1976

The Effects of Chronic Anaerobic Infection on Atherosclerosis in Rabbits

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THE EFFECTS OF CHRONIC ANAEROBIC INFECTION ON ATHEROSCLEROSIS IN RABBITS

BY

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B.S., Radford college, 1972

Thesis
Submitted in partial fulfillment of the requirements for the Degree of Master of Science in the Department of Pathology at the Medical College of Virginia
Virginia Commonwealth University
Richmond, Virginia
May, 1976

This thesis by Janet Smitheran Bissell is accepted in its present form as satisfying the thesis requirement for the degree of

Master of Science

Date: Approved:

4/13/76 .....

[redacted]

Advisor, Chairman

4/13/76 .....

[redacted]

4/13/76 .....

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Approved: [redacted]

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ACKNOWLEDGEMENTS

For his guidance and advice during the preparation of this thesis, I wish to sincerely thank my graduate advisor, Dr. Marvin J. Allison.

I also express special appreciation to David Klurfeld for allowing me the use of some of his techniques in the study of this animal model and for his advise and assistance.

Appreciation is expressed to the members of my thesis committee, Dr. Harry Dalton, and Dr. Herbert Welshimer for their helpful suggestions and interest.

The staff of the Microbiology Laboratory of MCV and W. E. Chandler are also thanked for their help in furnishing specimens and aiding me with this study.

I wish to express a special thanks to my husband Bob, for his patience over the last two years and also for his helpfulness and encouragement in this study.

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INTRODUCTION

Arterial disease is virtually a universal entity in man. Some cultures, such as the African Bantus have very little in the way of vascular lesions, while in the United States and other industrialized nations atherosclerosis affects a large segment of the population. With this in mind it is not hard to understand why ischemia, or arteriosclerotic heart disease, is second only to cardiovascular diseases in general as the major cause of death in the United States (4, 42).

Ruffer (112) noted the presence of arterial lesions in Egyptian mummies dating back 3,400 years. The presence of atherosclerotic lesions has also been reported in a 2100 year old Chinese mummy (36). Though atherosclerosis is an ancient disease it is seen more and more often in our culture in vascular disease.

The early sixteenth and seventeenth century anatomists were the first to make note of arterial lesions and guess at their origins. Over the centuries came theories and definitions that are at the least confusing. Virchow in 1856 (6, 7, 45) was the first to propose the theory of mechanical damage to the intima of the artery to account for the entry of blood plasma into the arterial wall. The lesions were attributed to chronic irritation. Marchand in 1904 coined the term atherosclerosis. At the time the dominant concept was one of arterial lesions

forming after there was an increase in sub-intimal connective tissue due to irritative or mechanical forces. Today much more is understood about the processes of arteriosclerosis, but definitions still vary among authors.

Arteriosclerosis is the broad generic term which literally means "hardening of the arteries" and involves the processes of thickening and loss of elasticity to arterial walls (42, 108, 109). Three distinct morphological entities are grouped under this broad heading; (1) medial sclerosis, (2) arteriolosclerosis and (3) atherosclerosis.

The medial or Monckeberg's sclerosis is characterized by calcifications within the media of medium to small arteries, particularly the femoral, tibial, radial and ulnar arteries.

Arteriolosclerosis is particularly common in hypertension and affects the small arteries and arterioles. It is distinguished by hyaline proliferation and fibrous and elastic hyperplasia of the media and the intima.

Atherosclerosis is the most commonly seen form of the arteriosclerotic diseases and when it occurs it has the most serious clinical consequenses. The aorta and other large elastic arteries of the extremities are the primary locations for atherosclerosis. The disease is characterized by patchy, nodular lesions due to a thickening of the

intima and a degenerative process in which lipid deposition occurs. The media may also become involved.

The earliest descernible lesion is termed a fatty streak. It presumably occurs over an area of stress to the vessels. Lipid is deposited in the site and soon macrophages and myocytes become filled with the fat droplets and form "foam cells". The intima is thickened and the elastic lamina is disrupted by extracellular fat. As the pathogenesis continues the arterial tissue attempts to repair itself and a fibrous capsule is layed down over the lesion thickening the intima even more. The term fibrous or pearly plaque now differentiates the lesion. The cells in the center of this lesion die due to a lack of oxygen and a necrotic mass, termed "gruel" is formed. From the vaso vasorum large capillaries or "sinusoids" invade the gruel and at the same time calcium is being deposited and the lesion can become calcified or "hardened". Up to this point the body has been doing though the usual healing and repair processes, but now destructive changes can occur. The cracking or breaking away of a plaque can cause hemorrhage or ulceration. These degenerative changes can be a grave threat when aneurysm or thrombus formation occurs.

It appears that this sequence of lesion formation must occur in waves throughout a lifetime since lesions of various size and age can be found side by side in the aorta.

In general however, there is agreement that in North America at least the fatty streaks appear during the first decade of life while pearly plaques are forming in the second and third decade. The complications and their consequences occur from the fourth decade on (16, 21, 72).

Atherosclerosis - Relationship with Infection

It has been established that inflammatory processes, whether mechanical or infective, can cause vascular lesions. Constantinides (21) and others (75, 86) give a long list of chemical and mechanical injury to the vessels which can help to contribute to arteriosclerotic lesions. Some of these include epinephrine, vitamin D, allylamine and freezing, surgery and post thrombotic arteritis. Researchers have found that bacterial endotoxins will produce lesions in rabbits (56, 57, 62). Gray (56, 57) found that endotoxin produced a degenerative arteritis in the small arteries characterized by endothelial vaculation, palisading and intiamal thickening. The pulmonary vasculature was the primary site of this involvement.

The early writings on vascular diseases make some standard remarks about certain infections leading to atherosclerosis (89, 101). Ophuls (101) in 1921 did a statistical study and showed that atherosclerosis was more prevalent in people who had had typhoid fever than in

those who had not. He concluded that arteriosclerosis is closely related to injury of the arteries resulting from various infections, and that these lesions reached their full development after the acute infection had long subsided.

Other researchers (46, 73, 90, 113, 130) have also attributed the possible formation of atherosclerotic lesions to infectious diseases. Some of these diseases include syphilis, rheumatic fever, typhoid fever, diphtheria and pneumonia. Typhoid fever has been shown to produce raised yellow plaques in the intima of the aorta and the coronary arteries (88).

Therefore the idea of atherosclerosis developing after acute or chronic infections is not new. The universiality of atherosclerosis in our society suggests that many other factors are also important in the pathogenesis of atherosclerosis. Some of these factors are hypertension, hyperlipidemia, obesity, cigarette smoking, and diabetes mellitus (42, 144).

Atherosclerosis is often thought of as an inevitable process of aging. With the increased human life span and therefore the increased geriatric population it is expected that an increase in atherosclerosis, it's complications and sequelae will occur. With this in mind this paper is interested not in infection as an initiating process in atherosclerosis, but in the effect that already present

atherosclerotic lesions have on an infective process. Does vascular disease enhance the infection and produce a cyclic reaction which then helps worsen the atherosclerosis?

There is evidence that atherosclerosis can act in the pathogenesis of disease. Rabbits fed an atherogenic diet show more permeability of their endothelial cells (135, 136). It is also established that the endothelial cells are capable of phagocytosis (40). With these findings in mind it can be postulated that bacteria could be capable of entry into a vessel through an atheroma or even be phagocytized and then initiate an infection. Theoretically microorganisms could become seeded in any area where there is injury to a vessel or associated structures such as the heart valves.

