COMPARATIVE STUDY OF SPONTANEOUS LESIONS IN THE HEARTS AND ARTERIES OF SOME IOWA WILD ANIMALS AND BIRDS

by

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INTRODUCTION

Diseases of the heart and blood vessels account for over half of all human deaths in the United States. For this reason, many researchers have endeavored to determine the exact causes and relationships involved in this disease complex. Experimental studies of atherogenesis in animals are essential to a better understanding of this problem in man.

Atherosclerosis is a general biological phenomenon which can be studied in domestic animals and birds. However, these studies have limitations because of the handicaps of prolonged inbreeding, an unnatural environment, and often specialized use. Investigations on wild animals and birds are needed to shed light on the actual causes of the specific differences in vulnerability to spontaneous atherosclerosis and to enlarge our present knowledge of the various conditions that are atherogenic.

The pheasant was selected as a species to be used to gain experimental information with respect to cardiovascular changes influenced by various diets. The results of these studied could be compared with changes observed in wild pheasants of comparable age. Factors which do not readily lend themselves to experimental control, such as crowding, exercise, social stresses, and diet were considered

collectively under the category of "wildness". Dietary protein was selected as the measurable variable in the experimental groups because of doubts as to the role of this important influence in avian atherogenesis. While the exact diets of the wild pheasants could not be known with precision, considerable inferential information exists relative to the diets consumed by these birds in the wild state.

Two reasons exist for choosing the ring-necked pheasant as an experimental model in this study. The pheasant is assumed to be very closely related genetically to the turkey. Both spontaneous and experimental atherosclerosis have been carefully characterized in the turkey by a number of investigators. Secondly, wild pheasants of an age which could be estimated and captive day old birds were readily available.

A comparative study of spontaneous lesions in the hearts and arteries of some Iowa wild animals and birds would be expected to provide not only information as to the incidence of lesions present and their nature, but might also suggest possible etiologies of cardiovascular lesions and perhaps information as to animal models with potential for further research.

REVIEW OF LITERATURE

It is customary to classify arterial diseases into two broad categories, degenerative and inflammatory (Boyd, 1961). In man, degenerative arterial disease is by far the more important of the two, while in animals its importance is considerably less (Smith and Jones, 1966). Within the group of degenerative arterial diseases, arteriosclerosis is the most important member, and it, in turn, is subclassified into three main forms: (1) atherosclerosis, a patchy lipoidal degeneration of the intima, by far the commonest and most important of the three; (2) medial calcification, commonly called Monckeberg's degeneration; (3) diffuse arteriolar sclerosis, a degenerative thickening of the intima of the smaller visceral arteries which may assume more than one form (Boyd, 1961).

Arterial degenerative changes in man as described by Clarkson (1963) involve not only the aorta, but also the renal, coronary and cerebral arteries. The most common site of aortic predilection is at the site of the bifurcation of the iliac artery and in the distal one-half of the aorta, with plaques rarely occurring in the aortic arch. The atherosclerotic lesion is believed to be progressive ranging from a "fatty streak" to a complicated atherosclerotic plaque with ulceration, calcification, hemorrhage into the

plaque and thrombus formation. The complicated atherosclerotic plaque in the artery may lead to partial or total occlusion of the affected vessel.

Boyd (1958) lists three theories to explain the accumulation of lipids and fibrosis or hyalinization of the intima of arteries undergoing degeneration. The first is the filtration or imbibition theory. According to this theory, lipids penetrate and accumulate in the intima when the blood level of lipid or cholesterol rises beyond a certain level. Second is the encrustation or thrombogenic theory. This involves formation of a film of fibrin on the intima which becomes covered with endothelium. This procedure is repeated and gradually an atherosclerotic plaque is built up. Last is the vascularization theory. Repeated occurrence of small petechial hemorrhages from intimal capillaries followed by organization of the clots results in the formation of fibrous tissue in this theory. The hemorrhage is an abundant source of lipids.

With regard to the heart, there are five main organic diseases as follows: (1) rheumatic heart disease; (2) bacterial endocarditis; (3) coronary artery occlusion; (4) hypertensive heart disease; (5) congenital heart disease (Boyd, 1961). The common inflammatory lesions of the heart (myocarditis, pericarditis, and endocarditis) often present in rheumatic heart disease and bacterial endocarditis, are

described as follows by Boyd (1958): Myocarditis is the presence of inflammatory foci in the myocardium. The inflammatory cells are primarily lymphocytes, plasma cells, and macrophages. Giant cells may be present, often associated with necrosis of muscle fibers. Pericarditis is usually an extension from and evidence of an underlying myocarditis. The microscopic picture of this condition reveals inflammation of a serous membrane. A diffuse exudate may be observed in the subendothelial tissue consisting mainly of lymphocytes and plasma cells, with an occasional polymorphonuclear leukocyte. Endocardial lesions may involve either the valves or the mural endocardium or both. The early microscopic lesions consist primarily of lymphocytes but later fibrin, fibrosis, and collagen formation may be observed along with the presence of mononuclear and polymorphonuclear cells. Coronary artery occlusion may be produced in a variety of ways such as atherosclerotic narrowing of the vessel, hemorrhage into an atheromatous plaque, rupture of a plaque into the lumen, or thrombosis in an already atheromatous artery (Boyd, 1961). Hypertensive heart disease results when the heart must accommodate itself to increased work that may be necessary due to narrowed coronary arteries, high blood pressure, and other conditions (Boyd, 1961). The most striking feature of prolonged hypertensive heart disease is marked hypertrophy of the left ventricle

(Boyd, 1961).

Spontaneous Cardiovascular Lesions in Animals

Most species of homeothermic animals that man has domesticated or confined in captivity may spontaneously develop degenerative lesions of the arteries. A brief review of published information dealing with such lesions follows according to species:

Non-human primates

While mild atheromatous changes are apparently observed in many, if not all species of primates, severe atherosclerosis with necrotic, lipid-filled plaques in the aorta have been reported in only a few species (Lindsay and Chaikoff, 1966). Chalmers and Gresham (1963) observed fatty streaks in 44 per cent of the non-human primates which they examined, and they considered this condition more common in primates than in other mammals. Fatty streaking is the main lesion in non-human primates and more advanced lesions are rare.

Swine

Skold and Getty (1961) studied the vascular system of old pigs which had been the subjects of a gerontological study, and described focal lesions composed of intimal thickenings with some lipid-containing cells in the thoracic and

abdominal aorta, iliac, and coronary arteries. Gresham and Howard (1963), working with pigs killed at about six months of age, found intimal lesions with fragmentation of the internal elastic lamella. They contend that these lesions present in the aorta provide evidence of serious and often irreparable damage to the vessel wall. Luginbuhl and Jones (1965) reported on 34 sows aged 8 to 12 years. The lesions observed were apparent migration and multiplication of smooth muscle cells of the media to the intima. They believed that elastin, reticulin, and collagenous fibers found in the intimal thicknenings were probably the products of altered smooth muscle cells. They also observed the development of fat storing foam cells from smooth muscle cells. Mainquet (1964) compared 50 wild boars and 50 domestic swine comparable in age and sex and found the incidence of coronary atherosclerosis was 8.3 per cent in wild boars compared to 38.5 per cent in the domestic boars. In a more recent study, Skold et al. (1966) examined 50 pigs, 1 to 8-1/2 years of age and found spontaneous atherosclerotic lesions in all of them.

Dog

Lindsay et al. (1952) found a high incidence of arteriosclerosis of the aorta in dogs. Most older dogs had evidence of cardiac disease, including coronary arteriosclerosis and

myocardial infarction or fibrosis. The principal spontaneous lesion was intimal fibrosis. Detweiler et al. (1961) also reported that fibroelastic plaques are common lesions in the aorta of old dogs.

Rabbit

A transitional type of aortic intimal "fatty streaking" occurs in rabbits during the suckling period and regresses after weaning (Clarkson 1963). Nuzum et al. (1930) observed six cases of spontaneous atherosclerosis among 190 old rabbits.

Cat

Lindsay and Chaikoff (1955) reported finding spontaneous atherosclerotic plaques in the abdominal aorta in several cats.

Cattle

Likar and Robinson (1966) examined the aortas of 200 cows and found gross lesions of atherosclerosis in 53.5 per cent of the aortas. A total of 97.2 per cent of the lesions present were in the abdominal aorta. Skold et al. (1967) reported that atherosclerotic plaques were found in the abdominal aortae of several of 30 cattle examined. However, no lesions were observed in the thoracic aortae except for fatty streaks in one animal. Likar et al. (1969) reported the presence of microthrombi in the left coronary arteries of 28 out of 51, 3 to 10-year-old Holstein-Frisien

cows. They observed all known stages of thrombosis from recent surface thrombus formation to complete incorporation of the thrombotic material into the arterial wall.

Zoo animals

Literature contains several reports of cardiovascular lesions in zoo animals. Lindsay et al. (1956) described intimal fibro-elastic plaques in a 47-year-old female elephant. Lesions in the arteries of the camel, buffalo, and yak have been reported in animals from a zoological garden by Vastesaeger and Delcourt (1962). Ratcliffe and Cronin (1958) reported that in necropsies on mammals at the Philadelphia Zoological Garden, the incidence of degenerative arterial lesions in carnivores was 12.5 per cent in raccoons (Procyonidae family), 8.2 per cent in mink, skunks, and badgers (Mustelidae family), 17 per cent in civet cats (Viveridae family), and 14.0 per cent in wolves, foxes, and coyotes (Canidae family). The overall incidence of lesions in the above species located in the same zoological garden prior to 1932 was 3 per cent. The authors suggested from their observations that social pressure, through an imbalance in adrenal secretion, was a major factor in the increased frequency of arteriosclerosis in these animals. Stout and Bohorquez (1969) reported on the examination of the aortas from 86 hoofed mammals dying in the Oklahoma City Zoo. They found lipid deposits in the aortas of 48.6

per cent of the individuals and fibrous or atherosclerotic plaques in 30.2 per cent.

Ratcliffe and Snyder (1962) compared arteriosclerosis in free ranging wood-chucks (Marmota monax) with a similar group in a zoological garden and found similar lesions of arteriosclerosis in both groups. They concluded that climate and food are not involved in the pathogenesis of coronary arteriosclerosis but that social interaction or the social environment is the main factor in the etiology of this disease.

Wild animals

Fox (1933) failed to observe any cases of arterial disease among wild rodents which included squirrels, wild rabbits, and porcupines. Sherwood et al. (1969) reported on necropsies of 90 opossums. Some of these animals were captured in the wild and housed in captivity for various lengths of time, and others were reared from birth in captivity. They reported lesions of myocarditis in 65 per cent of the animals. Beta hemolytic Streptococcus sp. was the most frequently isolated bacterial organism from these lesions. The opossums were found to have Capillaria sp. in the lungs in 47 per cent of the cases. Microlithiasis (small concretions) were found in the lungs of 81 per cent of the animals. Prathap et al. (1966) found fatty streak lesions in the aortas of 96 per cent of 23 non-captive antarctic seals.

McKinney (1970) reported a low incidence of hyaline arteriosclerosis in the splenic arteries of 40 wild herbivores and 17 wild carnivores from East Africa. The incidence of lesions was greater in the carnivores than the herbivores.

Reptiles

Among reptiles, coronary degenerative lesions are rare (Vastesaeger 1965). Ardlie and Schwartz (1965) examined 148 snakes and lizards and found that in spite of very high cholesterol levels in certain species, no aortic atheroma was seen. They did find a few aortic plaques and saccular aneurysms associated with parasitic invasion of the aortic wall.

Fish

Vastesaeger (1965) found degenerative lesions in the coronary arteries of numerous species of fish. The lesions consisted of focal accumulations of intimal sclerosis with abundant histiocyte-like lipid laden cells. Thacker and Wilber (1967) reported focal intimal proliferations in the ventral aortas of eight of ten species of fish from the Delaware Bay. All of the lesions observed were small, occluding less than 2 per cent of the lumen.

Spontaneous Cardiovascular Lesions in Birds

In birds, the whole series of degenerative vascular lesions seen in man varying from the early sudanophilic streak to the calcified fibrous plaque can be observed (Rigg et al. 1960).

Turkey

Atherosclerotic changes can be demonstrated in most five week old turkeys according to Gresham and Howard (1965). When the turkey is 20 weeks old, there are large lipid deposits in the aortic plaque (Kurtz, 1969). These lesions provide a point of weakness in the aortic wall where rupture and dissection of the aorta may occur (Ball et al. 1965). Turkey aortic alterations occur in both sexes but are more extensive in males according to Kurtz (1969).

Chicken

Atherosclerosis is recognized in the domestic chicken and was first reported in the literature by Dauber (1944). Lindsay and Chaikoff (1950) reported that domestic chickens develop atherosclerosis of the aorta under normal conditions of diet and environment. A comparison of the structural differences between the arteries of domestic chickens and the game cock was made by Steeves and Siegel (1968). They found more elastic fibers in the arterial tunica media in

the game cock which they believed helped prevent aortic rupture. They never observed the latter condition in game cocks, but observed it quite commonly in domestic cocks.

Pigeon

Prichard et al. (1964) described atherosclerosis in the White Carneau pigeon. The White Carneau suffers from a high incidence of atherosclerosis which cannot be related to age, sex, or diet as compared with other breeds of pigeons. Clarkson et al. (1965) found lesions of aortic atherosclerosis in 100 per cent of White Carneau pigeons at four years of age. He also reported a high incidence of coronary artery atherosclerosis and in 5 birds, myocardial infarcts were diagnosed as the cause of death.

Zoo birds

Zoo birds have also been examined for lesions of cardiovascular disease. Fox (1939) found lesions of atherosclerosis in 13.7 per cent of the birds examined at the Philadelphia Zoological Garden. Ratcliffe and Cronin (1958) reported a 20 per cent incidence of degenerative arterial lesions in birds at the same zoo. They believed that the increased incidence of lesions is due to a response of adequately nourished animals to population densities. Uncomplicated atherosclerotic lesions were observed in 21 per cent of the aortas of captive wild birds by Vastesaeger

(1968). Finlayson et al. (1962) observed that atheromas occurred with equal frequency in herbivorous, carnivorous and omnivorous birds in the London Zoological Garden. Ratcliffe et al. (1960) found lesions of atherosclerosis in 8 of 13 pheasants and quail, 6 of 11 hawks, and 13 of 28 ducks, geese, and swans.

Lelek et al. (1963) found that in the rook (crows) spontaneous atherosclerosis is very rare, even though these birds may attain the age of 80 to 100 years and have very high serum lipid values.

Induced Atherosclerosis in Mammals and Birds

Attempts to reproduce atherosclerosis experimentally have involved the use of many different species of animals and birds including rabbits, primates, swine, rats, dogs, chickens, pigeons, and turkeys.

Rabbit

Rabbits were the first animals in which fatty arterial lesions were experimentally induced (Saltykow, 1908). The production of predominantly "foam cell" lesions of the intima by cholesterol feeding has been reported by many workers (Clarkson, 1963). Constantinides et al. (1960) demonstrated that intermittent, rather than continuous, feeding of an atherosclerotic ration results in lesions in

the rabbit more typical of those observed in man.

Non-human primates

Attempts to reproduce atherosclerosis in primates have involved the use of squirrel monkeys, rhesus monkeys, cebus monkeys, baboons, and chimpanzees. These animals, when given various diets containing added vegetable oils or cholesterol, develop elevated serum lipid levels and atherosclerotic lesions (Kritchevsky, 1969).

Swine

Rowsell et al. (1958) compared the effects of egg yolk and butter on the development of atherosclerosis in swine and found that egg yolk produced the highest incidence of aortic atherosclerosis. Advanced lesions of atherosclerosis were produced by Rowsell et al. (1965) in swine fed diets containing either lard, cholesterol, or egg yolk. Greer et al. (1966) reported slightly elevated serum cholesterol levels in pigs fed diets containing 12 per cent protein compared to those receiving an 18 per cent diet. They did not find that protein level significantly affected the incidence of lesions. Baker et al. (1968) reported that serum cholesterol levels in finishing swine decreased as dietary protein increased, but were not affected by sex or by the addition of a diethylstilbestrol-methyltestosterone combination. They found lower serum triglyceride levels in barrows than in gilts. The

diethylstilbestrol and methyltestosterone combination caused a decrease of serum triglycerides in gilts but not in barrows.

Rat

The rat can tolerate high levels of cholesterol. However, Patex et al. (1963) produced coronary atherosclerosis in hypophysectomized rats.

Dog and cat

The dog, domestic cat and other Felidae are not promising subjects for the study of atherosclerosis. Extreme efforts such as thyroidectomy and intensive cholesterol feeding followed by the precautionary administration of I^{131} are needed before the dog will produce even the most meagre of lesions (Jordan et al. 1959). A recent survey of susceptible species did not rate this group of animals very high on the potential list of experimental animals for the production of atherosclerosis (Gresham and Howard, 1966).

Chicken

The cholesterol-fed chicken has received a great deal of attention from those who have studied atherosclerosis. Aortic and coronary atherosclerosis can be induced in cockerels within a short time (5 weeks) by feeding a diet containing cholesterol and fat (Pick and Katz, 1965). The lesions so induced within this time are pure uncomplicated atheromas,

but if the diet is maintained several months severe lesions of atherosclerosis may be observed. Nishida et al. (1958) reported that serum cholesterol and incidence of experimental atherosclerosis in chicks were more dependent on dietary protein than on dietary fat or cholesterol. A 10 per cent protein diet is much more atherogenic than a 20 per cent (or normal) protein diet is for the chicken (Pick and Katz, 1965). Rose and Balloun (1969) reported that serum cholesterol, serum lipids, and liver lipids were markedly increased when cockerels were fed diets containing added cholesterol. Each of these were increased by protein restriction and decreased by energy restriction when diets contained added cholesterol. Without added dietary cholesterol, serum cholesterol was not significantly affected by protein or energy restriction. Aortic atherosclerosis was more prevalent when the diets contained added cholesterol and more severe in the abdominal segment than the thoracic segment of the aorta regardless of dietary treatment. Protein or energy restriction had its greatest effect on atherosclerosis when the diets contained added cholesterol, and also affected the thoracic segment in these instances. Incidence of atherosclerosis in thoracic aorta was reduced in energy-restricted and increased in protein-restricted birds.

Estrogens protect against coronary atherosclerosis and lead to regression of previously induced lesions even in the

presence of a continuing atherogenic diet (Pick and Katz, 1965). However, Katz and Pick (1961) reported that when chickens are fed a high-fat, low-protein, cholesterol rich diet and then treated with estrogen, they develop ulcerated atheromatous lesions which probably represent the first stages of healing. Pick and Katz (1965) reported that oils of different fatty acid composition differ somewhat in their atherogenic action, but are all more atherogenic than solid vegetable fats of similar composition. Solid animal fats, e.g. butter, lard, or chicken fat are as atherogenic as oils. They also found that refined sugars are more atherogenic than complex polysaccharides.

Pigeons

Lofland (1961) reported that White Carneau pigeons fed diets high in protein developed more extensive atherosclerosis than a similar group fed a low protein diet when all birds received 0.25 per cent cholesterol in the ration. Prichard et al. (1962) noted that when cholesterol was not added to the diet of White Carneau pigeons, the dietary protein level did not have any effect on incidence of coronary artery atherosclerosis.

Turkey

Simpson and Harms (1969) induced aortic atherosclerosis in turkeys by feeding 2 per cent cholesterol from 6 weeks of

age to 32 weeks of age. The simplest lesion was characterized by lipid vacuoles in the aortic endothelium, non-membrane bound lipid between cells in the plaque, widening of the extracellular spaces, and fraying of the basement membranes of modified smooth muscle cells. An intermediate lesion contained foam cells and necrotic modified smooth muscle cells widely separated by fibrillar material and pools of lipid which appeared to be in the process of crystallization. The advanced atherosclerotic lesions contained foam cells, many of which were degenerative, and some were binucleated.

Serum Cholesterol and Triglycerides in Cardiovascular Disease

According to White et al. (1968), normal blood plasma in the postabsorbtive state in man contains some 500 mg. of total lipid per 100 ml., of which about one-quarter is triglyceride. There are 180 mg. or more of cholesterol, of which some two-thirds is esterified with fatty acids and one-third is present as free sterol. Phosphatides comprise about 160 mg. per 100 ml.

Serum cholesterol is derived primarily from dietary cholesterol and from synthesis in the liver (Johnson, 1963). The rate of synthesis of cholesterol by the liver is inversely related to the supply of dietary cholesterol thereby acting as a homeostatic mechanism (Johnson, 1963). Cholesterol is the probable parent compound from which progesterone,

testosterone, and the adrenocortical hormones are synthesized. Cholesterol can also be converted into D vitamins (Danielson, 1963). The main pathways for elimination of cholesterol are degradation to bile acids in the liver and excretion of neutral sterols in feces (Danielsson, 1963).

Triglycerides, from a quantitative standpoint, are by far the most important lipid in the diet (Johnson, 1963). Dietary fat is transported from the intestine to the blood mainly as triglycerides in chylomicrons (Malmos et al. 1965). Triglyceride fatty acids of the blood are important for the energy requirements of tissues and for the renewal of triglyceride stores of the body (Robinson, 1963). Triglycerides, which are stored in the fat deposits of the body are readily transferred as needed to the blood as free fatty acids where they are available to tissues (Robinson, 1963).

Plasma phospholipids, similarly to cholesterol, not only originate in the liver but are also removed from the blood and metabolized by the liver (Cantarow and Schepartz, 1962).

Plasma lipoprotein fractions can be separated by repeated high speed centrifugation after addition of salts. The low density fraction contains a proportionally high content of cholesterol and triglyceride, whereas phosphatids are in greater proportion in the high density fractions (White

et al. 1968). The B_1-lipoprotein (low density) portion is often present in plasma in increased amounts in atherosclerosis and the alpha-lipoproteins (high density) are lowered (White et al. 1968). Therefore, although the concentration of cholesterol in plasma may not be strikingly elevated, the ratio of cholesterol to phospholipids (c/p) may be of physiological significance in the development of atherosclerosis (White et al. 1968).

