive azotemia, acidosis, phosphatemia, and "uremia". At autopsy on the 6th post-opera-

tive day, each had about 300 cc of clear fluid in the peritoneal cavity; but neither dog had arterial lesions. Histological study of the kidneys showed early hemorrhage and necrosis about the angles of the calyces and early hydronephrosis. The collecting tubules were moderately dilated but the remaining tubules were remarkably well preserved.

Discussion. These experimental observations reaffirm the importance of the kidney in the pathogenesis of arterial lesions. All of our results to date indicate that some derangement of renal function is prerequisite to the development of arterial lesions. Other workers[4-6] have produced arterial lesions with various types of renal insufficiency, but none of these workers has found it necessary to control the diet of his experimental animals. Whereas, in our experience, "standard renal insufficiency" alone resulted in "typical arterial lesions" in only 5 of 130 dogs (4%), the combination of "standard high fat diet" and "standard renal insufficiency" resulted in "typical arterial lesions" in 36 to 40 dogs (90%). This discrepancy between our results incriminating a "dietary factor" and the results of others ignoring a "dietary factor" may, in part, be related to the definition of "typical arterial lesions", for we do not consider some of the hemorrhagic lesions illustrated in previous publications[4,5] "typical" of our experimental lesions which are predominately necrotizing in character and only occasionally associated with gross hemorrhage.

If, as the above results indicate, "standard high fat diet" and "standard renal insufficiency" are *both* necessary for the production of "typical arterial lesions," the problem simmers down to what role the kidney plays in the metabolism of the potentially noxious fatty substance or substances contained in the specified high fat diet. During the 2 months or more of high fat feeding, the kidneys—and especially the epithelial cells lining the loops of Henle along with those of the distal portion of the proximal convoluted tubules—become distended with sudanophilic material. This condition is still compatible with normal life, for the high fat diet can be fed indefinitely and no predictable changes in the vascular system are ever observed unless the kidneys are damaged. Anytime after 2 months of such a diet "standard renal insufficiency" is regularly followed by "typical arterial lesions". It can be surmised that the intact kidney elaborated something necessary for the metabolism of the noxious lipids and that when the kidney is severely damaged the noxious lipids (or metabolic by-products thereof) pile up to "explosive" levels as manifested by the arterial lesions.

The factor common to all the methods used for the production of "standard renal insufficiency" that have resulted in "typical arterial lesions" is massive "damage" to the proximal convoluted tubules in a relatively short period of time (massive coagulative necrosis with uranium nitrate and mercuric chloride and mass exclusion in the case of bilateral nephrectomy). The experiments reported in this paper show that severe damage to tissues and organs other than the kidneys does not precipitate arterial lesions in properly fed dogs and that bilateral ureteral ligation—despite degrees of azotemia, phosphatemia, acidosis, and "uremia" corresponding to those produced by "standard renal insufficiency"—is likewise ineffective in precipitating arterial lesions in properly fed dogs.

The simplest explanations that we have been able to formulate for these unanticipated findings are: (1) the proximal convoluted tubules elaborate something (lipase?) necessary for the proper utilization of certain lipid substances or (2) the integrity of the proximal convoluted tubules is necessary for the neutralization of certain toxic substances (amines?). These hypotheses are based in large part upon the fact that six days after bilateral ureteral ligation the epithelium lining the proximal convoluted tubules is fairly well preserved whereas six days after "standard renal insufficiency" this epithelium appears to

[4] Wintenitz, M. C., Myon, E., Walters, L. L., and Katzenstein, R., *Yale J. Biol. and Med.*, 1940, **12**, 623.

[5] Wintenitz, M. C., and Katzenstein, R., *Yale J. Biol. and Med.*, 1940, **13**, 15.

[6] Goldblatt, H., *Harvey Lectures*, 1937-38, **33**, 237.

be almost completely destroyed. If these experimental findings are confirmed and if our reasoning is valid, the identification of these hypothetical substances (lipases ?, amines ?) might help clarify the time-honored relation of the kidney to arterial lesions.

Summary. Previous studies have shown that the combination of "standard high fat diet" plus "standard renal damage" resulted in typical arterial lesions in 36 of 40 dogs (90%). The data presented in this paper indicate: (1) "Standard high fat diet" plus damage to organs and tissues other than the kidney (chloroform injury to the liver, turpentine abscesses of subcutaneum, and bacterial infections) failed to produce arterial lesions and (2) "Standard high fat diet" plus renal insufficiency produced by bilateral ureteral ligation also failed to produce arterial lesions. These studies re-emphasize the importance of the kidney in the pathogenesis of necrotizing arteritis and indicate that azotemia *per se* is not the "renal factor". They suggest the possibility that the epithelium of the proximal convoluted tubules elaborates one or more substances necessary for the proper utilization of certain lipid substances.

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Diurnal Variations in the Mating Behavior of Male Rats.* (17337)

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Female albino rats usually come into estrus and display their most active mating behavior during the night;[1] and animals maintained under artificial illumination with a reversed light-dark cycle become receptive during the solar day.[2] The present investigation tested the hypothesis that a comparable rhythm of sexual responsiveness occurs in males of the same species.

Methods. Twenty-seven adult, virgin males of the Sprague-Dawley strain were divided into 2 groups that were kept in different halves of a windowless room which was bisected by a black curtain. From 11 P.M. to 11 A.M. one half of the room was dark and the other was illuminated by 2 100 watt bulbs. From 11 A.M. to 11 P.M. the lighting conditions in the two halves of the room were reversed. For approximately 3 weeks before testing began and throughout the remainder of the experiment all animals lived under identical conditions save for the fact that for Group I it was "day" from 11 P.M. to 11 A.M. and for Group II this same period was "night."

The first 2 mating tests were conducted between the hours of 3 P.M. and 7 P.M. The tests occurred, therefore, in the middle of the "night" as far as Group I was concerned, and in the middle of the "day" for Group II. The third and fourth tests were made from 3 A.M. to 7 A.M., thus reversing the temporal placement employed in tests 1 and 2. Mating tests for each male were spaced 3 days apart to allow for complete recovery from effects of sexual exercise. The stimulus animals were spayed, Sprague-Dawley females brought into heat by injection of estrogen and progesterone.† Methods of testing sexual behavior were the same as those employed by the senior author in previous studies.[3] A test lasted for 10 minutes if there was no coital behavior, and for 10 minutes from the time of the first complete or incomplete copulation if either response occurred.

Results. The experimental results are sum-

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1 Ball, J., *Comp. Psychol. Monogr.*, 1937, **14**, 1.

2 Beach, F. A., *J. Comp. Psychol.*, 1938, **26**, 355.

† The hormone preparations were generously supplied by Dr. Edward Henderson of Schering Corporation, Bloomfield, N. J.

3 Beach, F. A., *J. Exp. Zool.*, 1944, **97**, 249.