Bacteremia is a prime method for this seeding. Bacteremias are a common occurance and the body's reticuloendothelial system normally has no trouble clearing the circulation of microorganisms. Bacteremias can be initiated by brushing the teeth or chewing. Invasion of bacteria into the body can also occur from lacerations, wounds and focal points of infection such as pnuemonia or abscess. An increase in iatrogenic bacteremias is being seen due to the increase in major and minor surgical procedures, and the use of indwelling catheters for intravenous fluids or hemodialysis (85). This increase in bacteremias is documented in a 1965 study at the Medical College of Virginia

where there has been an increase since 1950 from 6.9 cases per 1,000 admissions to 17.3 patients per 1,000 admissions with bacteremias (25). The risk of serious complications secondary to bacteremia is greatly increased when significant cardiovascular disease is present.

An infection of primary concern is endocarditis. Injury to a heart valve is usually acknowledged as a prerequisite for bacterial endocarditis (108, 109). In the past endocarditis was most often associated with rheumatic heart disease, congenital heart anomolies and syphilitic lesions (1,4). Antibiotics have helped to eliminate the complications from rheumatic fever and syphilis so that endocarditis is now shifting from a predominately young age group into an older, male group (3, 38, 133, 142). The average age is fifty years and there is an increase in the over sixty age group. Atherosclerosis in the endocardium and heart valves may predispose to infective endocarditis (10). Aneurysms could become infected in the same manner. Bennett (11) found that a majority of infected aneurysms are of atherosclerotic origin and are found in elderly male patients.

Anaerobic Infections and Atherosclerosis

In the last decade an increasing number of anaerobes have been isolated in all types of infections. The clin-

ician is becoming more aware of the need for proper transport and culture of specimens suspected of containing anaerobes and at the same time the laboratory is evolving more sophisticated techniques for culture and identification of anaerobic bacteria.

There has been a striking rise in the number of anaerobes, particularly intestinal anaerobes, being isolated from blood cultures (95, 120). In Sonnenwirth's review (120) he finds at one institution, the Jewish Hospital of St. Louis, that intestinal anaerobes were isolated from blood cultures in 5.6% of all positive blood cultures in 1960. By 1970 they had increased to 9.9%. A similar increase occurred at the Mayo Clinic with 11% positive blood cultures for anaerobes in 1968-1969. They rose to 14.2% in 1972. The intestinal organisms most often isolated from these bacteremias are from the Bacteroidaceae family (Table 1). When classification of the isolants was performed it was found that Bacteroides predominated. In bacteremias as well as other infections (35, 82) Bacteroides fragilis is the most common species of intestinal anaerobe isolated.

This increase in bacteremias due to Bacteroides or to other intestinal anaerobes is usually associated with intra-abdominal surgery (14, 35, 58, 91, 134). One study (82) shows that of 200 infections due to B. fragilis, 133 related to the intestinal tract and 56% of these patients

had undergone intestinal surgery. Chow (19) and Moore (95) believe this increase in anaerobic infection is due to impaired blood supply to the tissue, therefore decreasing oxygen tension and setting up conditions to initiate the spread of anaerobes. In Chow's study (19) of 112 patients with Bacteroidaceae bacteremia, atherosclerosis was considered the second most common underlying cause of anaerobic sepsis. Of these patients with atherosclerosis, 90% were felt to have vascular insufficiency severe enough to cause the bacteremia.

Experimental studies in rabbits have shown that oxygen tension within atherosclerotic plaques of the aorta is lower than those in normal aorta tissue (66). A decrease in oxygen tension is also seen closer to the lumen of the vessel than to the adventitia. This, coupled with lower oxygen potential in devitalized tissue, whether from atherosclerotic changes or other tissue damage, can predispose to anaerobic infections on atherosclerotic sites.

The present investigation seeks to study the effects an anaerobe has on existing atherosclerosis. B. fragilis sp fragilis has been choosen for this study. B. fragilis belongs to the family Bacteroidaceae (17). These organisms are obligate anaerobes and gram negative rods which are non-spore forming and non-motile (29, 96, 123). Ninety to 95% of intestinal flora are obligate anaerobes. They are predominately in the terminal ileum and colon.

B. fragilis is found at a concentration of 10^{11} per g in feces (69, 71). B. fragilis has five subspecies; B. fragilis sp fragilis, B. fragilis sp distasonis, B. fragilis sp ovatus, B. fragilis sp thetaiotamicron, and B. fragilis sp vulgatus. B. fragilis sp fragilis is the most common isolant from clinical specimens, but it is the least common subspecies found in the intestines of the normal North American (69, 120).

B. fragilis is not considered extremely invasive, and it does not wall itself off. This may be due to heparinase production (49, 50, 71). The thrombophlebitis often seen with B. fragilis may be due to this heparinase production (52). Bacteroides can produce an endotoxin but it generally does not appear to play a great role in the pathogenesis of the organism (68). However, from a study of 112 patients with Bacteroides bacteremia it was found that 8 had symptoms suggestive of disseminated intravascular coagulation which was attributed to an endotoxin (147).

Bacteroides will elicit an antibody response (26, 28, 79). This has been found to be true for wound infections and abscesses as well as septicemias. Distinct antibody formation with no cross reactivity has been found in a number of species of Bacteroides (28).

The clinical symptoms of Bacteroides infections are hectic fevers, rigors, diaphoresis, and chills (19, 35, 71) with leukocytosis, anemia, hematuria, proteinuria and

hyperbilirubinemia. Complications can involve thrombophlebitis, emboli and metastatic abscesses; often to the lungs, which is considered by Tynes (134) to be a hallmark in Bacteroides sepsis.

Bacteroides was first reported as an etiological agent in 1897 by Veillon, Zerber and Hall who found the organism the cause of inflammation of the appendix (134). Since then Bacteroides has been implicated in many infections including bacteremias, wounds, and respiratory tract infections. The portal of entry of Bacteroides in the pre-antibiotic era of the 1930's and 1940's was usually felt to be the upper respiratory tract, nasopharynx, and ear. From these sites a septicemia could occur (15, 58). Currently the site of infection and spread is generally from the lower gastrointestinal tract.

Anaerobic endocarditis is also being documented (14, 34). In a report by Felner (35) of 33 cases of anaerobic bacterial endocarditis, 7 patients had Bacteroides endocarditis. Six patients were reported as having arteriosclerosis. Of 7 patients that died, 5 were over 39 years of age and had B. fragilis endocarditis.

Use of the Rabbit Model

Arterial lesions have been demonstrated in a large number of animals as diverse as goldfish to elephants.

Zoos are reporting an increase in the last decade in the severity and prevalence of atherosclerosis in their animals (22, 105). Ignatowski first produced lesions in rabbits by feeding them cholesterol and saturated fats in the form of meat and eggs. But Anitschow in 1912 (5, 6) was the first to produce the typical lesions by feeding rabbits pure cholesterol in vegetable oil.

The rabbit is the most susceptible animal to the production of atherosclerosis through lipemia alone or coupled with arterial injury (5, 6, 21, 23). The structure of the heart and the valves in the rabbit is similar to mans (59). The rabbit also lends itself to a study of this nature because of the animals large size. The relatively large cardiovascular system facilitates observation of the gross lesions. The rabbit also has prominent marginal ear veins which are convenient for intravenous injection and collection of blood specimens.