The technique generally used to experimentally produce atherosclerosis is the feeding of large quantities of cholesterol (2 per cent of the diet). This causes lipemia including a high concentration of cholesterol and a mobilization of triglycerides in the plasma (White et al. 1968).

Fine Structure of Abdominal Aortic Atheromas

The ultrastructure of the normal chicken abdominal aorta is described by Moss and Benditt (1970). The intima consists of endothelial cells containing a nucleus, smooth and rough endoplasmic reticulum, pinocytotic vesicles, vacuoles, oval or round mitochondria, Golgi apparatus, and short fibrils. The next layer is the internal elastic lamina which is well defined in the abdominal aorta. The media is divided into an inner muscular zone and an outer elastic zone. The muscular portion comprises the inner two-thirds and the elastic area the outer one-third of the

media. The adventitia containing fibroblasts and closely packed collagenous fibers, merges gradually with the outer elastic media which it surrounds.

Simpson and Harms (1968b) examined abdominal aortas of turkeys with atherosclerotic plaques using electron microscopy. They observed spindle-shaped cells arranged in multilayered rows immediately above and perpendicular to the internal elastic membrane. They believed that these cells were modified smooth muscle cells. They also found cells that appeared to be lipid-bearing modified smooth muscle cells and lipid-laden fibrobalsts. These cells were separated by collagenous and elastic fibers. The endothelial cells contained lipid-like vacuoles. It was concluded that modified smooth muscle cells, a few fibroblasts, occasional leukocytes, extracellular substances, and lipid constitute the ingredients of the atherosclerotic plaque in turkeys.

Simpson and Harms (1968a) studied atherosclerosis in turkeys given diethylstilbestrol and observed that endothelial cells appeared to manifest injury induced by accumulations of metabolites in the vascular wall in the form of nuclear pyknosis, perinuclear vacuolization, the formation of cyst-like protuberances on the luminal surfaces, and vesiculation and lobations of nuclei. They believed that foreign material entered the arterial wall by way of enlarged intercellular boundaries between endothelial cells or through an area devoid

of endothelial cells.

McCombs et al. (1969) reported on the fine structure of atherosclerotic plaques of the aorta in the squirrel monkey. They found loosely-packed smooth muscle cells containing lipid, a normal appearing internal elastic lamina and extracellular lipid in plaques in the abdominal aorta. Smooth muscle cells found in the intima were oriented at right angles to the longitudinally running smooth muscle cells of the underlying media.

Besides these specific references, there are rather extensive reviews of atherosclerosis, including fine structure, such as a study of the development of the aorta in embryos and its role in atherogenesis by Fyfe et al. (1968) and an ultrastructural study of experimental atherosclerotic plaques in rabbits by Parker et al. (1963).

EXPERIMENTAL INVESTIGATIONS

Survey of Spontaneous Cardiovascular Lesions in Iowa Wild Mammals

The purpose of this experiment was to determine the incidence, severity, and nature of spontaneous lesions in the hearts and arteries of some Iowa wild mammals.

Methods of procedure

Animals available for this study consisted of badgers, civet cats, foxes, mink, muskrats, opossums, raccoons, and skunks. These animals were obtained by trapping during the trapping seasons between November 20, 1965 and February 15, 1967. They were obtained from eleven different counties: Audubon, Dickinson, Mahaska, Montgomery, Poweshiek, Sac, Story, Tama, Union, Warren, and Wright. The animals were identified as to sex and an attempt was made to determine if they were old or young by condition, hair coat, and dental wear. Examination of the eye lens using the method described by Lord (1959) and Sanderson (1961) was not used in this study but could be used for more precise age estimates.

The animals were brought to the Veterinary Medical Research Institute for necropsy. Heart, thoracic aorta, abdominal aorta, lung, liver, kidney, and skeletal muscle tissues were collected for histological examination. These tissues were fixed in 10 per cent phosphate buffered formalin solution, dehydrated in ethyl alcohol, cleared in xylene,

and embedded in paraffin. The sections were cut 8 microns in thickness and stained with Harris's hematoxylin and counter stained with eosin. Additional sections were stained using Verhoeff-van Gieson procedure.

Results

The sex, estimated age, location of capture and lesions present in the heart and arteries of each wild animal examined in this study are reported in the Appendix, Tables 7-14. The findings for each species examined is presented as follows:

Badgers The heart and arteries of 6 badgers were examined. A coronary artery in one animal revealed a mild lesion characterized by a small increase in the subintimal matrix and some reorientation of the subintimal cells. Examination of the thoracic and abdominal aortas did not reveal any lesions. However, a low grade perivasculitis was present in two animals. The cells present in these lesions were primarily lymphocytes and in one case the presence of fat necrosis suggested that trauma may have been the cause. In the other case on organism was observed which was believed to be Toxoplasma gondii, on the basis of its morphological appearance.

Civet cats Tissues from a total of seven civet cats were examined and no lesions were found in the arteries.

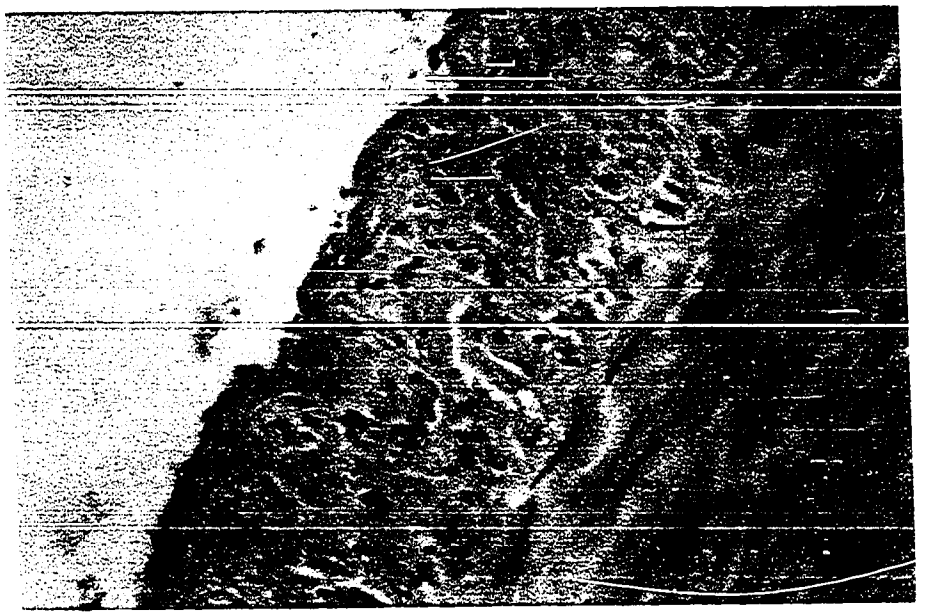
However, mild lesions of myocardial degeneration with focal infiltration of lymphocytes were found in the heart of one animal. The presence of lungworms was observed in one of the seven civet cats.

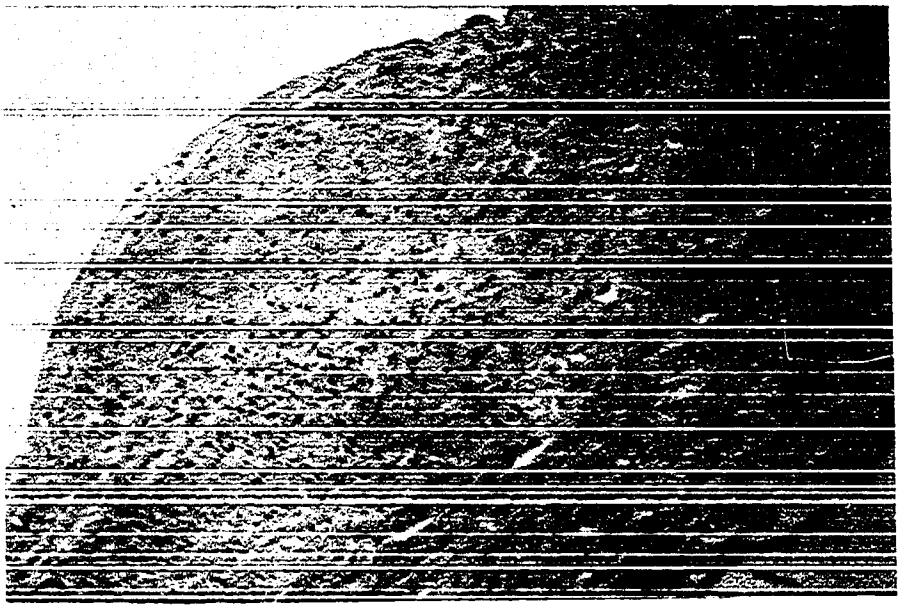
Foxes Tissues from 33 foxes were examined microscopically. Mild lesions of connective tissue infiltration of the intima of the coronary artery were present in one animal. No lesions were found in the thoracic aorta. Microscopic lesions in the abdominal portion of the aorta were found in two old males. The most severe lesion was a plaque involving the intima with reorientation of smooth muscle cells as pictured in Figure 1. The small lesion in the other animal also revealed reorientation of smooth muscle cells in the intimal layer. No abnormalities of the myocardium were found.

Mink Microscopic examination of the arteries from 39 mink did not reveal any lesions of atherosclerosis. Thoracic and abdominal sections of aortae revealed small intimal thickenings consisting primarily of increased amounts of smooth muscle cells and connective tissue cells that were believed to be normal anatomical areas frequently found at the aortic trifurcation. Figure 2 presents one of these raised areas. No lesions were observed in the myocardium except the presence of sarcocysts in one young

Figure 1. Section of the abdominal aorta from an old male fox, No. 206, with a fibrous plaque of the intima. Hematoxylin and eosin stain. X 250

Figure 2. A thickened area of the intima in the thoracic aorta from a young female mink, No. 183, believed to be normal anatomical structure at an aortic trifurcation. Hematoxylin and eosin stain. X 100





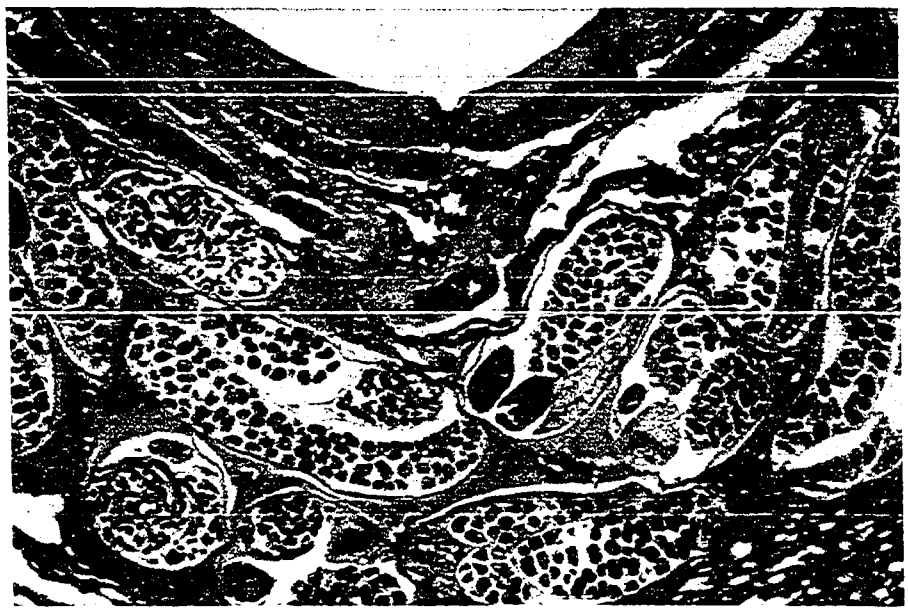
male. Five of the animals revealed the presence of lungworms on microscopic examination. Figure 3 reveals a foci of lungworms surrounding a bronchus. Microscopic examination of sections of skeletal muscle revealed Sarcocystis sp. in 10 animals (Figure 4). A nematode was observed in the skeletal muscle in one mink, presumably Trichinella spiralis, because of its morphological characteristics.

Muskrats Tissues from a total of 17 muskrats were examined histologically. Examination of the coronary arteries did not reveal any lesions. Two male animals revealed typical atherosclerotic plaques in the thoracic aorta which involved the intima. No lesions were found in the abdominal portion of the aorta. Examination of the myocardium did not reveal any lesions. Sarcocysts were found in the skeletal muscle of one of the muskrats examined.

Opossums Tissues from 72 opossums were examined microscopically. Thickening of the intima due to reorientation of smooth muscle cells was observed in the coronary artery of one opossum. The thoracic aorta of one old male contained changes in the media consisting of smooth muscle cell proliferation and mild scarring. This animal also had lesions of periarteritis with infiltration of neutrophils and lymphocytes. Lesions of periarteritis were also present

Figure 3. Section of the lung from a young female mink, No. 151, with a foci of lungworms adjacent to a bronchus. Hematoxylin and eosin stain. X 40

Figure 4. *Sarcocystis sp.* in skeletal muscle of a young male mink, No. 185. Hematoxylin and eosin stain. X 250



in two old females and one other old male. Microscopic examination of the abdominal portion of the aorta did not reveal lesions in any of the oppossums. Areas of degeneration and cellular infiltration were found in the myocardium of 16 opossums. Figures 5, 6, and 7 are examples of the lesions found in the myocardium, pericardium, and endocardium. The cellular infiltrations were largely mononuclear cells, but some polymorphonuclear cells were also present. Table 1 lists the relationship of sex and age to the incidence of myocarditis found in opossums.

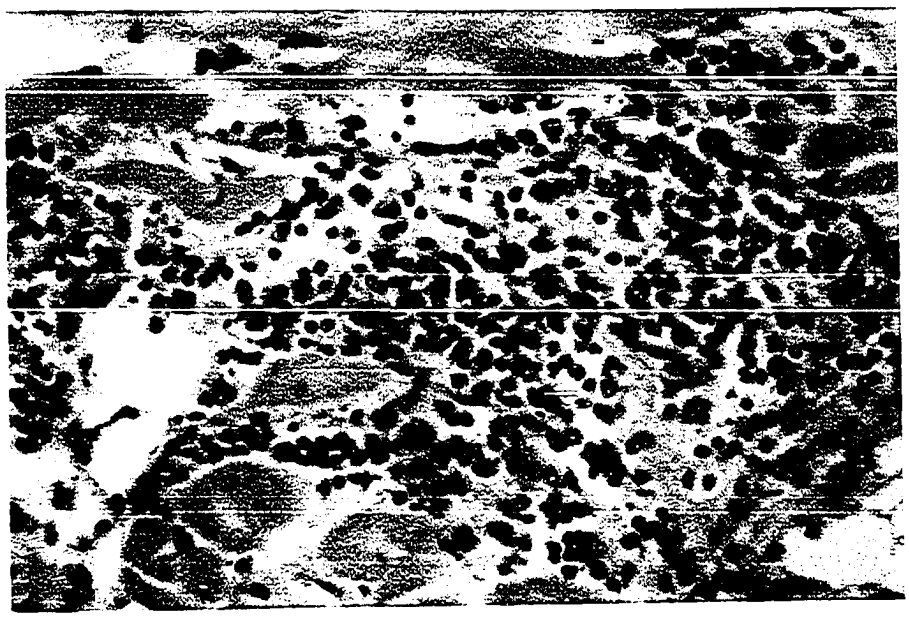
Table 1. Relationship of sex and age to incidence of myocarditis in opossums

Sex	Age	Total	Positive	% incidence
Female	Young	8	1	12.5%
Female	Old	18	6	33.3%
Male	Young	17	2	11.8%
Male	Old	28	7	25.0%

The lungs of the opossums contained several lesions. The small pulmonary arteries (Figure 8) were often greatly thickened. This thickening of the media was observed in over half of the lungs examined. Lesions of microlithiasis were found in 9 animals. Figures 9, 10 and 11, reveal possible stages from early microlithiasis to the final calcified lesion.

Figure 5. Section of the heart from an old male opossum, No. 254, with a chronic focal suppurative myocarditis. Hematoxylin and eosin stain. X 250

Figure 6. Chronic diffuse pericarditis in an old female opossum, No. 67. Hematoxylin and eosin stain. X 40



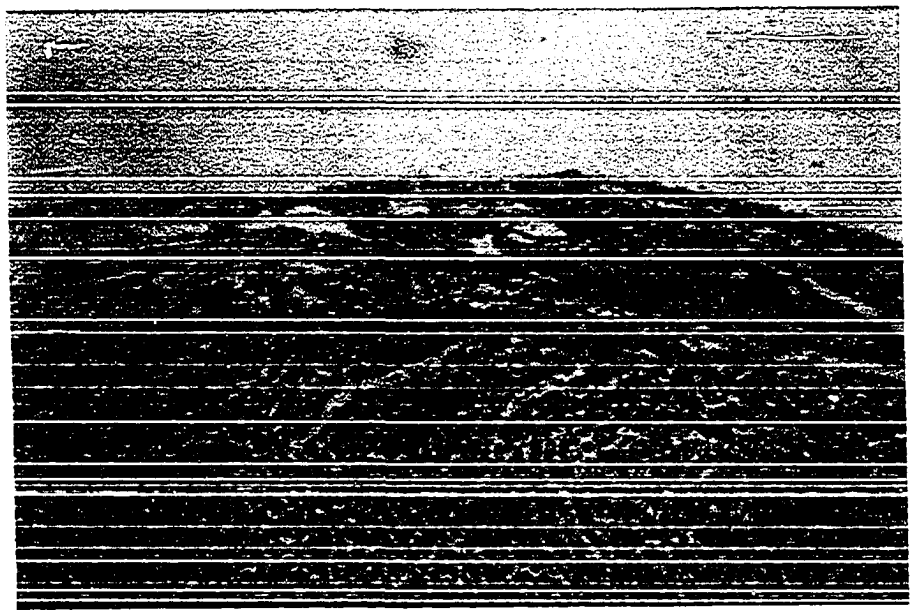


Figure 7. Focal suppurative endocarditis in an old male opossum, No. 254. Hematoxylin and eosin stain. X 40

Figure 8. Section of the lung from a young male opossum, No. 148, with a greatly thickened media in a small pulmonary artery. Hematoxylin and eosin stain. X 100

Figure 9. A possible early developmental stage of a microlith in the lung of an old female opossum, No. 83. Hematoxylin and eosin stain. X 100

Figure 10. Typical microlith in the lung of an old male opossum, No. 251. Hematoxylin and eosin stain. X 100

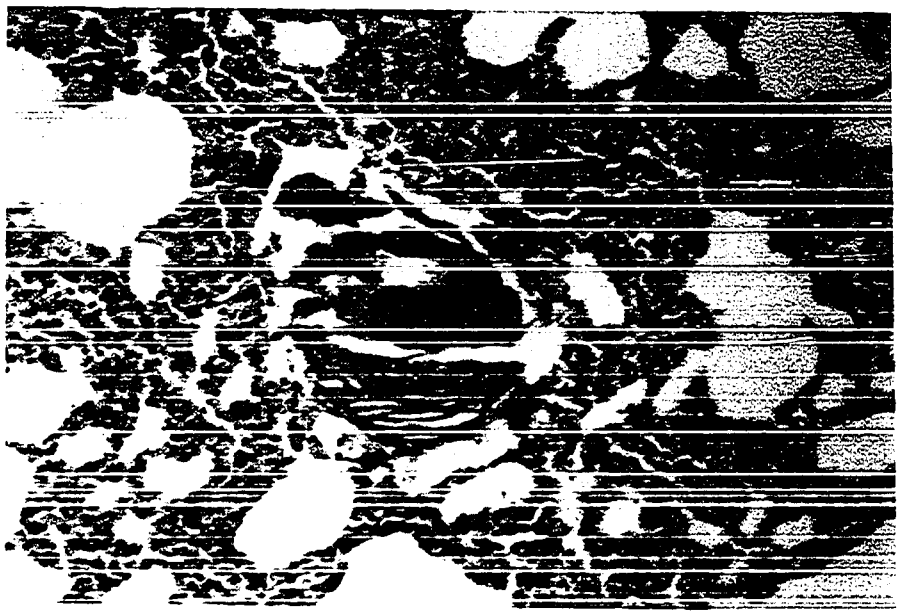
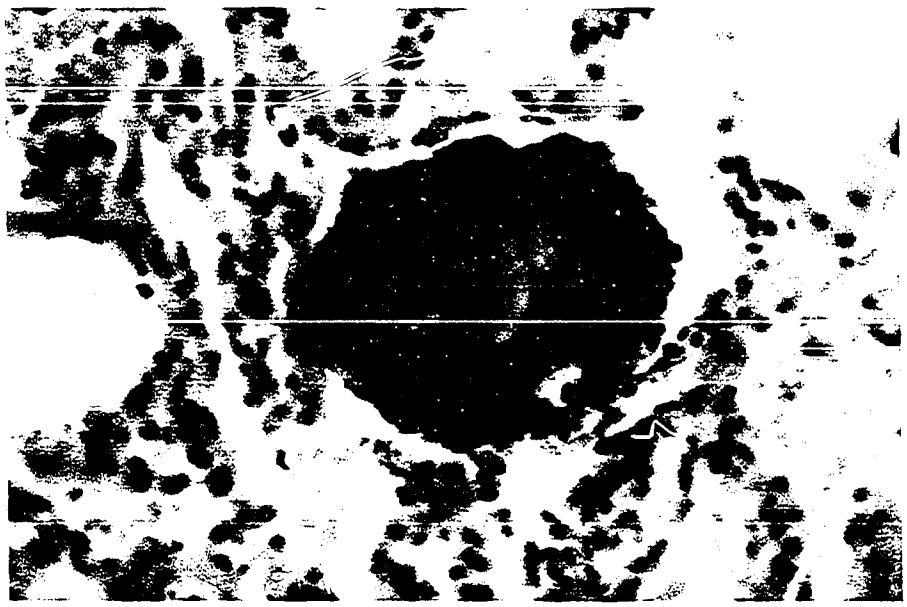
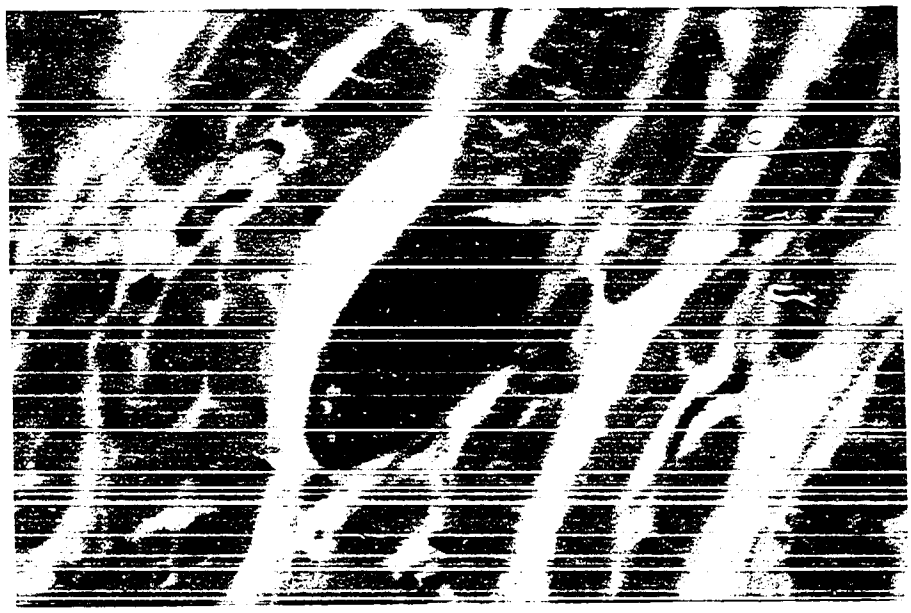


Figure 11. Section of the lung from an old male opossum, No. 251, with a typical advanced stage microlith. Hematoxylin and eosin stain. X 250

Figure 12. *Sarcocystis* sp. in the myocardium of an old female raccoon, No. 19. Hematoxylin and eosin stain. X 400





Raccoons The tissues from 112 raccoons were examined microscopically. The coronary arteries were not found to contain any lesions. Lesions were observed in the thoracic portion of the aorta in one old male raccoon. These were severe fibrosis of the media. No lesions were found in the abdominal portion of the aorta. A very severe myocarditis was present in one young male raccoon. Many neutrophils and mononuclear cells were present in the myocardium. Sarcocysts were observed in the muscle of the heart in 12 of the raccoons. A typical sarcocyst is illustrated in Figure 12. Table 2 indicates the relationship of sex and age to the incidence of raccoons having sarcocysts in their heart muscle. Sarcocysts were also found in the skeletal muscle of 23 of the raccoons.