Many different diets by themselves or coupled with mechanical or chemical injury have been used over the years to produce atheromas (21,22). These include a wide range of chemicals and combinations of fats, eggs and other lipids. Cholesterol alone however will elicit a large degree of atherosclerosis in a short time. This is due to the rabbits ability to absorb very large amounts of cholesterol though their intestinal mucosa while at the same time they are not able to metabolize the substance. This

leads to a very high level of cholesterol in their serum, typically in excess of 1000 mg/100ml of serum. This causes the widespread deposition of cholesterol in the reticuloendothelial system and the vascular system (43, 44).

Some authors critize the use of the rabbit as a model in atherosclerosis and make the objections that the lesions produced in rabbits bear little resemblance to the lesions seen in man. Pollack (104, 105) points out that lesions seen in the rabbit resemble the "fatty streak" type lesion which is only found in the very early stages of atherosclerosis in man. Further, these lesions rarely evolve into the complications of ulceration and hemorrhage. But recently the use of a cyclic feeding schedule has ended some of these objections. It was found that by feeding rabbits a cholesterol diet for two months; then a normal diet for two months and then reverting back to a cholesterol diet; advanced lesions very similar to human atheromas could be produced (21, 22, 23). Furthermore, electron microscope studies of the morphology of these lesions bear out the similarities between the rabbit and human lesions (18, 121).

Specific Aims of Experiment

The first goal in this experiment is to produce a consistent atherogenesis in the rabbit model. At a time

when most of the animals are expected to have some degree of atherosclerosis, anaerobic bacterial suspensions will be administered intravenously. These transient bacteremias will be repeated several times in an effort to establish a non-fatal, but chronic infection in the animals. In this way it will be shown that atherosclerosis can be a predisposing factor to anaerobic infections and that the infection in turn can then increase atherosclerotic pathology.

Since the atherosclerosis is expected to contribute to a more severe infectious process, it is hoped that this model will also serve to study Bacteroides infection. In the literature there is no mention of an animal model in which a severe Bacteroides infection can be produced systemically. To date only abdominal abcesses have been produced by inserting capsules of various mucin, feces and bacterial combinations into the peritoneum of experimantal animals (67, 102, 141).

This paper will discuss the pathogenesis of anaerobic infection in atherosclerotic rabbits and will discuss the model in relationship to the increase in Bacteroides infections, particularly in the elderly and in conjunction with atherosclerosis.

METHODS and MATERIALS

Animals

Twenty eight female, albino, New Zealand rabbits were used in this research. Thirteen of the animals were obtained from York Animal Farm, Richmond, Va. While the other fifteen were from Pel-Frez, Rogers, Arkansas. The animals had an initial weight of approximately 2.5 kg.

The rabbits were divided into three groups. The first group initially consisted of 18 rabbits which were fed the cholesterol diet and were injected with B. fragilis. These animals will be referred to as group I. The first 8 animals in this group were from York Animal Farm and will be referred to as group Ia. Animals 6 and 7 of group Ia died before the bacterial treatments started and therefore will be excluded from the results. The total number of animals in group Ia is 6.

The second 10 animals to receive the cholesterol diet and the bacterial injections came from Pel-Frez. These animals will be referred to as group Ib. All of these animals survived the cholesterol feeding and will be included in the results.

Included in this study were two control groups. Five animals received the cholesterol diet but no injections of Bacteroides. These animals are in group II. Two drug

control animals are included in this group. The second control group consisted of 5 animals maintained on a normal diet and who recieved the Bacteroides injections. These animals will be referred to as group III. Table 2 summarizes the treatments of the animals.

Care, Feeding and Conditions of Animals

The animals normal food consisted of Purina Laboratory Rabbit Chow 5301 (Ralston Purina Co., St. Louis, Mo.). This food contains no antibiotics nor antibacterial agents and is composed of plant products, iodized salt, minerals and vitamins. Table 3 lists the ingredients of the rabbit chow.

The cholesterol diet was produced by the addition of 1% cholesterol to the regular diet. A powdered cholesterol (Sigma Chemical Corp., St. Louis, Mo.) was dissolved in diethyl ether in a ratio of one gram of solute to five ml of solvent. After pouring this solution over the food and thoroughly mixing, it was spread in shallow trays to allow the ether to evaporate. Upon drying, the pellets were a paler color than the untreated food. Due to the large volume of food that needed to be prepared it was convenient to mix batches of 1,200 g of food pellets and a cholesterol solution of 13.2 g of cholesterol dissolved in 65 ml of ether.

A previous study (76) showed that the rabbits ate the

cholesterol food as readily as the untreated diet. Therefore each animal was given the normal or the treated diet ad libitum according to his treatment schedule. Tap water was also supplied ad libitum.

The animals were housed in individual cages with wire floors measuring 50 cm by 35 cm by 55 cm. On receiving the animals theywere maintained on a normal diet for one week to check the general health of the rabbits as well as to let them acclimatize to the animal room.

Rabbits on the cholesterol diet were maintained on it for eight weeks and then returned to a normal diet to allow the accumulated cholesterol to be removed from their reticuloendothelial system. From a previous study (76) it was found that this eight week regimen usually produced atherosclerosis in at least the aorta, pulmonary, and coronary arteries.

The health of the animals was apparently not affected by factors other than experimental treatments. Some of the animals developed foot papillomata on the underside of their paws. Most animals fed the cholesterol diet showed varying degrees of alopecia, believed to be caused by cholesterols deposition in the skin. Neither the papillomas nor the alopecia appeared to outwardly affect the health of the rabbits.

During the course of the experiment two animals (6 and 7) died of unknown caused while still on the cholesterol

diet. These animals are not included in the study.

Several of the animals used in this study demonstrated a parasitic infection known as coccidiosis. A sporozoan, Eimeria stiedae, is the etiological agent. The parasite is endemic in rabbit populations and there is no effective treatment for it. The bile ducts of the liver are usually affected, but only mildly, and the health of the animals is not visibly impaired. Only on microscopic examination of the liver was any evidence of coccidiosis seen in these animals. There was usually fibrosis and proliferation of the bile ducts.

Bacteriologic Techniques

For this experiment a strain of B. fragilis sp fragilis was used. It was obtained and identified by the Anaerobic Laboratory of Virginia Polytechnical Institute, Blacksburg, Virginia. The organism had been isolated from a lip wound and found to exhibit the following biochemical reactions; H_2S +, nitrate +, indole -, glucose +, manitol -, rhamnose -, and trehalose -. The organism was maintained on blood agar plates (BAP) in anaerobic conditions supplied by a Gas-Pak jar (BBL, Baltimore, Md.). The organism was transferred to Pre-Reduced Anaerobically Sterilized Brain Heart Infusion (PRAS-BHI) supplemented broth tubes (Scott Laboratories, Fiskevile, Rhode Island), and incubated at 35 C

to obtain a 16 to 18 h culture of the Bacteroides. These cultures were used for injecting the rabbits by first pooling the broth from the tubes and centrifuging at 5000 revolutions per minute for 10 min. The supernatant was decanted and the bacteria washed and resuspended using a 0.9% sodium chloride solution (TRavenol, Deerfield, Ill.) and then shaken on a Vortex Genie Mixer (Scientific Products, Evanston, Ill.) for 10 sec. The PRAS-BHI tubes originally had 5 ml of broth in them, and an equal amount of saline was added for the washing process. The bacterial suspension was centrifuged a second time and the supernatant again decanted to remove all media and soluble toxins. The organisms were mixed with the normal saline in a ratio of 1 ml of saline for every 5 ml of the original broth and resuspended on the mixer for 1 min to break up any clumps of bacteria that could possibly block small caliber pulmonary blood vessels. After making serial dilutions of this final suspension and planting 0.1 ml of each dilution on BAP for overnight incubation; 1 ml of the final suspension was injected into the marginal ear veins of the rabbits. It was found that the final innoculum contained a range of $1,0 \times 10^8$ to 9.0×10^9 colony forming units with an average of 2.0×10^9 colony forming units.

Since this study involves the effects of long term chronic infection, the rabbits were given injections at three different intervals with five injections in each

interval. Fever was used as the parameter to monitor the disease process. Group Ia received injections first, so that when a rectal temperature of 40 C or more was exhibited by one of the rabbits in this group, the injections were stopped and all the animals were placed on oral doxycycline until the fever went down. For this reason all of the rabbits did not receive the total fifteen bacterial injections.

Drug Treatment and Studies

Doxycycline (Vibramycin, Pfizer Laboratories, New York, N. Y.), one of the newer derivatives of tetracycline was used to treat the severly infected animals. From a previous study (12) using an anaerobic agar dilution technique, it was found that the strain of Bacteroides used was susceptible to doxycycline at a minimal inhibitory concentration (MIC) of less than 0.5 ug/ml.

This antibiotic is only available in oral or intravenous injection. For convenience the oral suspension (doxycycline calcium) was used. This is distributed in a raspberry apple flavored syrup with a concentration of 5 mg of drug for every ml of the syrup. The drug dosage was calculated at double a pediatric dose, which is 4.4 mg per kg of body weight. The rabbits were administered the drug by licking it from a graduated pipette. Since the

syrup was thick and the rabbits apparently liked the taste, they took the drug with no difficulty.

To evaluate the drug levels being achieved, blood samples were initially drawn from rabbits in the cholesterol fed, infected group (Ia) at 3 h, 24 h, and 3 days. This was accomplished by pinching off the marginal ear vein and puncturing it with a 25 gauge needle. On removing the needle about 1 to 2 ml of blood was allowed to drip into a small test tube. After clotting, each sample was spun in a serofuge (Clay-Adams, New York, N. Y.). The serum was removed and stored at -70 C until a time when the specimens could be run.

When these specimens were tested by using a micro assay technique employing seeded agar plates it was found that no drug level could be detected. Other trials run using normal rabbits that were not fed a cholesterol diet, gave the same results.

The intravenous drug (doxycycline hyclate) was then tested to see if it would produce a detectable drug level. Initially a normal, non-cholesterol fed rabbit was given a 4.4mg/kg of body weight dose of the doxycycline and the serum obtained at 5 min, 30 min, and 1 h. The drug was reconstituted with 10 ml of normal saline and the calculated dose given using the marginal ear vein and injecting with a syringe. The drug was administered at about 1 ml/min to help minimize thrombosis to the vein. The rabbits showed

no ill effects. When serum samples from this trial were run, detectable drug levels were found. The two drug control rabbits (1 and 8) were then given the recommended pediatric dose of doxycycline and samples were taken at 30 min, 1 h, 2 h, 4 h, and 8 h. Detectable levels were achieved, therefore the remainder of the study employed the doxycycline hyclate (intravenous) when an animal exhibited a fever of 40 C or higher.

To measure the antibiotic serum levels a microtechnique using agar diffusion was adapted with a modification of techniques used by Schering Corp. (116), Warren (138) Washington (139) and Stroy (122). The technique involves seeded agar plates on which paper discs impregnated with known standard or test serum are placed. After incubation the diameters of the zones of inhibition are measured. The standard curve zones are plotted on semilog paper and the zone diameters of the test serum are extrapolated from the standard curve to get the serum level of the drug.

The indicator organism used was a Staphylococcus epidermidis obtained from the Clinical Microbiology Laboratory at the Medical College of Virginia. This organism was tested and found to have an MIC of less than 0.312 ug/ml. An overnight culture of the Staphylococcus grown in Tryptic Soy Broth (TSB) was diluted 1:20 and 2 ml of this dilution were added to 198 ml of molten Tryptic Soy Agar (TSA). Plates were then poured with 10 ml of agar

being evenly distributed in 200 ml petri dishes. These plates were good for 1 week if kept refrigerated.

To prepare standards for the assay, normal rabbit serum was obtained by cardiac puncture and tested alone on a seeded plate to insure there would be no inhibition from the serum alone. Standard serums of 10 ug/ml, 5 ug/ml, 2.5 ug/ml, and 1 ug/ml were then prepared from a stock standard of 1,000 ug/ml of doxycycline and normal rabbit serum. The standard sera and the test sera were than saturated on one-half inch blank paper discs and placed on the seeded plates. The plates were incubated overnight in a 35 C incubator. Each plate contained two discs for each standard and two discs for each test serum. The plates were run in duplicate, and the zones of inhibition were measured and averaged for each specimen and standard.

Consistently clear, reproducable zones could be obtained with the 10 ug, 5 ug and 2.5 ug standards. At times the 1 ug standard gave a hazy zone that was difficult to measure.

Autopsy

Autopies were performed on all the animals, either when they were found dead or within thirty minutes of being sacrificed with an intravenous injection of 2 ml of sodium pentabardital (Diabutal, Diamond Laboratories, Des Moines,

Iowa). Approved autopsy procedure was followed as closely as possible (2).

After an external examination to note any pathology, a ventral, midline incision from throat to pelvis was used to open the skin. Blunt dissection was used to separate the skin from the fascia. The thoracic cavity was then opened and inspected for adhesions or effusions. At this point heart's blood was taken for culture. The right ventricle was seared to dryness with a scalpel heated in the flame of a bunsen burner. Five to ten ml of blood was then removed from the seared area with a sterile needle and syringe and placed in blood culture bottles. The bottles consisted of slants of TSA (Difco) and 90 ml of Brain Heart Infusion (BHI) semisolid broth (Difco) with para-aminobenzoic acid and penicillinase. A rubber diaphram acts as closure and the route of innoculation for the blood, by using the needle and syringe. Cultures of lung were taken by again searing the organ surface and then, with a sterile scapel blade, making an incision within the bounds of the seared area. A sterile swab was then inserted into the incision to collect the specimen and the swab placed in a tube of TSB.

Next the abdomen was opened and the liver and spleen cultured in the same manner as the lung. Urine cultures were obtained using the method for blood cultures and then all tubes were placed in a Gas-Pak jar and incubated.

After cultures were completed, abdominal organs were removed, weighed and multiple slices made to look for any pathology. Sections of each organ were placed in 10% buffered formalin to fix for later histiologiclal evaluation. The formalin was prepared by adding 4.0 g of sodium phosphate monobasic and 6.5 g of sodium phosphate dibasic (Merck and Co., Inc. Rockway, N. J.) to each liter of formalin.

The brain was removed intact from the calvarium and fixed in formalin without sectioning the organ first. The heart, lungs, pulmonary artery and aorta were removed intact down to the iliac bifurcation so that the atherosclerosis and other pathology could be studied in more detail. The heart was cut in the usual manner to open the chambers of the organ for observation. The aorta was left attached to the heart but was opened with a cut down its entire length. When significant pathology was found the heart and aorta were pinned flat on cardboard and floated upside down in formalin for 24 h. This prevented the aorta from curling and better facilitated evaluation of the vessel for atherosclerosis.