Table 2. Relationship of sex and age to incidence of *Sarcocystis sp.* in the cardiac muscle of raccoons

Sex	Age	Total	Positive	% incidence
Female	Young	30	5	16.7%
Female	Old	24	4	16.7%
Male	Young	27	2	7.4%
Male	Old	25	1	4.0%

Skunks A total of 46 skunks were examined in this study. Lesions in the coronary arteries were observed in 2 animals and these were primarily arteritis and periarteritis with many plasma cells (Figure 14). An atherosclerotic plaque present in the thoracic aorta of an old female skunk is noted in Figure 15. Examination of sections of the abdominal portion of the aorta did not reveal any lesions. Lesions of a severe periarteritis were present in 3 skunks. Focal myocarditis and pericarditis was observed in 6 skunks and plasma cells were present in high proportions in these lesions. Sarcocysts were found in the skeletal muscles of 8 of the skunks and nematodes, probably Filaroides sp. (Figure 16), were present in the lungs of 15 skunks.

Discussion

The results of this survey of lesions in the hearts and arteries of 332 wild animals are summarized in Table 3. Lesions were observed in the arteries of 3.6 per cent of the animals examined. This is considerably below the 8 to 17 per cent reported for similar animals held captive in the Philadelphia Zoological Garden for various periods of time, as reported by Ratcliffe and Cronin (1958). However, prior to 1932, the above authors found vascular lesions in only 3 per cent of the animals in the same zoological garden. They believed that the lower incidence was due to less social pressure present at that time and to a higher incidence of

Figure 13. Section of tissue adjacent to the thoracic aorta of a young female raccoon, No. 7, with what appears to be a protozoan parasite, possibly Besnoitia sp., as evidenced by its size and morphological features. Hematoxylin and eosin stain. X 250

Figure 14. Arteritis and periarteritis in a section of a coronary artery in a young male skunk, No. 271. Hematoxylin and eosin stain. X 40

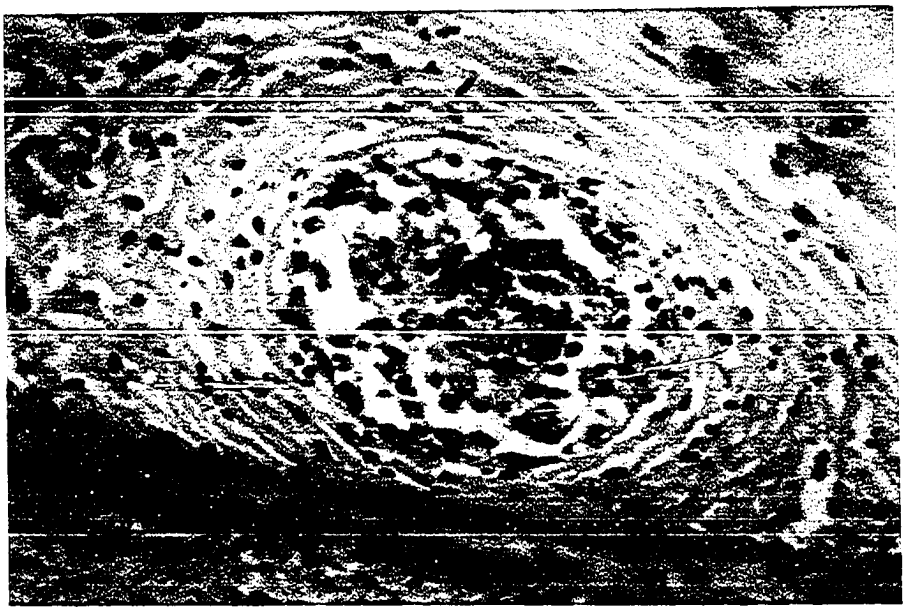


Figure 15. An atherosclerotic plaque in the thoracic aorta of an old female skunk, No. 133. Verhoeff-van Gieson stain. X 100

Figure 16. Section of the lung from a young male skunk, No. 192, with lungworms. Hematoxylin and eosin stain. X 40

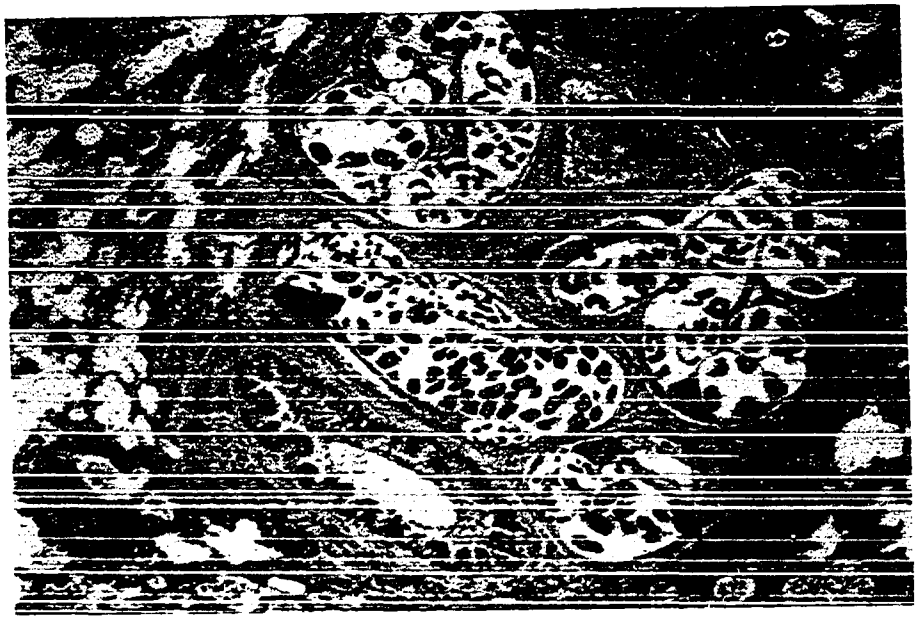


Table 3. Summary of the number of lesions present in the hearts and arteries of some Iowa wild mammals

	Total examined	Fibrous Plaques			Myocarditis	Sarcocysts in myocardium
		Coronary artery	Thoracic aorta	Abdominal aorta		
Badger	6	1	0	0	1	0
Civet cat	7	0	0	0	1	0
Fox	33	1	0	2	0	0
Mink	39	0	0	0	0	1
Muskrat	17	0	2	0	0	0
Opossum	72	1	1	0	16	0
Raccoon	112	0	1	0	1	12
Skunk	46	2	1	0	6	0
Total	332	5	5	2	25	13

conditions such as infectious diseases and malnutrition. Page (1954) postulated that the low incidence of lesions in species such as the dog and cat was due to a relatively low beta-lipoprotein content of the plasma. This might also explain the extremely low incidence of even rudimentary atherosclerosis in the animals examined in this study, most of which were wild carnivores or omnivores. Of those animals in the survey reported with lesions, 92 per cent were males and 90 per cent were old animals suggesting that old males were more likely to have lesions than females or young males.

Lesions of myocarditis were present in 8 per cent of the animals. The incidence of myocarditis was 22 per cent in opossums and 14 per cent in skunks. Sherwood et al. (1969) reported a 38 per cent incidence of myocarditis in opossums. However, many of these animals had been maintained in captivity for several months. The lesions observed in opossums were suggestive of a type caused by bacterial organisms. This finding is consistent with the finding of Sherwood et al. (1969). The lesions in skunks were characterized by the presence of many plasma cells and appeared similar to the condition in mink called Aleutian disease. Myocarditis did not appear to be related to sex, but 72 per cent of the cases were in old animals suggesting that this problem increases with age.

Table 2 indicates the relationship of sex and age to the incidence of sarcocysts in the cardiac muscle of raccoons. This did not reveal a relationship to age but did indicate that a female was about 3 times as likely to be affected as a male. Sarcocysts were observed in the skeletal muscles of mink, muskrats, opossums, raccoons, and skunks.

Lungworms, probably Filaroides sp., were present in lungs of many of the skunks, civet cats, and mink. Microlithiasis was found in 13 per cent of the animals. This incidence was considerably less than the 81 per cent reported by Sherwood et al. (1969). The etiology of these lesions is not known, but histologic examination of possible early developmental stages suggest that they develop in bronchi or blood vessels.

The reason for the thick, muscular media in the small pulmonary arteries of many of the opossums was not determined. Similar arterial lesions are observed in the lungs of cats (Smith and Jones, 1966). In the bovine, Alexander and Jensen (1963) reported that the media of pulmonary veins is composed of a heavy muscular coat and may be confused with pulmonary arteries.

Survey of Spontaneous Cardiovascular Lesions in Iowa Wild Birds

The purpose of this study was to determine the incidence, severity, and nature of lesions in the hearts and arteries of some Iowa wild birds.

Methods of procedure

The hawks, owls, and crows were obtained during the spring of 1965 and the spring of 1966 from Warren County, Iowa. These birds were brought to the Veterinary Medical Research Institute for necropsy.

The wild pheasants were captured alive in Union County by Iowa State Conservation Commission personnel in September, 1969. The procedure used to capture these birds is called "spotlighting". This involves driving a vehicle along fence rows and other places where pheasants are likely to be roosting. The birds are temporarily blinded with a spotlight providing the opportunity to throw a circular net over them.

The approximate age of the birds was determined by the method described by Waehler (1953) where by the feather length ranges of certain primary feathers are used to determine the weekly age of juvenile pheasants. Thirteen of them were sacrificed about 12 hours after their capture and the remaining 12 birds were utilized in the cholesterol feeding experiment described later in this thesis.

Hearts and blood vessels were collected from all birds and placed in 10 per cent buffered formalin. Tissues were prepared for histological examination by methods described in the previous experiment on wild animals. However, in addition to hematoxylin and eosin, Masson's Trichrome and Verhoeff-van Gieson stains, other tissues were cut 10 microns

in thickness using frozen section techniques and stained with oil red O.

Results

The lesions observed in the heart and arteries of each wild bird examined in this study are reported in the Appendix, Tables 15-18. The findings for each species examined are presented as follows:

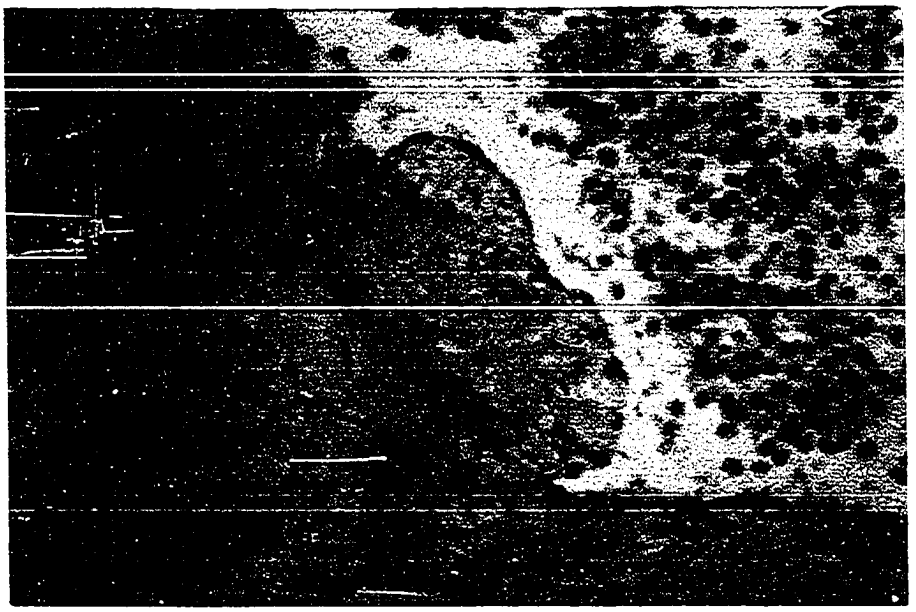
Crows Three crows were examined in this study. A small fibrous plaque of the intima was found in the thoracic aorta of one bird.

Hawks Examination of 8 hawks revealed aortic lesions in 2 of them. Microscopic examination of these plaques indicated that the intima was primarily involved and that the smooth muscle cells have been reoriented and are pushing out into the lumen of the blood vessel (Figure 17).

Owls A histologic examination was made on the hearts and arteries of 29 owls. Small lesions involving the thoracic portion of the aorta were found in 3 birds. Two of these birds and one other also had plaques in the abdominal aorta. These lesions were fibrous proliferative changes of the intima. Oil red O stained sections did not reveal the presence of lipids. A mild myocarditis was found in one owl. Lymphocytes were the primary cell type involved in this lesion.

Figure 17. Abdominal aorta from a hawk, No. 287, with a small fibrous plaque. Hematoxylin and eosin stain. X 250

Figure 18. Section of the thoracic aorta from an approximately 18-week-old, wild-reared rooster pheasant, No. 3289, with an atheromatous plaque. Frozen section, oil red O stain. X 40



Pheasants Thirteen young rooster pheasants were examined. A lesion was present in the thoracic aorta in one bird (Figures 18, 19). Oil red O stains revealed considerable lipid at the edges and base of this plaque. Lesions were found in the abdominal aorta of 3 of the 13 birds examined (Figure 20). These plaques involved primarily the intima, and proliferation and hypertrophy of smooth muscle cells was evident at the base. Sections stained with oil red O did not indicate the presence of excess amounts of lipid. Examination of the hearts did not reveal any pathologic changes.

Discussion

The results of this survey of lesions in the hearts and arteries of 53 Iowa wild birds are summarized in Table 4. Lesions of atherosclerosis were found in 19 per cent of the wild birds examined. This incidence of lesions is similar to the 20 per cent incidence reported by Ratcliffe and Cronin (1958) in birds in the Philadelphia Zoological Garden and a 21 per cent incidence in zoo birds reported by Vastesaeger (1968). The number of wild Iowa birds with atherosclerotic changes was considerably higher than the 3.6 per cent incidence found in wild Iowa mammals. Page (1954) reported the birds have higher plasma beta-lipoprotein levels than animals, and this might cause a higher incidence of atherosclerosis in birds.

Figure 19. Higher magnification of Figure 18. Note red staining lipid at edge and base of lesion. Frozen section, oil red O stain. X 100

Figure 20. Atheroma in the abdominal aorta of an approximately 18-week-old, wild-reared rooster pheasant, No. 3294. Masson's trichrome stain. X 40

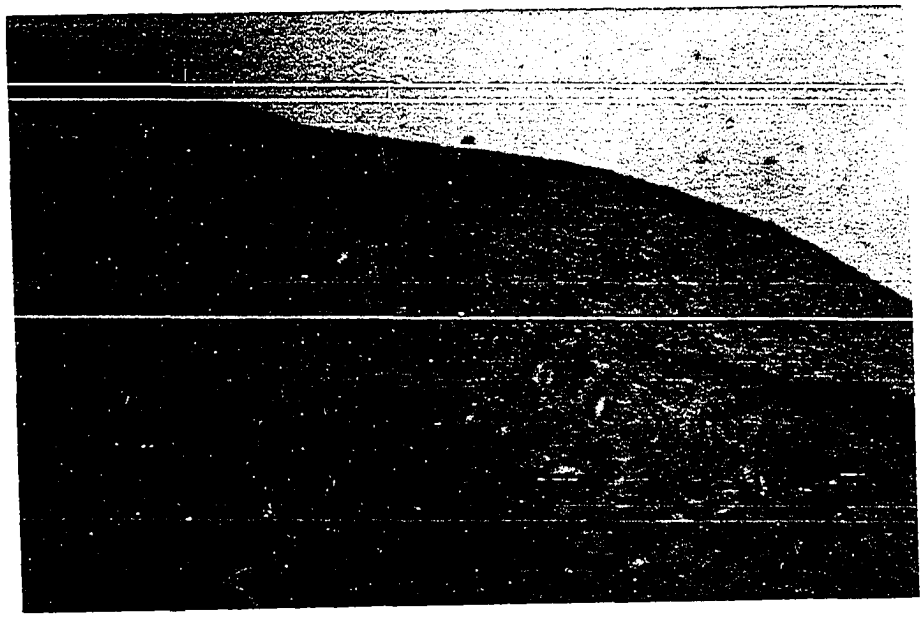


Table 4. Summary of the number of lesions present in the hearts and arteries of some Iowa wild birds

	Total examined	Fibrous plaques			Myocarditis
		Coronary artery	Thoracic aorta	Abdominal aorta	
Crow	3	0	1	0	0
Hawk	8	0	2	0	0
Owl	29	0	3	3	1
Pheasant	13	0	1	5	0
Total	53	0	7	8	1

The lesions found in birds in this study were generally quite small. The lipid content was low and the lesions appeared to be primarily proliferation, reorientation, and degeneration of smooth muscle cells. They correspond to what many authors refer to as fibrous plaques. However, lesions observed in the wild pheasants contained more lipid and were typical atheromas.

Myocardial degeneration changes were not a problem in birds examined in this study and sarcocysts were not observed. Arterial lesions in the lungs were not found.

Effect of Captivity and Dietary Protein on Atherogenesis in the Pheasant

The purpose of this experiment was to determine the influence of captivity and dietary protein on atherogenesis in pheasants.

Methods of procedure

Day old rooster pheasant chicks were obtained May 27, 1969 from the State Game Conservation Commission pheasant hatchery at Boone, Iowa. The breeder flock from which these birds were hatched had been captured the fall before in Union County, Iowa.

The 150 chicks obtained were randomly allotted to 6 brooder batteries. The birds were removed from the batteries when 6 weeks of age and each group placed in a 9' x 12' pen. Two pens received a low protein diet, two an intermediate protein level generally considered optimal for pheasants by commercial companies supplying pheasant rations, and two pens a high protein diet. The different protein levels were produced by varying the amounts of corn and soybean meal. The low protein ration consisted of a 20 per cent protein ration during the first 6 weeks followed by a 16 per cent protein ration to the conclusion of the experiment at 18 weeks of age. The middle protein level group received 28 per cent and 22 per cent protein rations, and the high protein rations were 36 per cent and 30 per cent for

the starter and grower rations respectively.

The difference in dietary net metabolizable energy for each ration are presented in Table 20. Energy levels were slightly higher in the low protein and slightly lower in the high protein rations as compared to the intermediate protein level.

The ration ingredients were purchased at a local elevator and blended as indicated in the Appendix, Table 19. Appendix Table 20 lists the calculated analysis of the rations.

The pheasants were weighed as a group at one day of age and weekly thereafter for 6 weeks. Following this, the birds were weighed every 2 weeks until reaching 18 weeks of age. Feed was provided free choice and feed consumed was determined each time the birds were weighed.

The experiment was terminated when the pheasants were 18 weeks of age. Birds were sacrificed by dislocation of the atlanto-occipital joint. Tissues for histological examination were collected and processed as described previously in this manuscript.

Results

Feed consumption, weight gains, and amount of feed consumed per pound of gain during the duration of this experiment are presented in the Appendix, Tables 21, 22, and 23. Pheasants on the low protein diet grew more slowly and consumed less feed than those on the intermediate and high

protein diets. The roosters on the intermediate protein diet were slightly more efficient in the utilization of their feed than those on the high protein diet. Both of these groups converted their feed to gain better than the low protein group.

The average weight of the roosters on each diet at 18 weeks of age along with the weights of approximately 18-week-old birds captured from the wild can be noted in Figure 21. The birds fed the low protein diet were considerably smaller than the intermediate and high protein fed groups. The birds grown in the wild outgained all of the captive reared birds.

The number of deaths occurring in birds fed low, intermediate, and high protein diets are listed in Table 5. The number of deaths were higher during the first 2 weeks in the intermediate and high protein groups. The birds were moved from brooders to pens at 6 weeks of age, and this appeared to result in several deaths probably from stress of handling and transportation. The birds on the low protein diets did very poorly during the 9th week when several died suddenly without apparent illness. Necropsy and bacteriological culture of tissues did not indicate the presence of known infectious diseases.

Figure 21. Average weight of 18-week-old, captive-reared rooster pheasants on low, intermediate, and high protein diets and of approximately 18-week-old, wild reared rooster pheasants

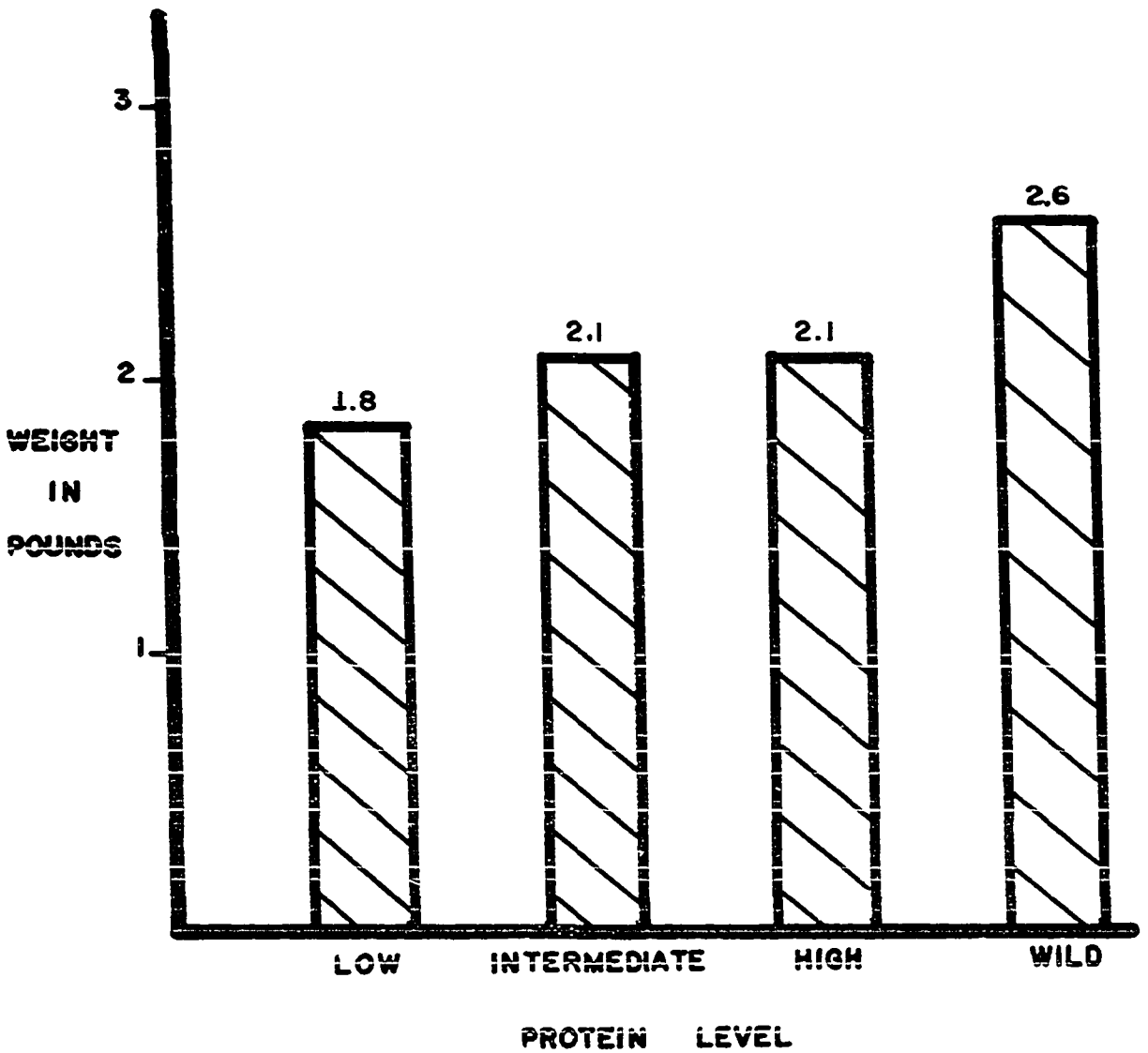

3
2
1
WEIGHT
IN
POUNDS
1.8
2.1
2.1
2.6
LOW
INTERMEDIATE
HIGH
WILD
PROTEIN LEVEL

Table 5. Deaths from hatching to 18 weeks of age in captive reared pheasants on low, intermediate, and high protein diets

Age of birds Week	Low protein[a]		Intermediate protein[b]		High protein[c]	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
1	0	0	2	2	2	2
2	0	0	0	1	1	0
3	1	2	0	0	0	0
4	1	0	0	0	0	0
5	0	0	0	0	1	1
6	1	0	0	1	1	1
7 & 8[d]	2	3	3	1	2	1
9 & 10[e]	6	5	0	1	0	1
11 & 12	2	1	1	2	0	0
13 & 14	0	1	0	0	1	0
15 & 16	0	0	0	0	0	0
17 & 18	0	0	0	0	0	0
Totals	13	12	6	8	8	6

[a]Low protein (20% first 6 weeks, 16% thereafter).

[b]Intermediate protein (28% first 6 weeks, 22% thereafter).

[c]High protein (36% first 6 weeks, 30% thereafter).

[d]Birds moved from brooder to pens, 7 of these dead next day.

[e]Apparent nutritional crises around 9th week for group on low protein levels.

The results of the histologic examination of the hearts and aortas of each bird in the experiment are listed in the Appendix, Tables 24, 25, and 26. The atheromatous lesions were graded according to size as follows: a one-plus lesion was small and occupied a small portion of the intimal wall; a two-plus plaque involved 10 to 25 per cent of the intimal surface examined in that bird; the three-plus lesion involved 25 to 50 per cent of the intimal surface examined, was also thicker and was present in all sections examined for that particular bird; and a four-plus lesion was still larger in size being thicker and involving over half of the artery.

Microscopic examination of sections of the hearts did not reveal any lesions in any of the birds.

Lesions were present in the thoracic portion of the aorta in one or more of the birds in each group. Sections stained with oil red O revealed lipid material at the edge and on the surface of these plaques (Figures 22, 23). Cells at the base of the lesion appeared to be reoriented perpendicular to the arterial wall. These cells were believed to be modified smooth muscle cells.

Intimal plaques (Figures 24, 25, 26, 27) were observed in the abdominal aorta of many of the birds examined in each group. Figure 24 illustrates a plaque that has bulged inward and caused a thinning of the intimal wall. The other plaques bulge out into the lumen of the artery; the latter situation

Figure 22. Atheroma in a section of the thoracic aorta from an 18-week-old pheasant rooster, No. 3874, reared on a low protein diet. Frozen section, oil red O stain. X 40

Figure 23. Higher magnification of Figure 22. Note red staining lipid material on the surface. Frozen section, oil red O stain. X 100

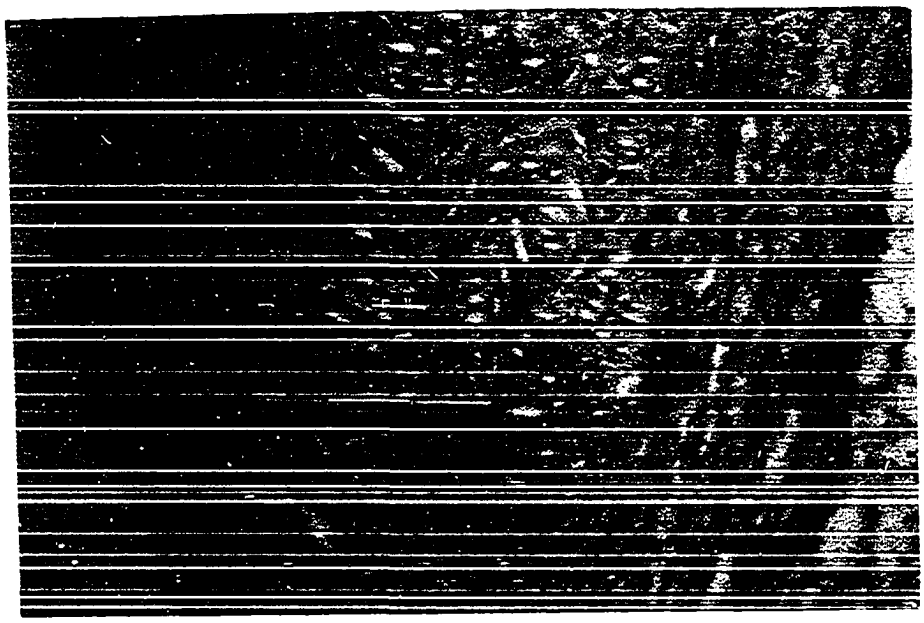
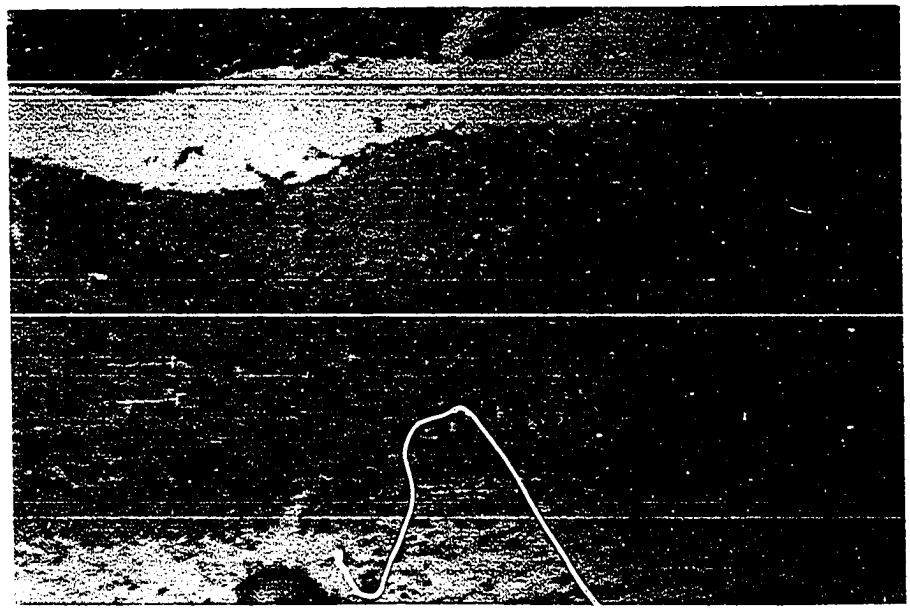


Figure 24. Atheromatous plaque in the abdominal aorta from an 18-week-old rooster pheasant, No. 3608, reared on an intermediate protein diet. Hematoxylin and eosin stain. X 100

Figure 25. Portion of an atheroma in the abdominal aorta of an 18-week-old rooster pheasant, No. 3642, reared on a high protein diet. Toluidine blue stain. X 250



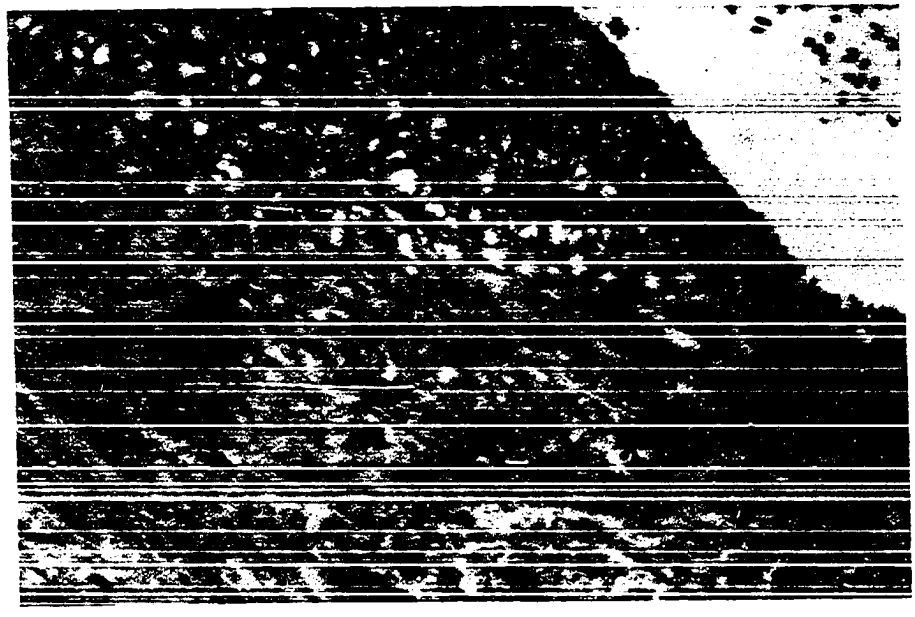
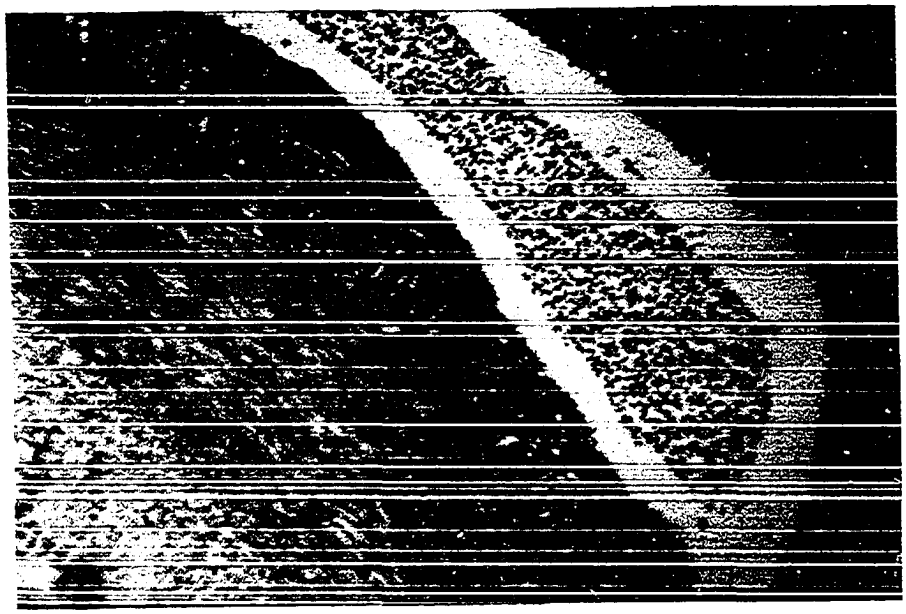


Figure 26. Atheroma in the abdominal aorta from an 18-week-old rooster pheasant. No. 3646, reared on a high protein diet. Verhoeff-van Gieson stain. X 40

Figure 27. An atheroma in the abdominal aorta from an 18-week-old rooster pheasant, No. 3646, reared on a high protein diet. Toluidine blue stain. X 100



is more typical. Fat stains did not reveal the presence of appreciable lipid in these plaques. Generally a reorientation of cells perpendicular to the arterial wall was observed at the base of the lesion (Figure 27). These cells are probably modified smooth muscle cells. A small amount of fibrosis was present in some of the lesions.

Discussion

The rations currently utilized to raise many pheasants in captivity consists of a 28 per cent starter and a 22 per cent grower ration. The results of this study do not indicate any better growth from higher protein rations and if the ration is somewhat lower in protein, a higher death rate and a poorer growth rate can be expected. Therefore, the currently used protein content of rations for pheasants appears to be optimal.

Pheasants raised in captivity in this experiment did not grow as well as birds in the wild. Infectious diseases and parasites were not observed in these birds and were not believed to be factors. The diet consumed by wild young pheasants in the summer is believed to be primarily insects and weed seeds according to Bengtson (1962). However, Severin (1933) reported that the pheasant is essentially a granivorous or seed eating species. He found that during September, seeds of green and yellow foxtail made up the greater portion of the pheasants diet. A few insects

including flies, grasshoppers, crickets, and beetles were also consumed. This diet would be expected to be of relatively high protein content. The intermediate and high protein diets are believed to contain all of the protein and nutrients required for maximum growth and thus should not be involved in poorer growth rates in captive birds. However, stress problems created by confinement and crowding were very likely factors. The birds were quite nervous and would fly around the pen when a person entered the pen or a noise was heard. A peck order was established in each pen as one rooster in each pen was feathered out very nicely while others had various amounts of feathers pecked out.

The number of pheasants with aortic intimal plaques are summarized in Table 6 for 18-week-old birds raised on low, intermediate, and high protein rations and approximately 18-week-old birds reared in the wild. Lesions were observed in 68 per cent of the birds reared on low protein, 78 per cent of the birds on the intermediate protein, and 79 per cent of the birds on the high protein ration. This did not indicate that protein level affected the incidence of atheromas in the pheasant. This finding differed with the results reported by Rose and Balloun (1969) which indicated an increased incidence of atheromatous plaques in protein restricted cockerels.

Table 6. Summary of lesions present in the aortas of captive reared 18-week-old pheasants on low, intermediate, and high protein diets and in approximately 18 week old wild pheasants

Protein level	No. of birds	No. with lesions	Per cent
Low[a]	19	13	68.4
Intermediate[b]	32	25	78.1
High[c]	28	21	78.6
Wild diet[d]	13	3	23.1
Totals	90	62	68.9

[a]Low protein (20% first 6 weeks, 16% thereafter).

[b]Intermediate protein (28% first 6 weeks, 22% thereafter).

[c]High protein (36% first 6 weeks, 30% thereafter).

[d]Expected to consist of primarily weed seeds along with some insects.

Intimal plaques were observed in only 23 per cent of the wild reared pheasants as compared to an overall incidence of 76 per cent in captive-reared birds. We can only speculate on the reason for this. Diseases and parasites were not believed to be factors. Diet in the case of the intermediate and high protein rations was considered to supply all nutrients needed for optimum growth. However, substances could be present in the wild diets such as in the chitin of insects that could inhibit atheroma formation. The stress of confinement and crowding appear to be the most likely reason

for atheroma formation. The presence of a peck order suggests that this might be a factor, but number and severity of lesions were not related to weight of the birds. However, stress is not only placed on the individuals at the bottom of the peck order, but is also present in those attempting to maintain a position at the top. Wild-reared pheasants may also be affected by stresses including weather and predators, most often red-tailed hawks, great horned owls, raccoons, skunks, opossums, badgers, foxes, mink, and weasels (Klonglan, 1962). Ratcliffe and Cronin (1958) believed that an increased incidence of lesions in animals and birds in the Philadelphia Zoological Garden was due to a response of adequately nourished animals to population density. Prolonged exercise by wild pheasants during flight might also cause these birds to have fewer aortic lesions than captive-reared pheasants. However, it is questionable that exercise alone could prevent injury to a vessel wall with subsequent atheroma development.

Induction of Atherogenesis in the Pheasant by Dietary Cholesterol

The purpose of this experiment was to determine if wild 18-week-old pheasants would develop degenerative vascular lesions if placed in captivity and fed a high cholesterol diet for an 8 week period.

Methods of procedure

Procurement of the 12 wild rooster pheasants used in this experiment is described previously in this thesis. These birds were weighed and placed in a 10' x 14' pen. Free access to water was allowed and the ration fed was a 16 per cent protein ration to which 4 per cent by weight of the ration of cholesterol was added and thoroughly mixed. The composition of the ration is listed in the Appendix, Table 19, and the calculated analysis is listed in the Appendix, Table 20. The birds were weighed at the end of 8 weeks and the experiment concluded. The pheasants were sacrificed and tissues collected and processed for histological examination in the manner described previously in this text.

Results

Each pheasant consumed an average of 8.1 pounds of feed with added cholesterol during the 8-week feeding period. During this time, they gained an average of 0.4 lb. each, as listed in the Appendix, Table 27.

The lesions in the aorta of each bird are presented in Table 27 in the Appendix. They were not found in the coronary arteries of any of the birds.

Two birds had lesions in the thoracic portion of the aorta. They were classified as intimal plaques (Figures 28, 29 and 30). The plaques contained considerable amounts of lipid as evidenced by sections stained with oil red O.

Figure 28. Larger atheromatous plaque in a section of the thoracic aorta from a 26-week old rooster pheasant, No. 3286, following 8 weeks treatment of 4 per cent cholesterol added to the diet. Masson's trichrome stain. X 25

Figure 29. Higher magnification of Figure 28. Reorientation of smooth muscle cells perpendicular to the lumen is present in the base of the lesion. Masson's trichrome stain. X 100

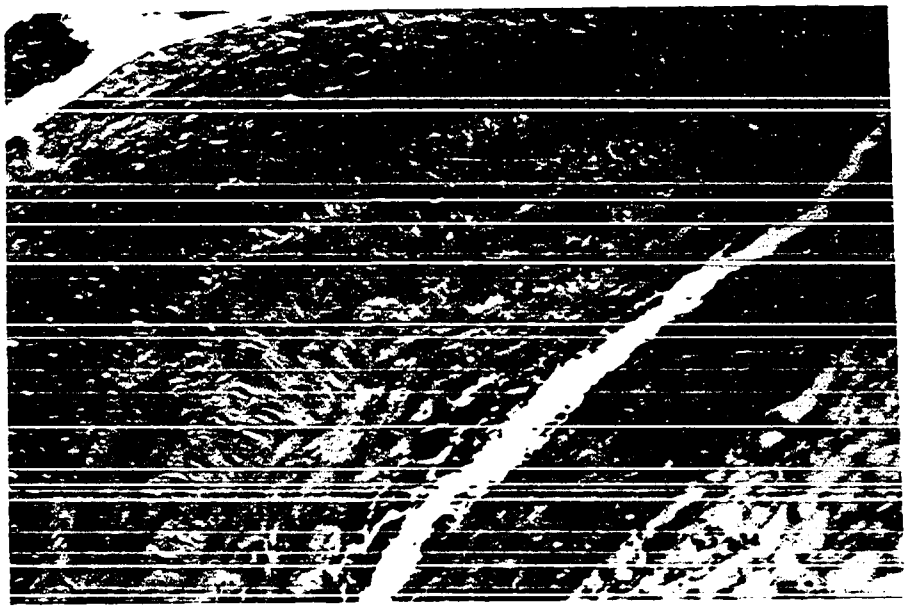


Figure 29 illustrates some evidence of reoriented smooth muscle cells at the base of the plaque.