Grading of Aortic and Pulmonary Lesions

Since the rabbit aorta is relatively small, the aorta and heart were stained using a fat stain to enhance

the lesions on the great vessel and heart valves. The procedure used was a modification of Holman (69). The formalin fixed tissue was rinsed in 70% isopropal alcohol and then placed in a 1% solution of Sudan IV (Hartman-Leddon Co., Philadelphia, Penn.) in 70% isopropal alcohol. Staining was complete in 15 to 20 min followed by a rinsing period in the alcohol of approximately 5 min. The tissue was agitated periodically and removed from the alcohol after the unaffected vessel wall retained no red color. The Sudan IV gave any fat tissue a bright red color and gave atheromas a pale pink to red color, and revealed subintimal fat deposits which were not visible on gross examination before the staining.

After staining, each aorta was then evaluated for its extent and type of atherosclerosis using a modification of a technique used by Gore and Tejarda (55). The traditional grading of atherosclerosis to determine the extent or quantity of intimal involvement is given in table 4. The letters on the left are the grouping symbols Gore and Tejarda use. Therefore a letter 0 represents a 0-5% area of the aorta as being affected by atherosclerosis. A represents 6-15% of the aorta; B represents 16-33%; C represents 34-50% and D represents 51-100% of the aorta as being affected.

To evaluate the type of lesions seen, four categories are employed. These are in table 5 and are considered to be in the order of their significance as well as their dev-

elopment. These are probably the most widely used and traditional categories or grades. Grade I represents the least complicated lesions the fatty streaks, spots or patches. Grade II are the elevated fibrous plaques; grade III is used to denote plaques with necrosis, ulceration, or hemorrhage. The final grade, grade IV, represents the calcified plaques. By using these criteria in tables 4 and 5, the extent and type of atherosclerotic lesions yield an "atherosclerotic profile." A group letter is assigned to the extent of involvement of the lesions and is followed by a number of four digits which corresponds to one of the grades of lesions. Therefore the first digit corresponds to grade I, the second digit to grade II and so on to the fourth digit. Each digit may be a number from 1 to 10, but the four digits must add up to 10 unless the are 0, where no atherosclerosis is present. A profile for example would be D(1,2,5,2). The "D" represents a severely diseased aorta with more than half of its intima involved while the digits tell that 1/10 of the lesions are grade I, 2/10 are grade II, 5/10 are grade III, and 2/10 are grade IV.

To help evaluate this profile it is changed to an "atherosclerotic index". This involves assigning weights to the factors involved. Table 4 gives a linear progression of weighting based on the area or extent of involvement. The inference is that the extent of atherosclerosis is directly related to its clinical importance. It is felt how-

ever that the physiologic process of growth and regeneration is of semilogarithmic relationship and better reflects the differences between the fatty streaks and ulcerated plaques. Table 6 shows the proposed weighting for the grade of type of lesions. Grade III and IV are grouped together because there is no basis for placing calcified lesions as more important than ulcerated or necrotic plaques. So using the example cited by Gore and Tejarda (55), a profile of D(1,2,5,2) would give an index of:

$30(1/10 \times 1 + 2/10 \times 10 + 5/10 \times 100 + 2/10 \times 100) =$

$30 \times 72.1 = 2163.$

Since the theoretical range is from 0 to 300, the use of a constant 1/30 reduces the range to 0-100, and makes the final index of this example :

$2163 \times 1/30 = 72.1$

An attempt was made to also evaluate the extent of atherosclerosis in the pulmonary arteries. A histological slide of the lung was chosen at random on each animal and the arteries on the entire section were taken into consideration. The arteries were then assigned a percentage number according to the overall extent of atherosclerosis. The values could range from 0-100%; 0 being no atheromatous changes being seen in any of the arteries, while 100% would indicate complete occlusion of vessels.

Histological Evaluation

All tissue sections obtained were submitted for histological examination. The following stains were used; hematoxylin eosin for cellular structure, Giemsa's stain for hematologic evaluation, gram stain for bacteria, and Movat's stain for mucosubstances.

RESULTS

Atherosclerotic Pathology

All but one of the rabbits in this study who were fed the cholesterol diet showed some degree of atherosclerosis. Rabbit 8, a drug control animal who recieved the cholesterol diet but no Bacteroides (group II), showed no fat deposition in its aorta, even after staining with Sudan IV. There were some signs of small areas of lipid deposition in the adrenal and liver, but no atheromatous lesions could be found in any of the vessels in any organ system.

All of the other cholesterol fed rabbits had some degree of atherosclerosis in their aortas. For gross evaluation these plaques were intensified by staining with Sudan IV (fig 1). The extent and type of atherosclerosis is given in table 7, along with the atherosclerotic index and profile and the extent of involvement of the aortic valves. A majority of animals showed lesions on their aortic valves (fig 2 and 3).

There is no correlation between aortic and pulmonary atherosclerosis, but for this study an attempt was made to grade the atherosclerosis in the pulmonary arteries. The pulmonary arteries showed the typical foam cell lesions (fig 4), but these lesions did not appear to evolve into fibrous plaques to the same extent as the lesions in the

aorta. An interesting finding in this study involved the pulmonary veins. Of the animals in the cholesterol fed-infected group (I), 5 out of 16 or 31% of the animals had foam cell lesions in their pulmonary veins (fig 5 and 6). These venous lesions were not found in the cholesterol fed non-infected animals (group II) or the non-cholesterol fed, infected rabbits (group III). Table 8 lists the rabbits with the venous lesions and gives the extent of pulmonary artery atherosclerosis of each animal. Four of the animals in group I showed areas of questionable lesion formation in their pulmonary veins. In these areas the vessel wall was thickened and fibrosed.

The only other organ to show consistent atheromatous changes in the vessels was the heart. In this study the term coronary vessel is used to denote any vessel seen in the epicardium, myocardium or endocardium. Figure 7 shows the atheromas in a large coronary artery. A number of animals showed the most severe coronary vessel lesions in the papillary muscles of the heart. These lesions severely diminished vessel diameter and at times occluded the vessel (fig 8). Because the lesions in the papillary muscles were severe and in 5 animals were the only coronary vessels involved, these lesions were considered separately on table 9. Of all the cholesterol fed animals (groups I and II) 13 out of 21 or 68% had atheromas in the coronary vessels or the vessels of the papillary muscles.

The aortas displayed the most advanced lesions. Macroscopically the lesions were pale yellow and slightly elevated and appeared initially at sites where the vessels branched and where the hydrostatic pressure was greatest, for example at the root of the aorta and over the arch. A majority of the animals had fatty streaks even at the iliac bifurcations. Lesions spread from these initial sites to involve wider areas of the intima. In areas where fibrous caps had formed, the lesions took on an intense white color and the lesions were elevated.

Microscopically the lesions were all very similar in appearance. Typically they were a foam cell lesion (fig 9) where the cells of the intima took on a foamy homogenous appearance from lipid deposition. After cessation of the cholesterol diet the lesions progressed to fibrous plaques (fig 10 and 11). Rabbit 10 exhibited a sterile abscess in the aorta (fig 12 and 13). This gruel formation is from the death and necrosis of the foam cells at the bottom of the plaque. Rabbit 2 exhibited calcium formation in the aorta (fig 14).