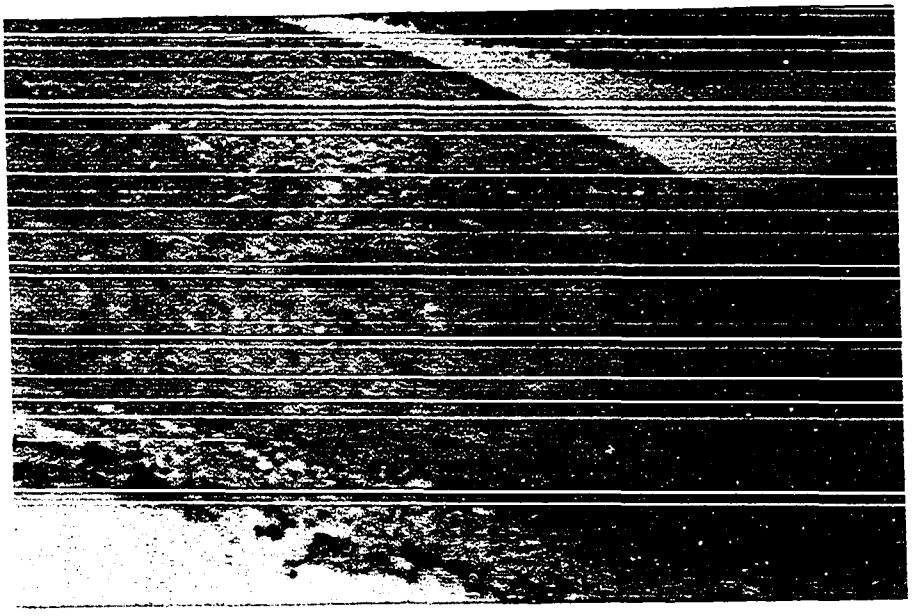
Eleven of the 12 birds examined were found to have lesions in the abdominal portion of the aorta. These intimal plaques contained considerable amounts of lipid material and were either typical fatty streak lesions or atheromas. Examples of these atheromas are illustrated in Figures 31, 32 and 33.

Discussion

Lesions were observed in the aortas of 23 per cent of 18-week-old wild rooster pheasants reported elsewhere in this manuscript. In this experiment after feeding a similar group of birds a diet containing added cholesterol for 8 weeks, the incidence of aortic lesions was 92 per cent. These findings suggest that feeding cholesterol results in increased intimal plaque formation in the aorta of rooster pheasants. The lesions are primarily atheromas and not those of complicated atherosclerosis. These findings are similar to those of Pick and Katz (1965), using cockerels, and Simpson and Harms (1969), using turkeys. The incidence of plaques is much greater in the abdominal portion of the aorta than in the thoracic portion. Similar results were reported by Rose and Balloun (1969) working with cockerels. The atheromas contained considerably more lipid material when cholesterol

Figure 30. An atheroma in the thoracic aorta from a 26-week-old rooster pheasant, No. 3286, following 8 week treatment of 4 per cent cholesterol added to the diet. Hemorrhage is present at the base of the lesion. Hematoxylin and eosin stain. X 25

Figure 31. Typical atheromatous plaque in a section of the abdominal aorta from a 26-week-old rooster pheasant, No. 3283, following 8 week treatment of 4 per cent cholesterol added to the diet. Hematoxylin and eosin stain. X 100



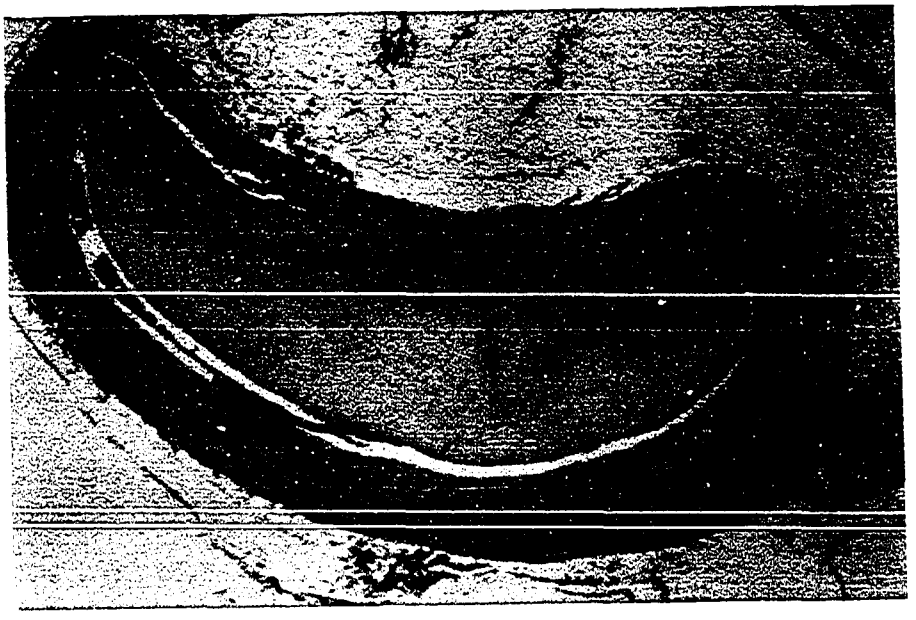
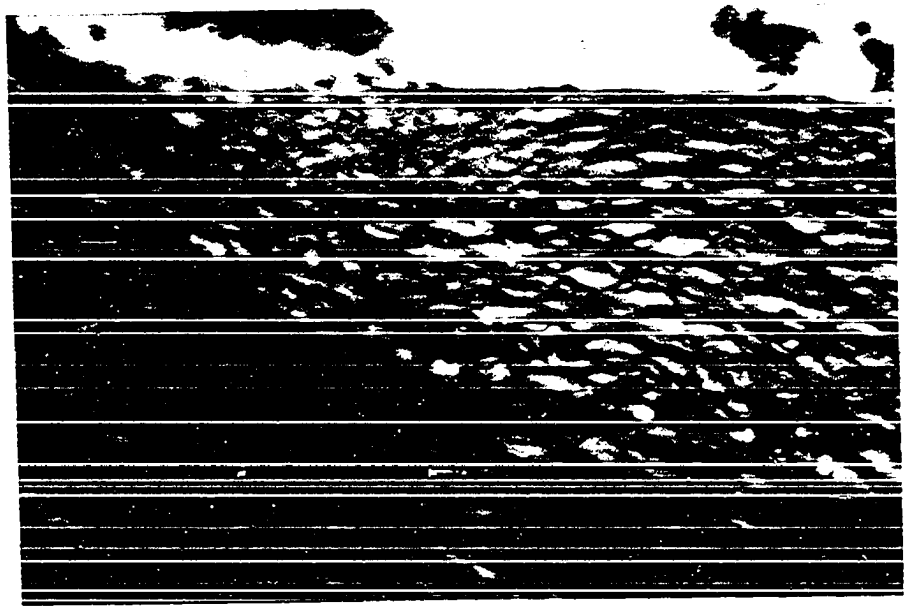


Figure 32. An atheroma in the abdominal aorta of a 26-week-old rooster pheasant, No. 3283, following 8 week treatment of 4 per cent cholesterol added to the diet. Masson's trichrome stain. X 100

Figure 33. Higher magnification of Figure 32. Masson's trichrome stain. X 250



was added to the diet than was observed in atheromas in birds not fed cholesterol.

Serum Cholesterol and Triglyceride Levels in Wild Pheasants and in Pheasants on Experimental Treatments

This experiment was conducted to compare serum cholesterol and triglyceride levels between wild pheasants and captive-reared pheasants and to determine the effect of low, intermediate, and high protein diets and the effect of added dietary cholesterol on these serum lipids.

Methods of procedure

Blood samples were collected from the internal jugular vein following a 20 hour fast. Samples were obtained from all pheasants on low, intermediate, and high protein diets at 12 weeks of age and again at 18 weeks of age. The approximately 18 week old wild-reared pheasants were bled following capture and the birds fed cholesterol were bled prior to the onset and again following the 8 week period of feeding. Blood samples were allowed to clot at 4 C and the serum removed by centrifugation. The serum samples were stored at -20 C in stoppered glass vials.

Total serum cholesterol determinations were carried out using a Technicon Auto Analyzer[1] and as described in the

[1]Technicon Corporation, Tarryton, New York.

Technicon Auto Analyzer Methodology - Method File N-24a (1965). This quantitative procedure for the determination of cholesterol, described by Block et al. (1965), is based on the reaction of concentrated sulfuric acid and ferric chloride in acetic acid with steroids having the 5-ene, 3 beta - 01 grouping.

Total serum triglyceride determinations were also carried out using a Technicon Auto Analyzer and as described by Technicon Auto Analyzer Methodology - Method File N-70 (1968). This procedure is based on methods reported by Kessler and Lederer (1965). The serum sample is extracted with isopropanol in the presence of a slurry containing zerolite, copper lime, and Lloyd reagent. The lipid extract is then sampled into an air-segmented alcoholic potassium hydroxide solution and saponification of triglycerides to glycerol occurs on-steam in a 50 C heating bath. After saponification, periodate reagent is added to the reaction mixture to oxidize the glycerol to formaldehyde. This is followed by condensation with diacetylacetone and ammonia to give a fluorescent product 3, 5-diacetyl-1, 4-dihydro-lutidine. After heating, the reaction mixture enters the fluorometer where air is removed and the fluorescence activated.

The data collected from each experiment were statistically analyzed by variance methods described by Snedecor

(1956).

Results

Individual serum cholesterol and triglyceride determinations for each pheasant rooster included in this study are presented in Appendix, Tables 28-35. Summaries of serum cholesterol and triglyceride levels are illustrated in Figures 34, 35 and 36. The analysis of variance plans and observed mean squares are presented in Appendix, Tables 36-41.

The effect of dietary protein levels on serum cholesterol levels was significant ($P < .01$). Feeding a low protein ration resulted in significantly ($P < .01$) higher serum cholesterol levels than did the diet with an intermediate protein content and feeding an intermediate protein level ration resulted in significantly ($P < .01$) higher serum cholesterol levels than did the high protein content diet. These dietary effects on serum cholesterol levels were observed in the rooster pheasants at both 12 weeks and 18 weeks of age (Figures 34 and 35).

Serum cholesterol levels were not significantly related to either weight of the birds within each group or to the presence of aortic lesions.

The differences in serum cholesterol levels between captive-reared and wild-reared roosters were statistically significant ($P < .01$). Serum cholesterol levels were

Figure 34. Average serum cholesterol and triglyceride levels in 12-week-old captive-reared rooster pheasants on low, intermediate, and high protein diets

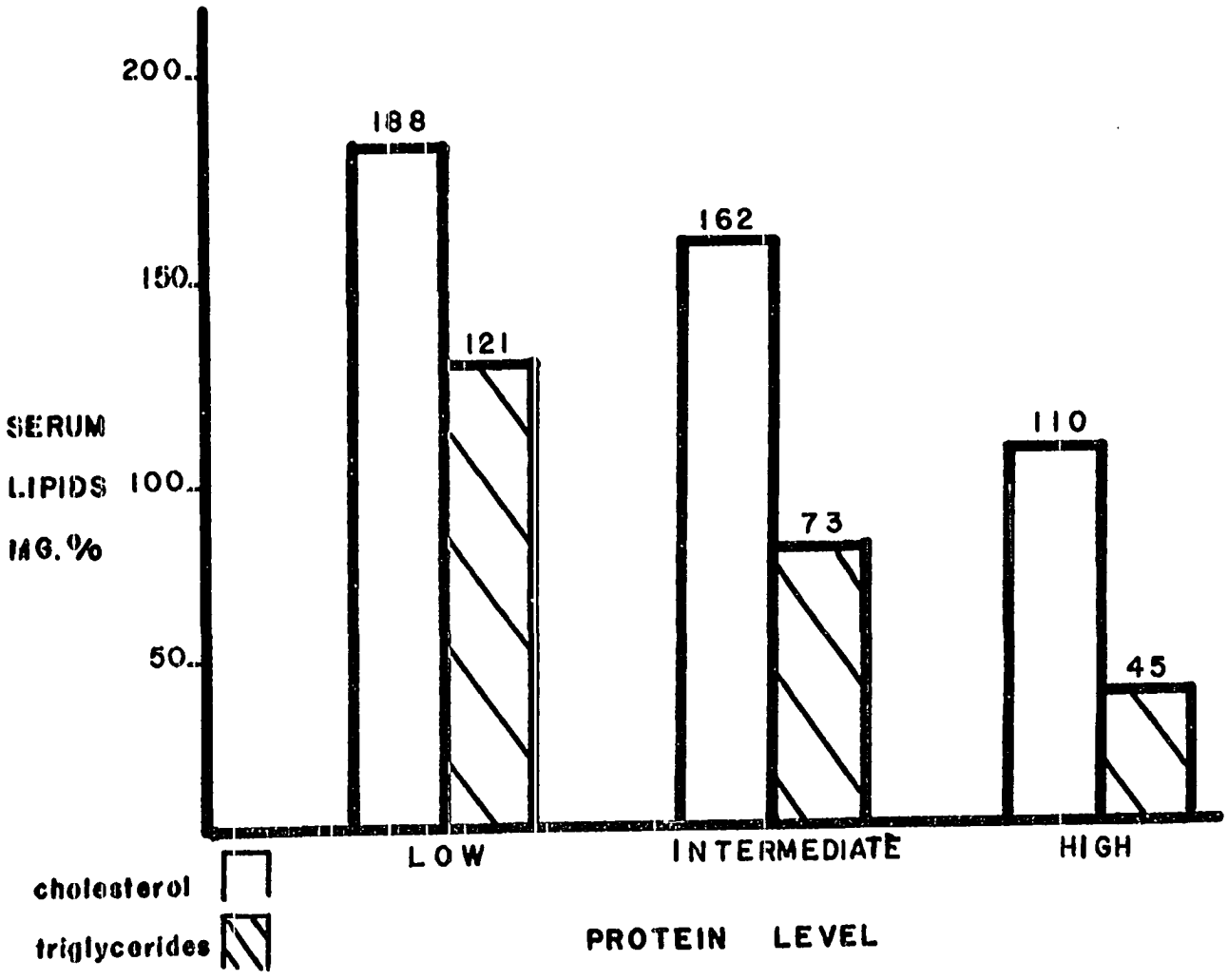
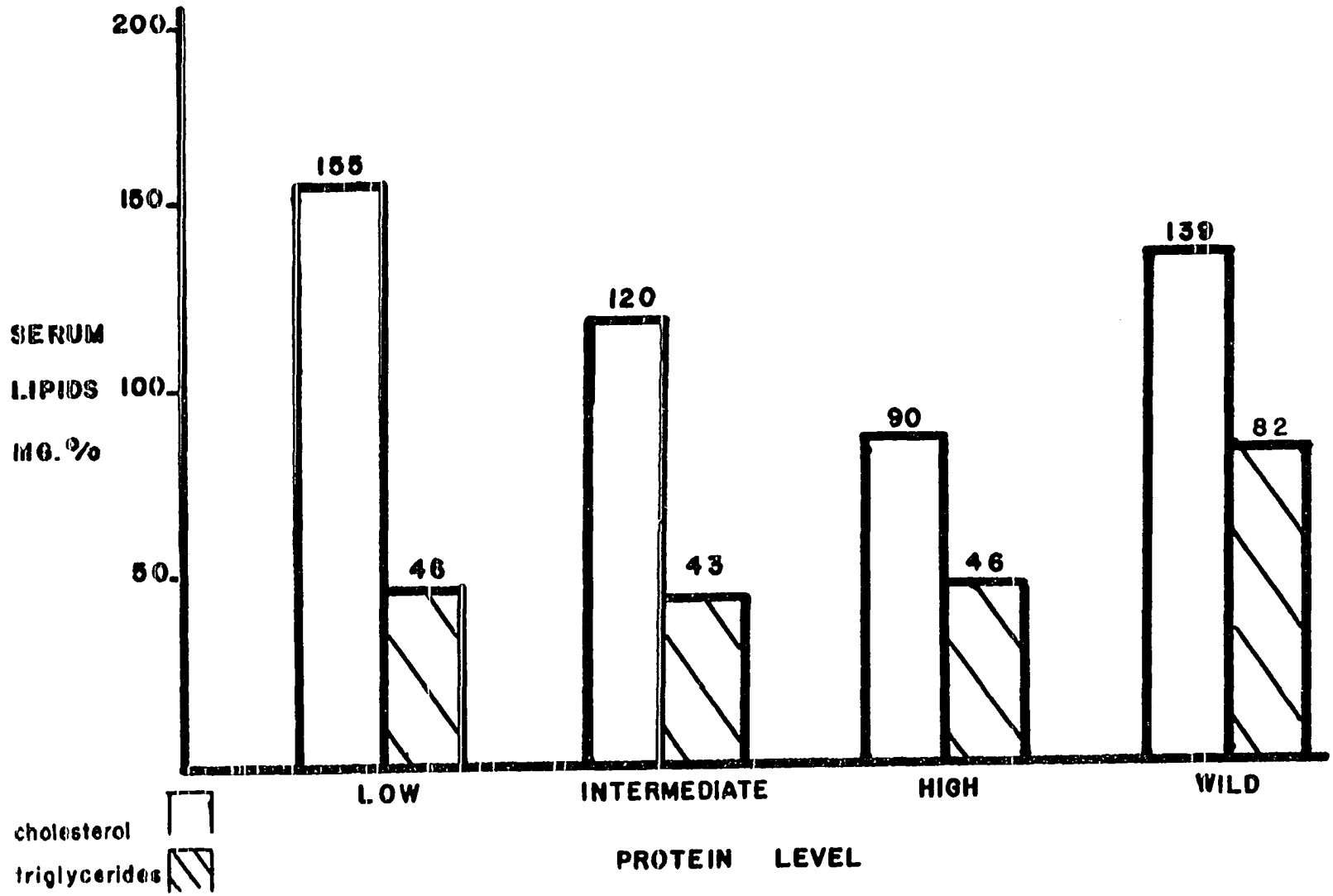

200.
150.
100.
50.
SERUM
LIPIDS
MG.%
188
121
162
73
110
45
LOW
INTERMEDIATE
HIGH
cholesterol
triglycerides
PROTEIN LEVEL

Figure 35. Average serum cholesterol and triglyceride levels in 18-week-old captive-reared rooster pheasants on low, intermediate, and high protein diets and in approximately 18-week-old, wild-reared rooster pheasants


200
150
100
50
SERUM
LIPIDS
MG.%
155
46
120
43
90
46
139
82
LOW
INTERMEDIATE
HIGH
WILD
cholesterol
triglycerides
PROTEIN LEVEL

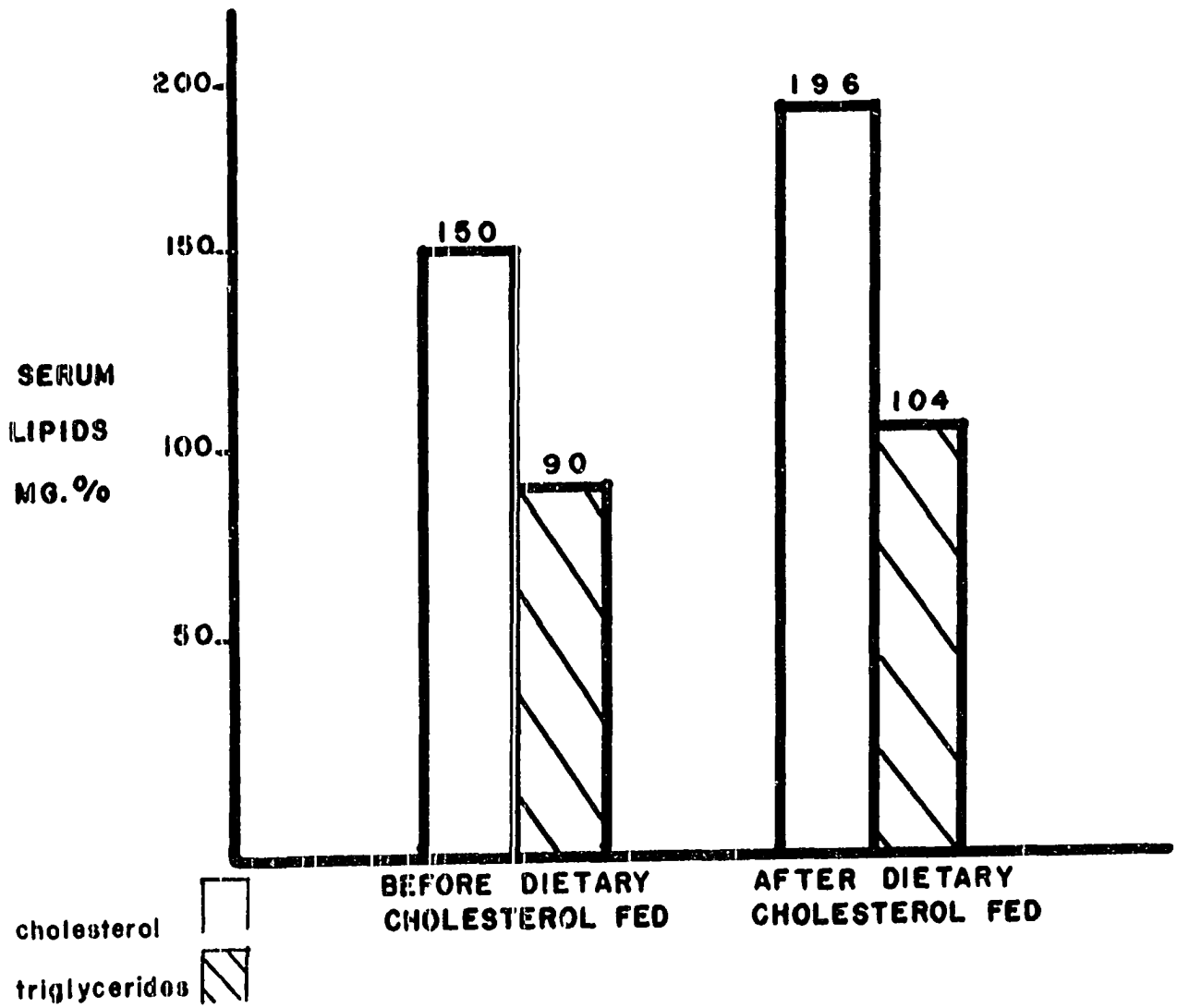
significantly ($P < .01$) higher in wild-reared birds as compared to all captive-reared birds in this study (Figure 35). However, when the serum cholesterol levels of wild-reared birds were compared to serum cholesterol levels of the captive-reared birds receiving a low protein diet no statistical significance was noted. Wild-reared birds had serum cholesterol levels significantly ($P < .01$) higher than captive-reared birds receiving either an intermediate or a high protein diet.

The serum cholesterol levels of wild-reared roosters were significantly ($P < .01$) increased by feeding a diet containing 4 per cent added cholesterol for 8 weeks (Figure 36).

The effect of dietary protein levels on serum triglyceride levels was significant ($P < .01$) in 12-week-old pheasants, but not in 18-week-old roosters. Feeding a low protein ration resulted in significantly ($P < .01$) higher serum triglyceride levels than did the diet with an intermediate protein content and feeding an intermediate protein level ration resulted in significantly ($P < .01$) higher serum triglyceride levels than did the high protein level ration in 12-week-old rooster pheasants (Figure 34). However, serum triglyceride levels were not statistically affected by dietary protein levels when the roosters were 18 weeks of age (Figure 35).

No significant correlation between serum triglyceride levels and weight of the birds within the group or incidence

Figure 36. Serum cholesterol and triglyceride levels prior to and following an 8 week period of 4 per cent cholesterol added to the diet


200
150
100
50
SERUM
LIPIDS
MG.%
150
90
196
104
BEFORE DIETARY
CHOLESTEROL FED
AFTER DIETARY
CHOLESTEROL FED
cholesterol
triglycerides

of aortic lesions was observed in this experiment.

Serum triglyceride levels were significantly ($P < .01$) higher in 18-week-old wild-reared birds as compared to 18-week-old captive-reared birds.

The serum triglyceride levels of wild-reared rooster pheasants were significantly ($P < .01$) increased by feeding a diet containing 4 per cent added cholesterol for 8 weeks (Figure 36).

Discussion

The results of this experiment agree with most of the reports in the literature, namely, that increased dietary protein results in lower serum cholesterol and decreased dietary protein results in higher serum cholesterol values. Such findings in the cockerel were reported by Stamler *et al.* (1958), Nishida *et al.* (1958), Chaikoff *et al.* (1961), and others, and in the pig by Greer *et al.* (1966) and Baker *et al.* (1968). However, Marion *et al.* (1961) and Rose and Balloun (1969) did not demonstrate a relationship of dietary protein and energy to cholesterol levels in the chicken unless cholesterol was added to the diet. The differences in serum cholesterol levels in this experiment may not be entirely due to differences in protein levels as the energy level of each ration differed slightly. Approximately 85 Kcal./lb. more energy was present in the low protein ration and 115 Kcal./lb. less energy in the high protein ration as compared

to the intermediate protein ration (Appendix, Table 20). Since Rose and Balloun (1969) determined that decreased energy decreases serum cholesterol values, part of the differences in serum cholesterol levels in the experiment being reported are probably due to dietary energy differences.

Information regarding serum cholesterol values in wild birds was not found in the literature except for a report of very high serum cholesterol in rooks by Lelek et al. (1963). The finding of significantly higher serum cholesterol values in wild birds compared to captive reared birds receiving intermediate and high protein diets was not expected. The size of the wild birds and the expected wild diet reported by Bengston (1962) suggest that pheasants from hatching to 18 weeks consume a high protein diet. Therefore, serum cholesterol levels of wild birds would be expected to be similar to birds reared on a high protein diet. On the other hand, the actual protein content of the wild pheasants diet may be less than is supposed.

The finding of increased serum cholesterol values in pheasants after feeding a cholesterol fortified ration is as expected and similar to findings in cockerels by Nichols (1963).

This study failed to demonstrate a relationship between serum cholesterol levels and aortic lesions except in the case of pheasants receiving dietary cholesterol. This indi-

cates that high serum cholesterol levels do not necessarily cause atheromas or indicate their presence. However, high serum cholesterol levels associated with feeding cholesterol apparently contribute to increased incidence of atheromas. Perhaps the concentration of cholesterol in the blood must be far above normal or be elevated for an extended period of time to initiate development of a lesion.

Few reports of serum triglycerides as related to atherosclerosis are found in the literature. The finding in this study of serum triglyceride levels inversely proportional to dietary protein levels in 12-week-old rooster pheasants is similar to higher total serum lipids in chickens on protein restricted diets as reported by Speers (1965) and Rose and Balloun (1969). Serum triglyceride levels perhaps reflect the birds requirement for energy as compared to protein. As previously stated,the low protein ration contained slightly more energy, and the high protein ration contained slightly less energy than the intermediate protein diet (Appendix, Table 20). This may account for some of the differences in serum triglyceride values. Twelve-week-old pheasants are growing rapidly and have considerable need for protein as compared to energy. Therefore, birds receiving low protein, high energy diets at 12 weeks of age would be expected to have excess energy which would be converted to triglycerides. However, at 18 weeks of age, pheasants are

considered mature and the need for protein is reduced and energy requirements are increased. This may account for the finding of no relationship between dietary protein and serum triglycerides in the 18-week-old pheasant in this experiment.

Significantly higher serum triglyceride levels were found in approximately 18-week-old, wild-reared pheasants than in 18-week-old, captive-reared birds. The diet of the wild birds may have contained weed seeds high in energy and perhaps oils which would account for higher serum triglyceride values. Another possible explanation for higher levels in wild pheasants is that these birds might have more functionally developed adrenocortical tissue as a response to adaptational needs. Burtis et al. (1966) found that hydrocortisone administered to sheep caused increased total plasma lipids, and a similar effect could occur in pheasants.

Increased serum triglyceride levels observed in pheasants in this experiment after feeding dietary cholesterol agrees with experimental reports. Dietary cholesterol results in a mobilization of triglycerides in the plasma (White et al. 1968).

This study did not demonstrate a correlation between triglyceride serum values and incidence of atheromas. This indicates that high serum triglyceride levels do not necessarily predict development of, or indicate the presence of atheromatous plaques.

Fine Structure of the Pheasant Abdominal Aorta

The purpose of this experiment was to determine the fine structure of the normal pheasant aorta and to compare these observations with fine structural changes observed in experimentally-induced atherosclerosis of the pheasant aorta. Also, a comparison was made with ultrastructural observations made in the aorta of wild pheasants.

Methods of procedure

Tissues were collected for electron microscopic studies from four wild 18-week-old pheasants, four 18-week-old birds on each of low, intermediate, and high protein diets and four 26-week-old birds fed a diet containing 4 per cent cholesterol for eight weeks following capture. A section of the abdominal aorta at the level of the adrenal glands was removed immediately after death and immersed in 2.5 per cent gluteraldehyde, then minced into 1 mm. segments. These were fixed in the gluteraldehyde for 45 minutes and stored in phosphate buffer at pH 7.4 prior to post-fixation in 1 per cent osmium tetroxide for 45 minutes. The material was dehydrated in the following grades of alcohol for 10 minutes each: 50, 70, 85, and 95 per cent, and twice in absolute alcohol. The tissue was embedded in Epon 812. Sections 500 to 700 Angstroms in thickness were cut with a diamond knife

on an LKB ultratome[1] and stained with uranyl acetate or lead citrate. They were examined on a Hitachi HS 8[2] electron microscope.[3]

Results

Electron micrographs of pheasant abdominal aortas are shown in Figures 37-42.

Figures 37 and 38 represent normal ultrastructure of the abdominal aorta in the pheasant. A single layer of endothelial cells is present in the intima. These cells contain a nucleus, mitochondria, and vacuoles. The next layer observed is the internal elastic lamina. The media contains smooth muscle cells and a connective tissue network, in which elastic fibers are prominent.

Myelin figures in endothelial cells were occasionally observed (Figure 39). When present in small numbers, these are of questionable pathologic significance. They probably represent the membranous breakdown products of organelle disintegration. The fact that these membranes are rich in lipid explains their high degree of electron density.

[1]L. K. B. Produkter A. B., Stockholm Sweden.

[2]Hitachi, Ltd., Tokyo, Japan.

[3]Available through the courtesy of Dr. D. Croghan, U.S.D.A., Ames, Iowa.

Figure 37. Electron micrograph of an aorta from an approximately 18-week-old wild-reared pheasant which represents normal structure. Endothelial cell (E), internal elastic lamina (L), smooth muscle cell (M), and elastic fibers (EF) are identified. X 7200

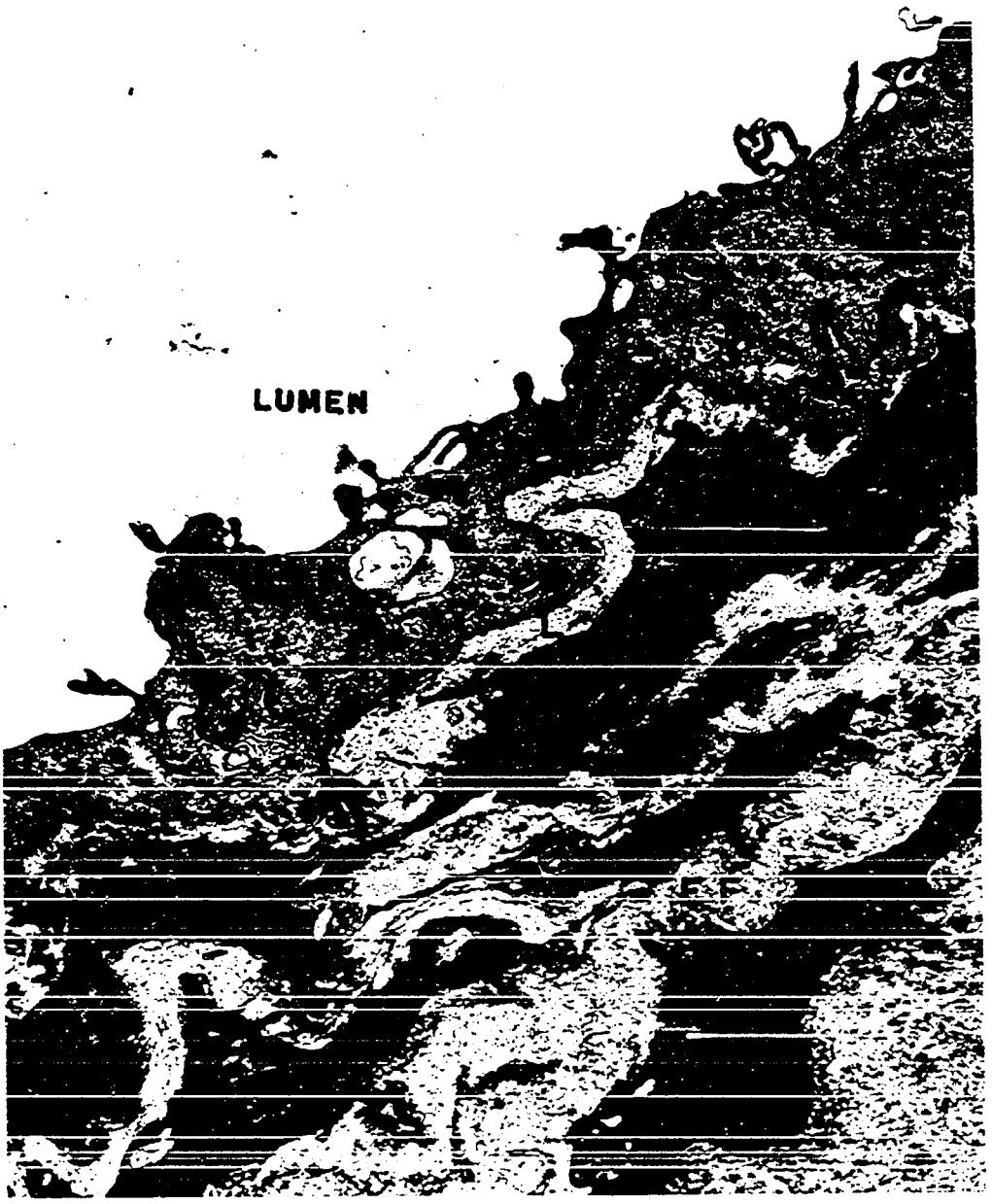

LUMEN

Figure 38. Characteristic normal aorta from an 18-week-old wild-reared pheasant illustrating endothelial cell (E), internal elastic lamina (L), smooth muscle cell (M), and elastic fibers (EF). X 14400

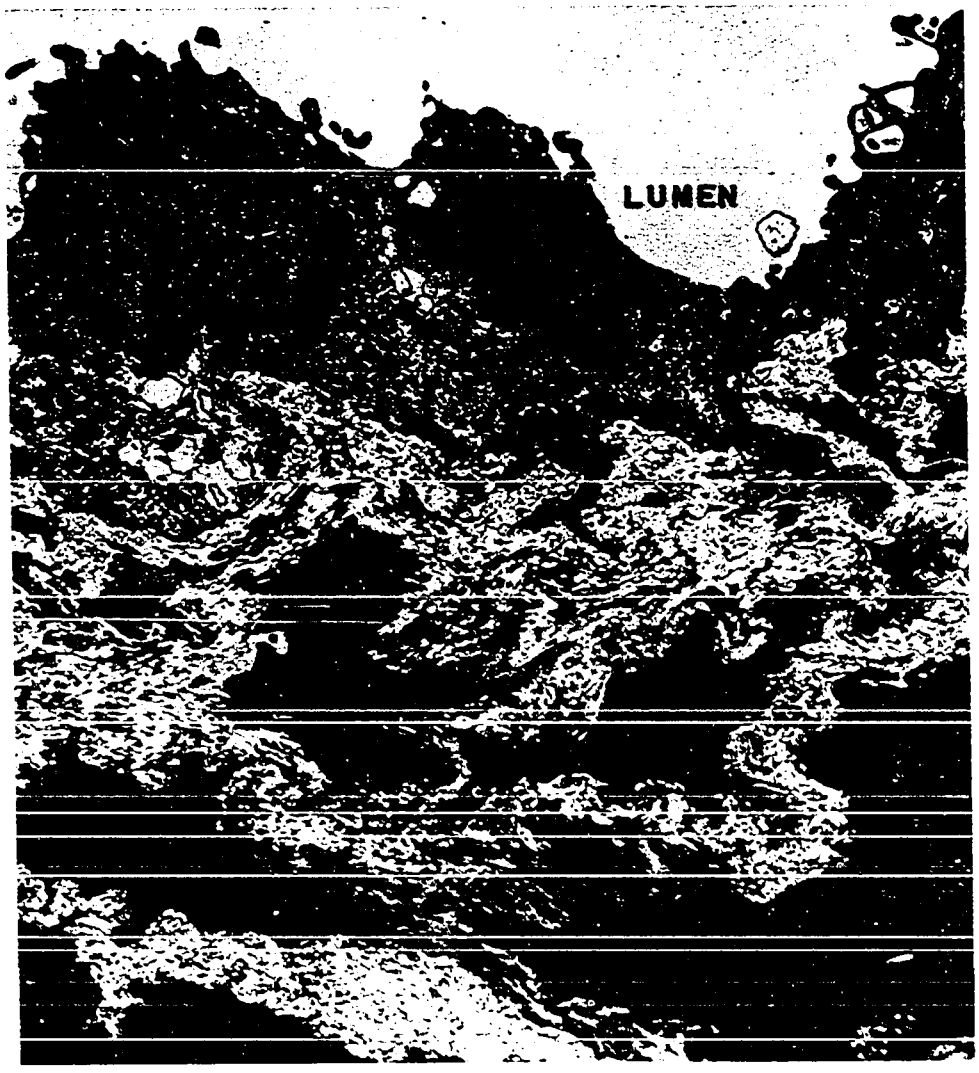
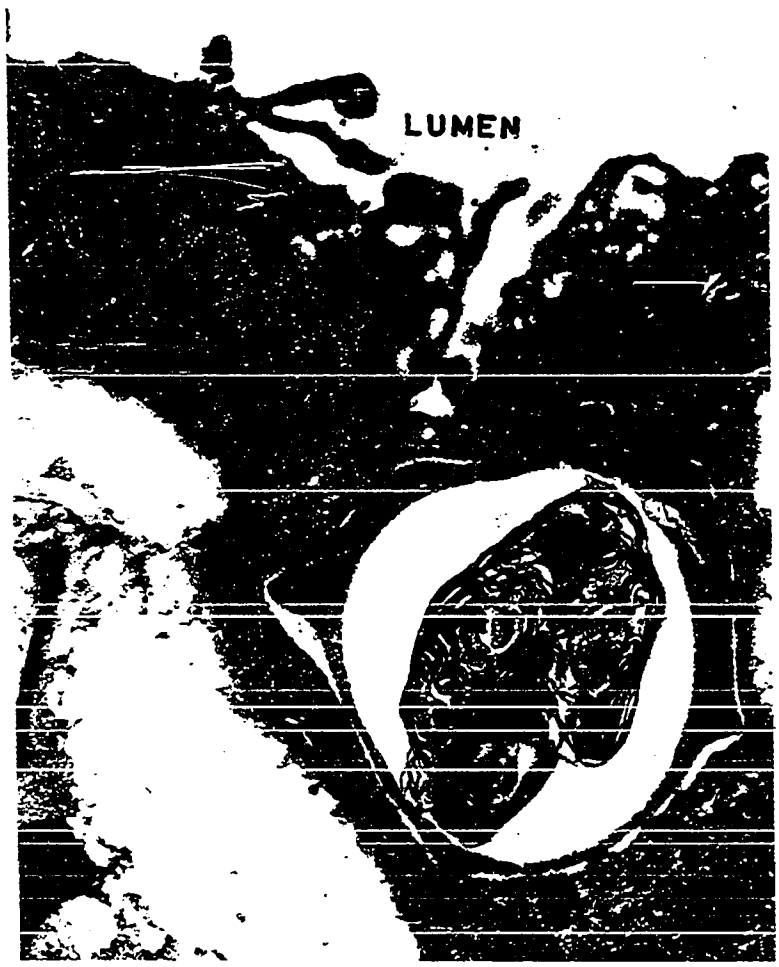

LUMEN

Figure 39. Abdominal aorta of an 18-week-old rooster reared on a high protein diet illustrating a myelin figure (MF) in an endothelial cell. X 14400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Figures 40 and 41 represent examples of degenerating smooth muscle cells which are believed to be involved in formation of the atherosclerotic plaque. The identification of the origin of the cells involved is based on finding gradations (transitional forms) between these cells and cells which could be clearly identified as smooth muscle cells. The morphology of the nucleus and cytoplasm, particularly by the localized areas of fusiform densities in the latter, is characteristic of smooth muscle cells.

One feature often mentioned by investigators as a characteristic of atherosclerosis in turkeys is a radial reorientation of smooth muscle cells in the inner media with apparent entry of some of the altered cells into the intima. These cells become incorporated in what ultimately becomes an intimal plaque, or if sufficient lipid is present, an atheroma. Part of these features are noted in Figure 42. While this area of aorta lacks many of the features of a well developed plaque, it does show the radial reorientation of smooth muscle cells and the apparent fraying or possibly splitting of the elastic network.

Discussion

The ultrastructural features of the normal pheasant aorta are similar to those found in the chicken as reported by Moss and Benditt (1970).

The ultrastructural findings of aortic lesions observed

Figure 40. Abdominal aorta of an 18-week-old pheasant rooster reared on an intermediate protein diet. A degenerating smooth muscle cell (SM) is illustrated. X 14400


LUMEN
SM

Figure 41. Abdominal aorta of an 18-week-old rooster pheasant reared on a low protein diet. A degenerating smooth muscle (SM) cell is illustrated below the internal elastic lamina. X 14400


LUMEN

Figure 42. Abdominal aorta of a 26-week-old rooster pheasant following 8 weeks of added dietary cholesterol. Smooth muscle cells (SM) appear to have become reoriented perpendicular to the lumen. X 7200

in pheasants used in this study were rather limited because advanced lesions failed to develop either in wild or experimental birds. The lesions which did occur were visualized mainly in the form of degenerative changes in modified smooth muscle cells and an occasional endothelial cell. It was expected that lipid droplets would be prominent in the intima of birds fed cholesterol and perhaps others in the experiment, but this was not observed.

The findings of this ultrastructural study in pheasants suggest that one of the primary cells involved in the formation of an early intimal plaque is the smooth muscle cell. This agrees with the report of Simpson and Harms (1968b), but not with the later report by Simpson and Harms (1969) in which they suggested that leukocytes from the blood were the origin of the foam cells found in plaques.

The lesions observed in pheasants in this study are believed to represent either early developmental stages of aortic plaques or perhaps plaques arrested in their development. Lesions fulfilling the criteria of atheroma were not observed under the electron microscope. Since no striking ultrastructural differences were observed between aortas of wild-reared pheasants and the experimental birds, no inferences could be drawn relative to the reduction in incidence and severity of atherosclerosis in the wild birds.