Not only the blood vessels showed the effects of the cholesterol diet, but several of the organs underwent change as well. The liver showed lipid deposition, particularly near the central vein, which gave the cells a lacy appearance, but no cholesterol clefts or foam cells were present. None of the spleens showed foam cell, but

rabbit 2 did have some atherosclerotic lesions in the central arteries.

The lungs of 2 of the animals (rabbit 2 and 5) showed foam cells throughout the lung tissue, with the largest accumulations on the periphery of the lobes.

The adrenals of all the cholesterol animals showed some degree of cholesterol deposition, primarily in the cortex. The cells showed marked swelling and patches of necrosis. Cholesterol clefts could be seen in these areas of necrosis.

The gall bladder of rabbit 2 had some cholesterol gravel, the largest piece being 1mm. No obstruction or stones were found in the ducts.

Drug Studies

The majority of the drug evaluations were done on the cholesterol fed-infected rabbits (group Ia). This included infected animals 2, 3, 4, 5, 9 and 10 as well as animals 1 and 8, the cholesterol fed, non-infected drug controls. During the first series of injections these rabbits were put on oral doxycycline when 2 out of 6 of the animals developed a fever. Serum levels were assayed at 3h, 24h, and at 3 days. At none of these times was a drug level detected. Since the system used was reliable at approximately lug at the lowest standard, it was felt that the oral drug was not being absorbed or was at levels to low to detect.

After switching to I V doxycycline a normal rabbit was given a pediatric dose of antibiotic, 4.4 mg/kg of body weight. Samples taken at 5 min, 30 min and 1 h all gave zone sizes. At 1 h the drug level was approximately 5 ug/ml.

The drug controls, rabbits 1 and 8, were then administered the drug to test for a detectable level in cholesterol fed rabbits. These rabbits were given pediatric doses of the doxycycline and serum taken at 30 min, 1 h, 4 h, and 8 h. At 30 min the levels were approximately 5-6 ug/ml but by 8 h the level had again fallen to less than 1 ug. Figure 15 gives the doxycycline standard curve for rabbits 1 and 8. The zone diameters along with the serum concentrations extrapolated from the standard curve are given in table 10.

For the remainder of the study only animals developing a fever of 40 C for more than one day were given the recommended pediatric dose of I V doxycycline. Only 2 animals, rabbit 2 and 17, fell into this category. Animal 2 recieved the I V antibiotic during the second series of injections when it appeared acutely ill with a fever of 41 C, and showed no response when it was approached or touched. Rabbit 17 developed a fever of 41 C during its first series of injections. It appeared to be having difficulty breathing and audible wheezing could be noted. Both animals showed marked improvement after treatment with the anti-

biotic. Rabbit 2 recieved the antibiotic for 2 days, while rabbit 17 recieved only one injection of drug to lower its temperature. Table 11 lists the animals who received antibiotic along with the amount and type received.

Bacteroides Infection

Only 4 animals from the cholesterol fed-infected animals (group I) showed a fever. Rabbit 2 had a rise in temperature at all three series of injections. At one point in the last series of injections this rabbit had a fever of 41 C. Since this was the last series of injectons no antibiotic was administered. The rabbit did not die and its fever went down by the third day of this last set of injections. Rabbit 5 also had a fever during the injections she recieved. Oral doxycycline was given, but no I V drug was administered since this rabbit was sacrificed to check the progress of the arterial disease and the infection. Two other rabbits, 17 and 9, showed fevers at only one series of injections and then only for an interval of 1 or 2 days. The remaining Bacteroides infected animals (group I and III) developed no fever or overt clinical symptoms of disease.

Initially 2 animals were sacrificed after they had recieved only two series of injections. This was to monitor the extent of the atherosclerosis and the infective process. Since the degree of clinical illness in the animals as a

whole was minimal it was necessary to see what degree of infection the Bacteroides was eliciting.

Rabbit 5 and 9 from group Ia were chosen for this purpose. On autopsy rabbit 5 revealed no remarkable pathology. The aorta was mildly affected by atherosclerosis as was the aortic valve. The lungs showed some consolidated areas with a few pale nodular areas. The liver showed very mild fatty changes. This rabbit had a fever of 40 C but 5 cultures were taken and all were negative for Bacteroides or any other organism. Histologically the lungs revealed no acute pneumonic process, however there was a mild bronchitis and some emphysema. The striking finding was the extensive pulmonary atherosclerosis in the arteries. Atheromas of the foam cell type were also found in pulmonary veins (fig 5). The heart showed only very minimal fat deposition and no vessel involvement. The only other organ with any pathology was the adrenal gland which showed marked cholesterol deposition and vessel involvement. The only other organ with any pathology was the adrenal gland which showed marked cholesterol depostion in the cortex with some cholesterol clefts. There were scattered chronic inflammatory cells and scattered calcium deposits.

Rabbit 9 on autopsy showed a very similar picture to 5; however the degree of aortic atherosclerosis was minimal and the aortic valve was not involved nor were the coronary arteries. This rabbit had had a fever on the first day

of the second series of injections and on culturing the blood, lungs, liver, spleen and urine, B. fragilis was grown from the spleen. Histologically the lungs again revealed no acute pneumonic process but some mild pleurisy, bronchitis and emphysema were noted. The pulmonary artery had some atherosclerotic lesions but the veins showed no atherosclerosis. The adrenals in this animal were very similar to the cholesterol fed, non-infected rabbits (group II). There were only very small areas of cholesterol deposition with no inflammatory cells or calcium deposits. The heart in this animal showed one small area of a resolving mural thrombus.

All but 2 of the remaining 14 cholesterol fed, infected animals (group I) finished the planned course of injections. Rabbit 10 was found dead in his cage 40 days after the last injection of Bacteroides and 44 days after cessation of the cholesterol diet. The rabbit had only received 3 injections. A blood culture was taken but grew no Bacteroides. On autopsy a thrombus in the heart was shown to be the immediate cause of death. The stomach was perforated and contained a hair ball but no suppuration was seen. There was an extensive pneumonia with edema and hemorrhage. The histology revealed pneumonia with edema, bronchitis and emphysema. An inflammatory exudate of mononuclear cells was present. The adrenals also showed large areas of hyperemia, necrosis with inflammatory cells and calcium deposits.

Rabbit 18 was also found dead in its cage. The rabbit had that morning recieved the third in the second series of injections. The rabbit had exhibited no fever and had shown no signs of destress. Of the routine cultures taken all grew B. fragilis. The immediate cause of death is unknown, but on autopsy the rabbit showed a consolidated pneumonia, with much mucous and fluid in the lungs. The stomach had perforated, but no supparation was seen. The aorta and pulmonary artery had evidence of atherosclerotic lesions. Histologically the lungs showed an extensive pneumonia with bronchitis, hemorrhage and areas of vasculitis (fig 16 and 17). The adrenals showed inflammation and large areas of necrosis and calcium deposition. In view of the extensive pneumonia it was felt that the animal died from its pneumonic process.

The remaining cholesterol fed-infected animals were all sacrificed and will be presented in a group along with the control animals (group II and III). Table 12 summarizes the number of days each rabbit in group I had been off the cholesterol diet along with the total number of injections it had received.

The yield of positive blood cultures from the infected animals was low. None of the non-cholesterol fed, infected animals (group III) grew Bacteroides, while only 5 out of 16 of the cholesterol fed-infected animals (group I) showed positive cultures. Table 13 lists the animals with positive

cultures. Two animals had positive blood cultures and 2 animals exhibited positive spleen cultures which would indicate bacteremias in these animals. Three of these animals (4, 11 and 12) had an active pneumonic process while rabbit 9 showed resolving areas of pneumonia with mild pleurisy, bronchitis and emphysema.