If the period for induction of atherosclerotic lesions

in the cholesterol-fed birds were prolonged, it might be worthwhile to re-explore this subject. It is also suggested that in any future studies of this nature more emphasis be placed on examination of the abdominal aorta since light microscopic studies indicate that more severe lesions could be expected in this area.

SUMMARY

The incidence and nature of spontaneous cardiovascular lesions in 332 Iowa wild mammals and 53 Iowa wild birds were determined. Further investigations were made in one species, the ring-necked pheasant. Young rooster pheasants were used to determine the influence of dietary protein levels and of added dietary cholesterol on the incidence and nature of cardiovascular lesions, and on serum cholesterol and triglyceride levels. Ultrastructural studies were made in order to increase understanding of the pathogenesis of plaque formation in the abdominal aorta of these birds.

Material for the study of spontaneous lesions was obtained from badgers, civet cats, foxes, mink, muskrats, opossums, raccoons, and skunks. The incidence of arterial lesions was 1.5 per cent in the coronary arteries, 1.5 per cent in the thoracic aorta, and 0.6 per cent in the abdominal aorta of the animals examined. Of the animals with lesions, 92 per cent were males and 90 per cent were animals judged to be old. Eight per cent of the wild animals were found to have lesions of myocarditis. This was primarily a problem in opossums with 22 per cent affected and in skunks with 14 per cent. A higher incidence of myocarditis was observed in old animals. Sarcocystis sp. was found in the myocardium of 11 per cent of the raccoons.

Tissues for the study of spontaneous lesions were taken from crows, hawks, owls, and pheasants collected in their natural habitat. Lesions were not found in any of the coronary arteries examined. However, 13 per cent of the birds were found to have small fibrous plaques in the thoracic aorta and 15 per cent in the abdominal aorta.

The incidence of aortic lesions in captive-reared, 18-week-old rooster pheasants was 68 per cent in those fed low protein, 78 per cent in those on intermediate protein, and 79 per cent in those given high protein diets. The incidence of lesions in wild-reared birds of a similar age was 23 per cent. From these results, protein content of the ration was not found to significantly affect the number of lesions. However, a significantly higher incidence of lesions was found in captive-reared as compared to wild-reared rooster pheasants.

Aortic lesions were found in 92 per cent of the rooster pheasants fed dietary cholesterol for 8 weeks. Therefore, added dietary cholesterol significantly increased the incidence of lesions.

Lesions found in all roosters examined were primarily fibrous plaques, some of which could be regarded as atheromas. The incidence of these plaques was higher in the abdominal as compared to the thoracic aorta. The size of lesions was not related to the diet or method of rearing.

Serum cholesterol levels in pheasants at 12 and 18 weeks of age were significantly increased by feeding the low protein diet and significantly decreased by feeding the high protein diet. Significantly higher serum cholesterol levels were found in wild-reared birds than in captive-reared birds fed the intermediate and high protein diets, but not the low protein diet. Serum cholesterol levels were significantly elevated in roosters after they were fed cholesterol for 8 weeks.

Serum triglyceride levels were significantly higher in 12-week-old, captive birds fed the low protein diet and significantly lower in those receiving the high protein diet. However, at 18 weeks of age protein had no apparent effect on serum triglyceride levels. Wild-reared birds had significantly higher serum triglyceride levels than captive-reared roosters at 18 weeks of age regardless of the dietary protein level. Serum triglyceride levels were significantly increased in roosters after cholesterol was fed for 8 weeks.

It was of interest to note that the incidence of lesions in the wild birds was relatively low compared to those fed high and intermediae protein levels, and yet the serum cholesterol-triglyceride levels of these wild birds were significantly higher. Also of interest is the poor correlation between incidence of aortic lesions in birds fed the various protein levels, and their serum cholesterol-

triglyceride levels. Perhaps extended feeding of these diets would have permitted the development of more clearly defined aortic lesions.

Ultrastructural studies of aortic changes revealed radial reorientation of smooth muscle fibers, the appearance of modified and degenerative smooth muscle cells, degenerative endothelial cells, and the occasional presence of myelin figures within endothelial cell cytoplasm. The pathogenesis of early plaque formation in the abdominal aorta of the rooster pheasant appears to involve modifications in smooth muscle and endothelial cells. The changes in the structure and functions of these cells account for the cellular pattern of response in early plaque formation. The role of cells derived from the circulating blood appears to be negligible.

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APPENDIX

Table 7. Table of lesions in the heart and arteries of badgers

No.	Sex	Age	County[a]	Coronary artery	Thoracic aorta	Abdominal aorta	Myocarditis
X	M	Y	85	-	-	-	-
54	F	O	30	-	-	-	+
156	M	O	85	+	-	-	-
190	F	Y	62	-	-	-	-
361	F	O	30	-	-	-	-
371	M	O	69	-	-	-	-

[a]Numerical code corresponds to alphabetical standing of counties within Iowa.

Table 8. Table of lesions in the heart and arteries of civet cats

No.	Sex	Age	County[a]	Coronary artery	Thoracic aorta	Abdominal aorta	Myocarditis
99	M	O	62	-	-	-	-
353	M	O	65	-	-	-	-
372	M	Y	65	-	-	-	-
378	M	O	79	-	-	-	-
383	F	O	99	-	-	-	-
392	F	O	88	-	-	-	-
402	F	Y	69	-	-	-	+

[a]Numerical code corresponds to alphabetical standing of counties within Iowa.

Table 9. Table of lesions in the hearts and arteries of foxes

No.	Sex	Age	County[a]	Coronary artery	Thoracic aorta	Abdominal aorta	Myocarditis
38	M	O	69	-	-	-	-
39	M	O	69	-	-	-	-
40	F	O	69	-	-	-	-
51	M	Y	30	-	-	-	-
147	M	Y	30	-	-	-	-
154	F	Y	85	-	-	-	-
162	M	Y	30	-	-	-	-
173	M	Y	91	-	-	-	-
206	M	O	69	-	-	+++	-
209	F	Y	69	-	-	-	-
210	F	Y	69	-	-	-	-
211	F	Y	69	-	-	-	-
212	M	O	69	+	-	-	-
225	M	Y	81	-	-	-	-
238	F	Y	5	-	-	-	-
239	M	Y	5	-	-	-	-
240	M	O	85	-	-	-	-
248	M	Y	85	-	-	-	-
249	M	O	85	-	-	-	-
250	-	O	85	-	-	-	-
257	F	O	85	-	-	-	-
268	F	Y	91	-	-	-	-
272	M	Y	91	-	-	-	-
275	M	O	91	-	-	-	-
324	M	Y	86	-	-	-	-
326	F	O	99	-	-	-	-
327	M	O	88	-	-	-	-
328	M	O	88	-	-	-	-
344	F	Y	81	-	-	-	-
355	M	O	30	-	-	-	-
387	M	Y	79	-	-	-	-
396	F	Y	86	-	-	-	-
397	M	O	62	-	-	+	-

[a]Numerical code corresponds to alphabetical standing of counties within Iowa.

Table 10. Table of lesions in the heart and arteries of mink

No.	Sex	Age	County[a]	Coronary artery	Thoracic aorta	Abdominal aorta	Myocarditis
136	M	Y	5	-	-	-	-
137	M	Y	5	-	-	-	-
138	M	Y	79	-	-	-	-
140	M	Y	79	-	-	-	-
145	M	Y	91	-	-	-	-
146	M	Y	5	-	-	-	-
150	M	O	91	-	-	-	-
151	F	Y	86	-	-	-	-
153	M	O	91	-	-	-	-
155	F	Y	86	-	-	-	-
157	M	Y	86	-	-	-	-
161	M	O	5	-	-	-	-
177	M	O	79	-	-	-	-
178	F	Y	88	-	-	-	-
179	M	Y	88	-	-	-	-
181	M	O	88	-	-	-	-
183	F	Y	88	-	-	-	-
184	M	Y	99	-	-	-	-
185	M	Y	99	-	-	-	-S[b]
186	M	O	88	-	-	-	-
187	M	O	99	-	-	-	-
214	M	Y	30	-	-	-	-
215	M	O	30	-	-	-	-
218	F	Y	30	-	-	-	-
219	M	O	30	-	-	-	-
221	M	Y	30	-	-	-	-
222	M	Y	30	-	-	-	-
224	M	O	30	-	-	-	-
244	M	O	85	-	-	-	-
264	M	Y	79	-	-	-	-
349	F	Y	79	-	-	-	-
350	F	Y	86	-	-	-	-
351	M	Y	79	-	-	-	-
352	M	Y	79	-	-	-	-
380	M	O	86	-	-	-	-
385	M	Y	81	-	-	-	-
398	M	Y	86	-	-	-	-
404	F	Y	30	-	-	-	-
406	F	O	79	-	-	-	-

[a]Numerical code corresponds to alphabetical standing of counties within Iowa.

[b]*Sarcocystis sp.*

Table 11. Table of lesions in the heart and arteries of muskrats

No.	Sex	Age	County[a]	Coronary artery	Thoracic aorta	Abdominal aorta	Myocarditis
135	F		88	-	-	-	-
143	M		88	-	-	-	-
180	M		88	-	-	-	-
182	F		88	-	-	-	-
188	M		88	-	-	-	-
216	M		30	-	-	-	-
217	M		30	-	-	-	-
220	M		30	-	-	-	-
223	M		30	-	-	-	-
242	F		79	-	-	-	-
245	F		5	-	-	-	-
247	M		91	-	-	-	-
259	M		85	-	+	-	-
261	M		91	-	-	-	-
401	M		81	-	+	-	-
405	F		86	-	-	-	-
407	F		85	-	-	-	-

[a]Numerical code corresponds to alphabetical standing of counties within Iowa.

Table 12. Table of lesions in the heart and arteries of opossums

No.	Sex	Age	County[a]	Coronary artery	Thoracic aorta	Abdominal aorta	Myocarditis
11	M	O	91	-	-	-	-
14	M	O	79	-	-	-	+
21	M	O	79	-	-	-	-
23	M	O	91	-	-	-	+
24	F	O	91	-	-	-	-
26	M	Y	85	-	-	-	-
35	F	O	81	-	-	-	+
41	F	O	65	-	-	-	++
45	F	O	65	-	-	-	-
53	M	O	30	-	-	-	-
55	M	O	30	-	-	-	++
63	F	Y	62	-	-	-	++
67	F	O	62	-	-	-	++++
77	F	O	5	-	-	-	-
83	F	O	86	-	-	-	++
96	M	O	81	-	-	-	-
97	M	O	85	-	-	-	-
98	M	O	85	-	-	-	-
101	F	O	81	-	-	-	-
102	M	Y	85	-	-	-	-
103	M	O	85	-	-	-	-
104	M	O	81	-	-	-	-
105	M	Y	85	-	-	-	-
106	F	O	81	-	-	-	-
109	M	Y	81	-	-	-	-
111	M	Y	5	-	-	-	-
113	F	O	81	-	-	-	-
114	M	Y	85	-	-	-	-
115	M	O	81	-	-	-	-
117	M	O	81	-	-	-	-
118	M	O	85	-	-	-	-
122	F	O	81	-	-	-	++++
130	M	Y	85	-	-	-	-
132	F	Y	5	-	-	-	-
134	M	O	5	-	-	-	-
139	F	Y	5	-	-	-	-
142	F	O	5	-	-	-	-
144	M	O	30	-	-	-	+
148	M	Y	99	-	-	-	-
149	M	O	30	+	-	-	-
152	M	O	99	-	-	-	-

[a]Numerical code corresponds to alphabetical standing of counties within Iowa.

Table 12 (Continued

No.	Sex	Age	County	Coronary artery[a]	Thoracic aorta	Abdominal aorta	Myocarditis
159	M	Y	30	-	-	-	-
160	M	O	99	-	+	-	-
176	M	Y	85	-	-	-	-
189	M	O	86	-	-	-	-
191	M	O	62	-	-	-	-
200	M	Y	62	-	-	-	-
203	F	Y	91	-	-	-	-
243	F	O	88	-	-	-	-
246	F	O	62	-	-	-	-
251	M	O	88	-	-	-	-
254	M	O	86	-	-	-	++++
255	M	Y	79	-	-	-	-
260	F	Y	88	-	-	-	-
262	F	Y	86	-	-	-	-
343	M	Y	81	-	-	-	+
357	M	Y	5	-	-	-	-
362	F	Y	5	-	-	-	-
363	F	O	85	-	-	-	-
365	F	Y	86	-	-	-	-
366	M	O	99	-	-	-	++
369	M	O	69	-	-	-	-
373	M	Y	86	-	-	-	-
374	F	O	85	-	-	-	-
375	M	O	86	-	-	-	+
377	M	Y	88	-	-	-	-
379	F	O	62	-	-	-	-
381	M	Y	91	-	-	-	++
382	M	Y	69	-	-	-	-
384	M	O	88	-	-	-	-
400	F	O	30	-	-	-	++
403	M	Y	69	-	-	-	-
408	M	O	79	-	-	-	-

Table 13. Table of lesions in the heart and arteries of raccoons

No.	Sex	Age	County[a]	Coronary artery	Thoracic aorta	Abdominal aorta	Myocarditis
1	F	O	86	-	-	-	-
2	M	O	86	-	-	-	-
3	M	Y	91	-	-	-	-S[b]
4	M	O	91	-	-	-	-
5	M	O	86	-	-	-	S
6	M	Y	86	-	-	-	-
7	F	Y	79	-	-	-	-
8	M	Y	79	-	-	-	-
9	F	O	79	-	-	-	-
10	F	O	91	-	-	-	-
12	M	O	79	-	-	-	-
13	F	Y	91	-	-	-	-
15	F	Y	88	-	-	-	-
16	M	O	88	-	-	-	-
17	F	Y	88	-	-	-	-
18	F	Y	88	-	-	-	-S
19	F	O	88	-	-	-	-S
20	F	O	88	-	-	-	-
22	F	O	91	-	-	-	-
25	M	Y	85	-	-	-	-
27	F	Y	85	-	-	-	-
29	M	Y	79	-	-	-	-
30	M	O	85	-	-	-	-
31	F	O	85	-	-	-	-S
32	M	O	85	-	-	-	-
33	M	Y	91	-	-	-	-
34	M	Y	79	-	-	-	-
37	F	O	69	-	-	-	-S
42	M	O	69	-	-	-	-
43	M	Y	69	-	-	-	-S
44	M	Y	69	-	-	-	-
46	F	O	30	-	-	-	-
47	F	Y	30	-	-	-	-S
48	M	Y	30	-	-	-	-
49	F	Y	5	-	-	-	-
50	F	O	30	-	-	-	-
52	F	Y	30	-	-	-	-
56	F	Y	5	-	-	-	-
57	F	Y	91	-	-	-	-

[a]Numerical code corresponds to alphabetical standing of counties within Iowa.

[b]*Sarcocystis sp.*

Table 13 (Continued)

No.	Sex	Age	County[a]	Coronary artery	Thoracic aorta	Abdominal aorta	Myocarditis
58	M	O	30	-	-	-	-
59	F	Y	5	-	-	-	-
60	M	Y	91	-	-	-	-
62	F	O	30	-	-	-	-
64	F	Y	62	-	-	-	-
65	F	Y	62	-	-	-	-
68	M	O	81	-	-	-	-
69	M	O	81	-	-	-	-
71	F	O	81	-	-	-	-
72	M	Y	86	-	-	-	-
73	M	Y	81	-	-	-	-
74	F	Y	81	-	-	-	-
75	F	Y	99	-	-	-	-
76	F	O	88	-	-	-	-
78	F	O	99	-	-	-	-S
79	F	Y	99	-	-	-	-S
80	F	O	99	-	-	-	-
81	F	Y	99	-	-	-	-
84	M	O	81	-	-	-	-
85	M	O	86	-	-	-	-
86	M	O	81	-	-	-	-
87	M	O	91	-	-	-	-
88	F	Y	81	-	-	-	-
89	M	O	86	-	-	-	-
90	M	O	62	-	-	-	-
91	F	Y	86	-	-	-	-S
92	F	O	81	-	-	-	-
93	F	O	62	-	-	-	-
94	F	O	62	-	-	-	-
108	F	O	85	-	-	-	-
110	F	Y	85	-	-	-	-S
112	F	Y	85	-	-	-	-
119	F	Y	86	-	-	-	-
165	M	O	5	-	-	-	-
166	F	O	5	-	-	-	-
167	F	Y	5	-	-	-	-
168	M	O	5	-	-	-	-
169	M	Y	5	-	-	-	-
170	F	Y	85	-	-	-	-
171	M	Y	5	-	-	-	-
174	F	Y	85	-	-	-	-
175	M	O	5	-	-	-	-
194	F	Y	99	-	-	-	-
195	M	Y	99	-	-	-	-
197	-	Y	88	-	-	-	-

Table 13 (Continued)

No.	Sex	Age	County[a]	Coronary artery	Thoracic aorta	Abdominal aorta	Myocarditis
198	M	O	69	-	-	-	-
199	F	Y	99	-	-	-	-
201	M	O	69	-	-	-	-
205	M	O	79	-	-	-	-
208	M	O	69	-	-	-	-
226	M	Y	99	-	-	-	-
227	F	O	30	-	-	-	-
228	M	Y	69	-	-	-	-
229	M	O	30	-	+++	-	-
230	F	O	99	-	-	-	-
231	M	Y	30	-	-	-	-
232	M	Y	88	-	-	-	-
233	F	O	99	-	-	-	-
234	M	Y	91	-	-	-	-
235	-	Y	69	-	-	-	-
236	M	Y	69	-	-	-	-
237	F	Y	99	-	-	-	-
338	M	Y	91	-	-	-	-
339	F	O	85	-	-	-	-
340	M	Y	62	-	-	-	-
341	F	Y	30	-	-	-	-
342	M	Y	99	-	-	-	+++
346	M	O	91	-	-	-	-
347	M	Y	69	-	-	-	-
356	F	Y	88	-	-	-	-
359	M	Y	69	-	-	-	-
360	M	Y	5	-	-	-	-
370	F	Y	91	-	-	-	-

Table 14. Table of lesions in the heart and arteries of the skunk

No.	Sex	Age	County[a]	Coronary artery	Thoracic aorta	Abdominal aorta	Myocarditis
36	F	O	81	-	-	-	-
66	F	Y	62	-	-	-	-
95	F	O	81	-	-	-	-
100	M	O	62	-	-	-	-
107	F	O	62	-	-	-	-
116	M	O	91	-	-	-	-
120	F	O	81	-	-	-	-
121	M	O	81	-	-	-	-
124	F	Y	81	-	-	-	-
125	M	O	81	-	-	-	+
126	F	O	81	-	-	-	-
127	M	O	81	-	-	-	+
128	F	O	81	-	-	-	-
129	M	O	85	-	-	-	-
131	M	O	62	-	-	-	-
133	F	O	79	-	+++	-	+
141	F	O	79	-	-	-	-
158	M	Y	30	-	-	-	-
192	M	Y	62	-	-	-	-
193	M	Y	62	-	-	-	-
204	F	O	79	-	-	-	-
205	M	Y	69	-	-	-	-
241	M	O	86	-	-	-	+
252	M	O	88	-	-	-	-
253	F	Y	88	-	-	-	-
256	M	Y	86	-	-	-	-
258	M	O	86	-	-	-	-
263	M	O	86	-	-	-	-
267	M	O	91	+++	-	-	-
269	F	Y	91	-	-	-	-
270	M	Y	99	-	-	-	-
271	M	Y	99	+++	-	-	-
273	M	Y	99	-	-	-	-
274	M	O	99	-	-	-	-
325	M	Y	91	-	-	-	+
330	M	Y	69	-	-	-	-
332	M	Y	85	-	-	-	-
333	M	O	86	-	-	-	-
345	F	O	30	-	-	-	-
348	F	Y	69	-	-	-	+
354	M	O	62	-	-	-	-

[a]Numerical code corresponds to alphabetical standing of counties within Iowa.

Table 14 (Continued)

No.	Sex	Age	County	Coronary artery[a]	Thoracic aorta	Abdominal aorta	Myocarditis
368	M	O	69	-	-	-	-
386	F	Y	86	-	-	-	-
388	M	Y	88	-	-	-	-
389	M	Y	85	-	-	-	-
399	M	Y	30	-	-	-	-

Table 15. Table of lesions in the heart and arteries of crows captured in Warren County, Iowa

No.	Coronary artery	Thoracic aorta	Abdominal aorta	Myocarditis
288	-	-	-	-
293	-	-	-	-
394	-	+	-	-

Table 16. Table of lesions in the heart and arteries of hawks captured in Warren County, Iowa

No.	Coronary artery	Thoracic aorta	Abdominal aorta	Myocarditis
28	-	+	-	-
82	-	-	-	-
172	-	-	-	-
265	-	-	-	-
266	-	-	-	-
276	-	-	-	-
287	-	+	-	-
296	-	-	-	-

Table 17. Table of lesions in the heart and arteries of owls captured in Warren County, Iowa

No.	Coronary artery	Thoracic aorta	Abdominal aorta	Myocarditis
61	-	-	-	-
123	-	-	-	-
163	-	-	-	-
164	-	-	-	-
196	-	-	-	-
277	-	+	-	+
278	-	-	-	-
279	-	+	+	-
280	-	-	-	-
281	-	-	-	-
282	-	-	-	-
283	-	-	-	-
284	-	-	-	-
285	-	+	+	-
286	-	-	-	-
289	-	-	-	-
290	-	-	-	-
291	-	-	+	-
292	-	-	-	-
295	-	-	-	-
297	-	-	-	-
298	-	-	-	-
300	-	-	-	-
301	-	-	-	-
302	-	-	-	-
303	-	-	-	-
304	-	-	-	-
305	-	-	-	-
306	-	-	-	-

Table 18. Table of lesions in the heart and arteries of young rooster pheasants captured in Union County, Iowa

No.	County	Coronary artery	Thoracic aorta	Abdominal aorta	Myocarditis
3281	88	-	-	-	-
3282	88	-	-	-	-
3284	88	-	-	-	-
3289	88	-	++	++	-
3291	88	-	-	-	-
3292	88	-	-	-	-
3294	88	-	-	+	-
3295	88	-	-	+	-
3296	88	-	-	-	-
3541	88	-	-	-	-
3542	88	-	-	-	-
3543	88	-	-	-	-
99W	88	-	-	-	-

Table 19. Composition of experimental diets[a]

Ingredient	Low protein		Intermediate protein		High protein	
	20%	16%	28%	22%	36%	30%
Ground yellow corn	66.7	78.0	43.9	61.0	21.2	38.2
Soybean oilmeal	26.3	15.0	49.1	32.0	71.8	54.8
Meat meal	2.0	2.0	2.0	2.0	2.0	2.0
Fish meal	2.0	2.0	2.0	2.0	2.0	2.0
Dehyrated alfalfa meal	2.0	2.0	2.0	2.0	2.0	2.0
Trace mineral salt	0.2	0.2	0.2	0.2	0.2	0.2
Mineral premix[b]	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin premix[c]	0.5	0.5	0.5	0.5	0.5	0.5

[a]Calculated analysis presented in Table 20.

[b]Super mineral concentrate HG 2 X 1, Protein Blenders, Inc., Iowa City, Iowa.

[c]VTM Link Pak, United Suppliers, Inc., Eldora, Iowa.

Table 20. Calculated analysis of the experimental diets

Item	Unit	Low Protein		Intermediate Protein		High Protein	
Protein	Percent	20	16	28	22	36	30
Metabolizable energy	Kcal./lb.	1341	1399	1225	1312	1109	1196
Calcium	Percent	.49	.46	.54	.50	.59	.55
Phosphorus	Percent	.52	.49	.60	.54	.66	.62
Vitamin A	I.U./lb.	4528.1	4753.7	4071.7	4413.7	3616.9	3957.6
Vitamin D	I.U./lb.	776.3	776.3	776.3	776.3	776.3	776.3
Riboflavin	Mg./lb.	2.5	2.4	2.7	2.6	2.9	2.8
Pantothenic acid	Mg./lb.	7.3	6.9	8.2	7.6	9.1	8.4
Niacin	Mg./lb.	19.5	19.3	20.2	19.7	20.8	20.4
Choline	Mg./lb.	523.1	410.1	751.1	580.1	978.1	808.1
Vitamin B_{12}	Mg./lb.	2.8	2.8	2.8	2.8	2.8	2.8

Table 21. Average amount of feed in ounces per bird consumed by pheasant roosters reared on low, intermediate, and high protein rations from hatching to 16 weeks of age

Age of birds Week	Low protein[a]		Intermediate protein[b]		High protein[c]	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
1	.88	.88	1.10	1.10	1.22	1.22
2	1.44	1.44	1.82	1.82	2.19	2.19
3	1.86	1.88	2.69	2.69	3.42	3.48
4	2.67	3.06	3.64	3.56	4.95	5.50
5	3.00	3.55	4.80	4.87	5.79	6.64
6	5.01	5.43	6.69	7.13	9.36	9.52
7 & 8	12.19	15.44	14.99	15.92	18.49	16.80
9 & 10	22.51	25.39	20.63	22.32	21.60	22.40
11 & 12	24.87	27.31	26.93	31.15	27.29	25.52
13 & 14	20.92	30.65	25.42	27.29	32.19	28.80
15 & 16	26.22	36.68	28.89	31.72	30.40	33.01

[a]Low protein (20% first 6 weeks, 16% thereafter).

[b]Intermediate protein (28% first 6 weeks, 22% thereafter).

[c]High protein (36% first 6 weeks, 30% thereafter).

Table 22. Average weight in ounces of rooster pheasants reared on low, intermediate, and high protein rations from hatching to 16 weeks of age

Age of birds / Week	Low protein[a]		Intermediate protein[b]		High protein[c]	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
0	.64	.64	.64	.64	.64	.64
1	1.00	1.00	1.17	1.17	1.22	1.22
2	1.60	1.54	2.04	2.11	2.33	2.30
3	2.37	2.18	3.20	3.27	3.78	3.76
4	3.33	3.14	4.87	5.04	5.60	5.98
5	4.33	4.73	6.64	6.64	7.85	8.36
6	5.91	6.40	9.09	8.84	9.92	10.29
8	8.99	9.28	12.97	12.80	14.49	15.76
10	12.37	13.44	17.77	17.68	19.56	21.05
12	16.49	18.74	24.36	23.72	25.87	26.11
14	20.55	23.02	27.73	27.76	30.12	29.64
16	25.23	27.08	32.09	32.09	33.60	33.01

[a]Low protein (20% first 6 weeks, 16% thereafter).

[b]Intermediate protein (28% first 6 weeks, 22% thereafter).

[c]High protein (36% first 6 weeks, 30% thereafter).

Table 23. Feed per pound of gain in pheasants on low, intermediate, and high protein diets

Age of birds Week	Low protein[a]		Intermediate protein[b]		High protein[c]	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
1	2.43	2.43	2.08	2.08	2.10	2.10
2	2.56	2.56	2.02	2.02	2.03	2.03
3	2.42	2.94	2.32	2.32	2.36	2.32
4	2.78	2.49	2.18	2.01	2.72	2.48
5	3.00	2.69	2.71	3.04	2.57	2.79
6	3.17	3.25	2.73	3.24	4.52	4.93
7 & 8	3.96	5.36	3.86	4.02	4.05	3.07
9 & 10	6.66	6.10	4.30	4.57	4.26	4.23
11 & 12	6.04	5.15	4.09	5.16	4.32	5.04
13 & 14	5.15	7.16	7.54	6.75	7.57	8.16
15 & 16	5.60	9.03	6.63	7.33	8.74	9.80

[a]Low protein (20% first 6 weeks, 16% thereafter).

[b]Intermediate protein (28% first 6 weeks, 22% thereafter).

[c]High protein (36% first 6 weeks, 30% thereafter).

Table 24. Lesions present in the thoracic and abdominal aorta of 18-week-old pheasants reared on a low protein diet (20% protein first 6 weeks, 16% protein thereafter)

Replication 1				Replication 2			
Bird No.	Wt. (lbs.)	Thoracic aorta	Abdominal aorta	Bird No.	Wt. (lbs.)	Thoracic aorta	Abdominal aorta
3676	1.5	-	+	3691	1.6	-	-
3677	1.9	-	-	3692	2.3	-	-
3680	1.4	-	-	3693	2.3	-	+
3681	1.7	+	-	3694	1.8	+	-
3682	1.7	-	+	3696	2.0	-	+
3683	2.1	-	-	3698	1.9	-	+
3684	1.9	-	+	3872	1.9	-	++
3688	1.5	-	++	3874	1.6	+++	-
3690	1.7	-	-	3875	1.8	-	++
				3900	1.9	-	+

Table 25. Lesions present in the thoracic and abdominal aorta of 18-week-old pheasants reared on an intermediate (normal protein diet (28% protein first 6 weeks, 22% thereafter)

Replication 1				Replication 2			
Bird No.	Wt. (lbs.)	Thoracic aorta	Abdominal aorta	Bird No.	Wt. (lbs.)	Thoracic aorta	Abdominal aorta
3601	1.9	--	+	3620	2.1	-	++++
3602	2.0	--	++	3621	1.8	-	++
3603	2.3	--	++	3622	2.1	-	-
3604	1.8	--	-	3623	1.9	-	++
3606	1.8	--	-	3624	2.0	-	-
3607	2.1	--	+	3625	2.2	-	+
3608	1.8	++	++	3630	1.9	-	+
3609	2.2	+++	+	3631	2.3	-	++
3610	2.1	+	-	3632	2.0	+	-
3611	2.2	+	+	3633	1.9	++	+++
3612	1.9	--	++	3634	2.0	-	-
3613	2.1	--	-	3635	2.6	-	+
3614	1.9	--	+++	3636	1.9	-	+
3616	2.1	--	+	3637	2.2	-	+++
3617	2.2	++	++	3638	2.2	-	++
3618	2.3	--	-				
3619	2.0	--	+				

Table 26. Lesions present in the thoracic and abdominal aorta of 18-week-old pheasants reared on a high protein diet (36% protein first 6 weeks, 30% thereafter)

Replication 1				Replication 2			
Bird No.	Wt. (lbs.)	Thoracic aorta	Abdominal aorta	Bird No.	Wt. (lbs.)	Thoracic aorta	Abdominal aorta
3658	2.1	-	+	3639	2.1	-	++
3659	2.2	-	-	3640	1.8	-	+
3660	1.8	-	+++	3641	2.1	-	-
3661	2.3	-	+	3642	1.9	-	++
3662	2.1	-	+	3643	2.4	-	++
3663	2.2	-	-	3644	2.4	-	+
3664	2.2	-	-	3645	2.1	-	+
3665	2.0	-	-	3646	2.1	-	+
3666	2.6	-	+	3647	2.4	-	+
3667	2.6	-	+	3648	2.1	-	+
3668	2.3	-	++	3649	2.0	-	+
3671	1.9	++	+++	3651	1.9	++	++
3673	1.7	-	+	3653	2.5	-	+
3674	1.8	-	+	3654	2.1	-	-

Table 27. Lesions in pheasants captured at approximately 18 weeks of age and placed on a diet containing 4 per cent added cholesterol for 8 weeks

Bird No.	Starting weight	Final weight	Thoracic aorta lesions	Abdominal aorta lesions
3280	2.8	3.2	-	+
3283	2.2	2.6	-	++
3285	2.9	3.1	-	+
3286	1.9	2.4	+++	+++
3287	2.2	2.8	-	++
3288	2.5	2.8	-	+
3290	2.6	2.9	-	+
3293	2.2	2.5	+	+
3297	1.7	2.0	-	++
3298	2.8	3.1	-	+
3299	2.4	2.9	-	++
3300	2.7	3.3	-	-
Average	2.4	2.8		

Table 28. Serum cholesterol and triglyceride levels in captive-reared 12-week-old pheasants on a low protein diet (20% protein first 6 weeks, 16% thereafter)

	Bird No.	Cholesterol (mg.%) Analysis 1	Cholesterol (mg.%) Analysis 2	Triglycerides (mg.%) Analysis 1	Triglycerides (mg.%) Analysis 2
Replication					
1	3676	230	232	136	135
	3677	200	200	156	157
	3680	205	206	107	104
	3681	200	202	100	100
	3682	165	167	120	119
	3683	191	188	116	110
	3684	218	221	114	115
	3688	124	124	90	83
	3690	210	209	129	128
2	3691	180	183	184	184
	3692	158	158	98	98
	3693	168	166	180	179
	3694	220	224	156	156
	3696	200	200	174	175
	3698	162	164	98	102
	3872	184	182	90	91
	3874	184	186	113	116
	3875	162	164	98	102
	3900	210	214	48	49
Average Replication 1		193.7	194.3	118.7	116.8
Average Replication 2		182.8	184.1	123.9	125.2
Overall Average		187.9	188.9	121.4	121.2

Table 29. Serum cholesterol and triglyceride levels in captive-reared 12-week-old pheasants on an intermediate protein diet (28% protein first 6 weeks, 22% thereafter)

	Bird No.	Cholesterol (mg.%) Analysis 1	Cholesterol (mg.%) Analysis 2	Triglycerides (mg.%) Analysis 1	Triglycerides (mg.%) Analysis 2
Replication					
1	3601	140	142	105	104
	3602	192	196	43	42
	3603	132	132	41	44
	3604	130	132	94	91
	3606	172	167	42	37
	3607	160	161	46	46
	3608	156	150	80	80
	3609	168	168	50	50
	3610	163	164	90	88
	3611	174	168	50	45
	3612	154	158	68	68
	3613	150	151	100	98
	3614	137	137	89	91
	3616	128	124	85	82
	3617	150	150	103	106
	3618	122	120	78	78
	3619	164	164	86	86
2	3620	164	164	109	107
	3621	136	136	50	49
	3622	186	182	56	58
	3623	158	160	62	62
	3624	163	163	84	82
	3625	188	188	58	56
	3630	170	168	65	67
	3631	164	161	65	63
	3632	209	219	120	116
	3633	195	190	56	50
	3634	216	218	50	50
	3635	154	158	94	90
	3636	160	164	87	88
	3637	160	162	78	80
	3638	145	159	68	71
Average Replication 1		152.5	152.0	73.5	72.7
Average Replication 2		171.2	172.8	73.5	72.6
Overall Average		161.3	161.8	73.5	72.7

Table 30. Serum cholesterol and triglyceride levels in captive-reared 12-week-old pheasants on a high protein diet (36% protein first 6 weeks, 30% thereafter)

	Bird No.	Cholesterol (mg.%) Analysis 1	Cholesterol (mg.%) Analysis 2	Triglycerides (mg.%) Analysis 1	Triglycerides (mg.%) Analysis 2
Replication					
1	3658	104	106	47	51
	3659	150	152	42	42
	3660	90	94	45	50
	3661	94	94	54	38
	3662	131	134	48	42
	3663	104	104	50	48
	3664	90	88	40	35
	3665	122	120	56	54
	3666	112	114	38	36
	3667	154	152	46	45
	3668	130	136	50	44
	3671	122	122	51	49
	3673	84	88	41	38
	3674	78	78	38	38
2	3639	120	120	44	45
	3640	91	95	48	46
	3641	100	105	34	35
	3642	138	146	48	51
	3643	126	124	42	44
	3644	110	110	46	44
	3645	160	159	44	42
	3646	74	77	42	46
	3647	81	86	26	30
	3648	66	66	31	32
	3551	90	91	46	53
	3553	113	112	48	53
	3554	118	117	68	68
Average Replication 1		111.8	113.0	46.1	43.6
Average Replication 2		106.7	108.3	43.6	45.3
Overall Average		109.3	110.7	44.9	44.4

Table 31. Serum cholesterol and triglyceride levels in captive-reared 18-week-old pheasants on a low protein diet (20% protein first 6 weeks, 16% thereafter)

	Bird No.	Cholesterol (mg.%) Analysis 1	Cholesterol (mg.%) Analysis 2	Triglycerides (mg.%) Analysis 1	Triglycerides (mg.%) Analysis 2
Replication					
1	3676	188	185	48	47
	3677	143	142	80	87
	3680	188	190	52	54
	3681	80	82	10	12
	3682	166	166	44	44
	3683	174	175	46	46
	3684	214	224	40	40
	3688	112	110	41	38
	3690	182	188	64	65
2	3691	126	122	27	30
	3692	196	200	60	61
	3693	148	150	52	53
	3694	154	154	32	32
	3696	154	156	38	39
	3698	96	98	29	29
	3872	150	152	79	76
	3874	158	164	56	57
	3875	130	130	30	30
	3900	165	165	42	42
Average Replication 1		160.8	162.4	47.2	48.1
Average Replication 2		147.7	149.1	44.5	44.9
Overall Average		153.8	155.4	45.8	46.4

Table 32. Serum cholesterol and triglyceride levels in captive-reared 18-week-old pheasants on an intermediate protein diet (28% protein first 6 weeks, 22% thereafter)

		Cholesterol (mg.%)		Triglycerides (mg.%)	
	Bird No.	Analysis 1	Analysis 2	Analysis 1	Analysis 2
Replication					
1	3601	68	64	10	15
	3602	136	138	36	35
	3603	118	118	44	42
	3604	90	92	44	44
	3606	100	98	22	20
	3607	56	58	10	9
	3608	68	68	23	18
	3609	140	142	54	54
	3610	90	84	44	72
	3611	123	122	46	55
	3612	80	80	20	20
	3613	120	120	50	48
	3614	140	142	28	28
	3616	148	150	48	47
	3617	134	126	42	42
	3618	180	188	95	96
	3619	100	104	48	52
2	3620	94	95	23	22
	3621	110	112	58	56
	3622	152	152	32	34
	3623	130	126	36	38
	3624	164	164	34	34
	3625	150	150	73	74
	3630	132	134	28	24
	3631	104	102	38	39
	3632	136	138	40	40
	3633	124	120	30	32
	3634	172	178	35	34
	3635	112	106	112	110
	3636	114	116	38	38
	3637	112	110	46	48
	3638	146	150	58	56
Average Replication 1		111.2	111.4	39.1	41.0
Average Replication 2		130.1	130.2	45.4	45.3
Overall Average		120.1	120.2	42.0	43.0

Table 33. Serum cholesterol and triglyceride levels in captive-reared 18-week-old pheasants on a high protein diet (36% protein first 6 weeks, 30% thereafter)

	Bird No.	Cholesterol (mg.%) Analysis 1	Cholesterol (mg.%) Analysis 2	Triglycerides (mg.%) Analysis 1	Triglycerides (mg.%) Analysis 2
Replication					
1	3658	66	67	16	15
	3659	100	98	37	40
	3660	104	101	50	50
	3661	82	82	44	44
	3662	88	84	38	37
	3663	90	89	35	31
	3664	86	84	88	89
	3665	80	80	61	63
	3666	102	103	65	66
	3667	102	100	102	108
	3668	84	88	60	58
	3671	68	69	42	42
	3673	50	50	10	13
	3674	80	78	40	41
2	3639	86	86	38	40
	3640	94	93	32	32
	3641	98	96	28	28
	3642	140	138	30	29
	3643	123	119	52	52
	3644	92	94	68	72
	3645	84	82	30	31
	3646	86	88	42	44
	3647	94	92	38	37
	3648	88	89	44	40
	3651	66	66	43	40
	3653	84	85	51	51
	3654	100	100	44	44
Average Replication 1		84.4	83.8	49.1	49.8
Average Replication 2		95.0	94.5	41.5	41.5
Overall Average		89.5	89.9	45.5	45.8

Table 34. Serum cholesterol and triglyceride levels in approximately 18-week-old wild pheasants

	Bird No.	Cholesterol (mg.%) Analysis 1	Cholesterol (mg.%) Analysis 2	Triglycerides (mg.%) Analysis 1	Triglycerides (mg.%) Analysis 2
Replication					
1	3281	134	134	136	134
	3282	124	124	61	60
	3284	142	144	112	112
	3289	130	129	75	75
	3291	80	80	95	97
	3292	105	106	92	92
	3294	156	160	138	138
	3295	144	148	107	102
	3296	120	121	99	97
	3541	142	146	51	53
	3542	118	118	36	34
	3543	136	132	40	40
	99W	138	136	40	41
2	3280	140	140	80	80
	3283	160	160	76	76
	3285	114	114	63	60
	3286	130	128	65	65
	3287	188	189	85	84
	3288	142	144	126	126
	3290	126	128	73	72
	3293	138	136	72	73
	3297	178	172	86	88
	3298	180	182	82	82
	3299	164	161	60	57
	3300	146	148	96	99
Average Replication 1		128.4	129.1	83.2	82.7
Average Replication 2		150.7	150.2	80.3	80.2
Overall Average		139.1	139.2	81.8	81.5

Table 35. Serum cholesterol and triglyceride levels in wild pheasants captured at approximately 18 weeks of age followed by 8 weeks feeding of a 16% protein diet with 4% added cholesterol

Bird No.	Cholesterol (mg.%)		Triglycerides (mg.%)	
	Analysis 1	Analysis 2	Analysis 1	Analysis 2
3280	248	250	142	142
3283	204	204	127	124
3285	136	132	75	72
3286	161	160	104	103
3287	222	226	134	138
3288	164	166	103	103
3290	184	182	92	92
3293	214	218	98	93
3297	308	318	80	81
3298	176	180	107	110
3299	162	160	80	85
3300	164	158	110	109
Average	195.3	196.2	104.3	104.3

Table 36. Analysis of variance plan and observed mean squares for serum cholesterol levels in 12-week-old rooster pheasants on low, intermediate, and high protein rations

Source of variation	d.f.	Mean squares
Replication	1	543
Protein level	2	37685**
Low vs. intermediate	1	8681**
Intermediate vs. high	1	38702**
Error	74	604
Total	77	1567

**Statistical significance at P = 0.01 or less.

Table 37. Analysis of variance plan and observed mean squares for serum triglyceride levels in 12-week-old rooster pheasants on low, intermediate, and high protein rations

Source of variation	d.f.	Mean squares
Replication	1	239
Protein level	2	32976**
Low vs. intermediate	1	27906**
Intermediate vs. high	1	11810**
Error	74	527
Total	77	1366

**Statistical significance at P = 0.01 or less.

Table 38. Analysis of variance plan and observed mean squares for serum cholesterol levels in 18-week-old rooster pheasants on low, intermediate, and high protein rations and in approximately 18-week-old wild-reared rooster pheasants

Source of variation	d.f.	Mean squares
Replication	1	3985*
Protein level	3	18850**
Low vs. intermediate	1	14230**
Intermediate vs. high	1	13908**
Captive diet vs. wild diet	1	8625**
Error	98	706
Total	102	1272

*Statistical significance at P = 0.05 or less.

**Statistical significance at P = 0.01 or less.

Table 39. Analysis of variance plan and observed mean squares for serum triglyceride levels in 18-week-old rooster pheasants on low, intermediate, and high protein rations and in approximately 18-week-old wild-reared rooster pheasants

Source of variation	d.f.	Mean squares
Replication	1	79
Protein level	3	8788**
Low vs. intermediate	1	162
Intermediate vs. high	1	147
Captive diet vs. wild diet	1	26144**
Error	98	498
Total	102	738

**Statistical significance at P = 0.01 or less.

Table 40. Analysis of variance plan and observed mean squares for serum cholesterol levels in pheasant roosters prior to and following an 8 week period of 4 per cent cholesterol added to the diet

Source of variation	d.f.	Mean squares
Treatment	1	13119**
Error	22	1485
Total	23	1991

**Statistical significance at P = 0.01 or less.

Table 41. Analysis of variance plan and observed mean squares for serum triglyceride levels in pheasant roosters prior to and following an 8 week period of 4 per cent cholesterol added to the diet

Source of variation	d.f.	Mean squares
Treatment	1	3504**
Error	22	396
Total	23	531

**Statistical significance at P = 0.01 or l