None of the rabbits showed any indication of an infection which had initiated on an atherosclerotic plaque, such as an endocarditis or a mycotic aneurysm. Three of the cholesterol fed-infected animals (group Ib)had arteritis in the lungs, but not in areas of plaque formation (fig 18).

The lungs contained the main area of pathology. A pneumonic process with mononuclear inflammatory cells was seen in 9 out of 16 or 56% of the cholesterol fed-infected animals (group I). Pleurisy, bronchitis and emphysema also were seen in a majority of these animals. From group Ib, pulmonary abscess was seen in two animals (fig 19); thrombophlebitis was seen in 3 animals and arteritis in 3 animals (fig 20). Of the cholesterol fed, non-infected animals (group II) only one showed a mild resolving pneumonia, while 2 of the animals showed pleurisy, 1 a mild bronchitis and 3 an emphysema (fig 21). One of the drug controls (animal 8) exhibited an arteritis. None of the cholesterol fed, non-infected controls showed thrombophlebitis.

Of the non-cholesterol fed, infected controls (group

III) showed a mild resolving pneumonia and bronchitis. One animal had emphysema and another pleurisy. None of these controls showed abscess, arteritis or thrombophlebitis.

There is no significant difference in the non-cholesterol fed, infected controls (group III) and the cholesterol fed-infected animals (group I) as to the number with pneumonia. But the controls showed a milder pnuemonia and fewer complications. Table 14 is a summary of the lung pathology seen in the animals. It would also appear that the second group of cholesterol fed-infected animals (group Ib) show a more severe pneumonic process than group Ia. Group Ia showed less active pneumonia and pleurisy and they also showed no arteritis, thrombophlebitis or abscess formation.

Animal 14 showed a small area of hemorrhage and inflammation in the left ventrical of the heart and a mild vasculitis. The animal also showed a bronchopneumonia with thrombophlebitis in the lungs.

The only other organ to show any significant pathology was the adrenal gland. The non-cholesterol fed, infected animals (group III) showed no areas of necrosis and fat deposition comperable to that seen in the cholesterol fed rabbits. However, all the adrenals in the cholesterol fed groups (group I and II) showed mild inflammatory response with infiltration of chronic inflammatory cells; mostly lymphocytes.

The cholesterol fed, non-infected controls (group II) showed areas of lipid deposition in the cortex with slight necrosis and cholesterol clefts. Of the cholesterol fed-infected animals, (group I), 3 showed a process similar to the cholesterol fed, non-infected controls,but the other 13 animals all showed abundant cholesterol deposition as well as inflammation with mononuclear inflammatory cells (fig 22). Four of the rabbits also showed calcium deposits in the adrenal cortexes (fig 23 and 24), as well as large areas of necrosis, hyperemia and inflammation. (See table 15).

In summary the major significant findings were those of venous pulmonary lesions only in the cholesterol fed-infected animals (group I). The animals showed a pneumonic process identical to that in humans infected with B. fragilis. The adrenals of these rabbits also showed large amounts of cholesterol deposition and necrosis along with inflammatory cell response and in some instances focal calcium deposits.

DISCUSSION

The cholesterol diet fed to the animals in group I and II followed by a waiting period of approximately two and a half months before autopsy, gave a degree and type of atherosclerosis similar to that seen in more advanced human disease. Fibrous cap formation was seen in a majority of the animals and in some instances gruel and even calcium deposits were noted. No hemorrhagic or ulcerative lesions were seen. Before this cyclic feeding regimen was instituted by Constantinides (21, 23) one of the objections to the use of the rabbit as a model for human atherosclerosis was the fact that it produced primarily xanthoma tuberosum type lesions (104). These foam cell type lesions were reminiscent only of the very early human lesions; the fatty streak.

A majority of the animals in this study also developed atheromas on their aortic valves. These atheromas were of the foam cell type. No previous mention of valve involvement in cholesterol fed rabbits can be found.

The degree and type of atherosclerosis that can be produced in the rabbit is subject to many variables. Pollack (104, 105) points out that the age and breed of the rabbit is a factor that must be considered. Older aniamals generally develop more extensive and advanced lesions. The time of year and even the amount of excersice the

animals are permitted will affect the degree of atherosclerosis. The type of atherogenic diet itself can cause variations. Often a liquid oil (corn oil, etc.) is the vehicle used to administer the cholesterol, but researchers often do not report on the type or amount of cholesterol ingested or the age and conditions of the rabbits. It is often difficult to compare results when the variables of a study are not outlined.

There were only a few references found in the literature in which a cholesterol diet was administered in the same manner as this study, and both showed comparable results (23, 65). They found the pulmonary artery and its branches to be severly affected by atherosclerosis. The coronary arteries, particularly those supplying the papillary muscles were partially or totally occluded. The reticuloendothelial system, particularly the liver, was affected. The liver showed lipid deposition, primarly around the central viens.

Heptinstall (65) reported that the animals in his study showed enlarged adrenal glands which were white in color and the cortical cells, particularly in the zona fasciculata, were swollen. Scattered eosinophils were present in the cortex.

Nowhere in the literature could any reference be found that mentions atheromatous lesions occuring in the pulmonary veins of the rabbits fed the cholesterol diet.

Constantinides (21, 22) specifically states that in his experience, lesions of the veins do not occur in rabbits. This agrees with our study of cholesterol fed rabbits alone, but in cholesterol fed and infected animals venous lesions were found.

In man, the lesions of atherosclerosis are irregularly distributed. The aorta, coronary arteries and cerebral arteries are generally the primary sites of involvement. The aorta is most heavily involved at the arch and along the intercostal branches and the abdominal branches down to and including the illiac bifurcation. The large arteries of the lower limbs are more effected than those of the upper limbs. The main epicardial branches of the coronary arteries have a higher incidence of atherosclerosis than the smaller intermural branches. Patchy distribution of lesions is seen in the cervical and cerebral vessels, with a concentration of the lesions at the bifurcations of these vessels (42). Pulmonary atherosclerosis bears no relation to the severity of the disease in the aorta (80). Pulmonary hypertension can be involved where it produces medial hypertrophy, intimal thickening and an acceleration of atheroma formation. In these cases atherosclerosis is felt to be secondary to the hypertension and its related causes. The lesions in pulmonary atherosclerosis do not advance to as severe lesions as in the aorta.

The distribution of atherosclerosis in the rabbit is

similar to that in man but with a few variations (51). Pulmonary artery atherosclerosis in the rabbit is more extensive, but the advance stage lesions still are not seen. The cerebral arteries of the rabbit only rarely develop atheromas, and no animals in this study, who were on the cholesterol diet, showed atheromas in cerebral vessels.

The coronary arteries also show a difference in distribution. In man the main coronary arteries are the primary sight of involvement. However, in the rabbit the smaller myocardial branches and the papillary vessels are more often affected. This is probably why rabbits rarely have heart attacks. Constantinides (21) has reported cases of fibrosis, scarring and eventual atropy of papillary muscles due to the occlusion of the vessels feeding them.

It appears that atherosclerosis in the rabbit is influenced by many of the same variables that have been implicated in atherosclerosis in the human. Diet, stress, environment and hereditary all combine to play a part in the atherogenesis.

Drug Studies

When this research was undertaken it was decided to use a newer derivative of tetracycline to treat the *B. fragilis* infection. Tetracycline had been the drug of

choice for a number of years in the treatment of B. fragilis (39). But more recent studies have shown anywhere from 40-80% of B. fragilis isolants resistent to tetracycline (19, 20, 35, 37). Doxycycline is reported to be more effective than tetracycline because it is more lipid soluble and more readily absorbed. It reaches higher serum levels faster and does not chelate to free ions such as calcium or magnesium as readily as tetracycline does (32, 81, 111, 143, 145, 146). Reports also indicate that doxycycline may be more effective than tetracycyline against B. fragilis and other anaerobes (20, 24, 87, 124, 125, 126, 129).

A review of some of the properties of the tetracycline shows that their absorption is affected by chelating to divalent cations such as Ca^{++}, Fe^{++}, Mg^{++}, Cu^{++}, and Al^{++} (61, 74, 127). Different vitamins can also affect the absorption. Meyers (94) states that the slowly excreated tetracyclines, such as doxycycline, are less reliably absorbed and produce lower levels.

With no previously achieved levels for comparison it is difficult to determine why so little absorption of doxycycline was achieved in the rabbits. Looking at the list of ingredients in the rabbit food, it is noted that a number of cations and vitamins are present that could affect absorption of the drug. There are B vitamin supplements, iron sulfate and a number of cations, most notable Ca^{++}, Mg^{++}, Mn^{++}, and Cu^{++}. A study by Neuvonen (97) in which he

gave oral doxycycline and 200mg of ferrous sulphate to humans showed that the iron had a highly significant effect on the absorption of the antibiotic. When doxycycline was administered alone the serum level rose to 3.0 ug/ml, while with the iron sulfate the serum level never exceeded o.6 ug/ml.

Once the doxycycline is absorbed, the rabbit's metabolism and serum components must be taken into account in considering the achieved drug level. The calcium level is high in the rabbit, and the cholesterol level in a majority of the animals exceeds 1000 mg/100 ml when they are on the cholesterol diet. These levels remain elevated for three to four months or longer (43, 44). The serum is quite lipemic and could interfere with the drug. These factors appear to combine to give limited absorption, and lowered levels of activity in the rabbit.

Bacteroides Infection

Many of the pathological and clinical symptoms of a *Bacteroides* infection seen in man were also seen in the rabbits. Clinical findings in man are those of hectic fevers, sometimes as high as 41 C. There are chills, rigors, diaphoresis and in some cases jaundice and shock. Laboratory findings generally include a leukocytosis, anemia, hematuria, and protienuria (19, 35, 133). In humans the

complications of septicemia are those of thrombophlebitis, emboli, empyema and metastatic abscess, primarly in the lungs. Gorbach (53) found in his review that in Bacteroides septicemia 5-12% of the cases showed thrombophlebitis, while 10-28% showed septic and metastatic complications, usually pulmonary.

Pulmonary infections can be indolent, with abscess and empyemas that may take weeks to progress to a stage where the patient feels ill enough to seek treatment. In these cases the patient initially may not appear acutely ill or show a fever or leukocytosis (8, 9, 71).

A majority of the rabbits did not show acute clinical symptoms and in the 2 animals that did die of a fulminating pneumonic process, neither had a fever or showed any overt signs of illness.

The pathology of Bacteroides infections in the lungs of man shows pleural fibrosis with diffuse adhesions, and a bronchopneumaonia with scattered suppurative abscess. In some cases these abscesses may spread and cause large necrotizing areas in the lungs (93, 119). Microscopically the lung pathology is one of pleural fibrosis, and bronchopneumonia with a moderate diffuse interstial mononuclear cell response with the alveoli also filled with a mononuclear cell exudate. Abscesses and venous thrombophlebitis are present (107, 131).

The cholesterol fed, infected rabbits (group I) in

this study showed a very similar response. More than half of the animals had a bronchopneumonia and pleurisy with a mononuclear type inflammation. Some of the animals also had pulmonary abscess, thrombophlebitis, and arteritis. The 2 animals that died had severe bronchopneumonia with an inflammatory exudate of mononuclear cells and polymorphonuclear leukocytes. Their lungs showed edema and rabbit 18 had pulmonary hemorrhage.

From evaluation of the data a difference in the type and degree of infection between the two cholesterol fed, infected groups (groups Ia and Ib) can be seen. The animals in group Ib had a higher degree of an active pneumonia rather than the resolving, milder pnuemonia seen in group Ia. Animals in group Ib exhibited arteritis, thrombophlebitis and pulmonary abscess while group Ia showed none of these. Two of the rabbits in group Ib showed a small thrombus and a vasculitis in the heart wall. Rabbit 18 showed an area of inflammation in the septum. It is not known if these foci are due to the Bacteroides.

Each group (Ia and Ib) was obtained from different breeders and had been raised under different circumstances. Therefore a difference in susceptibility of these two groups may have played a role in their infection. Also the antibiotic administered to group Ia could have affected the out come of their infection. Even though detectable drug levels were never attained from the orally administed

drug , that does not mean that some absorption was not taking place. Since the Bacteroides was susceptible to the doxycycline at a low MIC the drug could have helped combat the first bout of infection in group Ia.

Atherosclerosis Predisposing to Infection

The atherosclerosis found in these animals had an affect on the degree of infection which was produced. In the case of this anaerobic infection, the atherosclerosis was a key factor in the establishment of the infection. A mild bronchopneumonia with no complications was the only finding in the non-cholesterol fed, infected controls (group III). All of the cholesterol fed, infected animals (group I) had some degree of pulmonary atherosclerosis which would hamper the blood flow to the lung and set up areas of lowered oxygen tension which could then more easily be seeded by the Bacteroides.

These same observations have been noted in human infections. An increase in more severe anaerobic infections in elderly or debilitated patients has been observed (19, 31, 34, 49, 93, 117). Trail (132) found that of thirteen autopsies of Clostridium welchii enterocolitis in nursing home patients, all showed advanced atherosclerosis which allowed insufficent blood supply to the bowel causing ischemia in the face of the infection. Bowel sterilization

using aminoglycosides often help to predispose to anaeribic infection; many of the anaerobes, particularly Bacteroides are resistent to these drugs.

There is also a higher mortality rate among the forty and older age group. The less than forty age group with Bacteroides infections tends to be obstetrical cases where the patients are in general good health. The mortality rate in these patients is low while the forty and older group are more often males with atherosclerosis and other health problems which lead to more serious complications and death (19, 49). With a rising incidence of atherosclerosis and the complications it can produce, it is expected that a rise in anaerobic infections will also be seen.

Infection Increasing Atherosclerosis

The question of a cyclic phenomenon between the atherosclerosis and the infection is an interesting one. In this study the atherosclerosis helped produce a more severe infection in the animals. The reverse also appears to be the case. In 5 cholesterol fed, infected animals (group I) there were foam cell plaques seen in the pulmonary veins. There are no references in the literature on venous lesions in the rabbit in relationship to infection. Constantinides (22) states that they do not occur in cholesterol fed animals alone.

For many years researchers have been listing vessel injury as an initiating factor in atheroma production. B. fragilis could easily lodge or even settle out in the veins and cause an injury to the endothelium which in the process of repair is infiltrated by lipophages or lipids accumulating in the vessel walls. The accumulations resemble the earliest fatty lesions in man. Thrombosis in rabbits has produced atheromas in the pulmonary arteries (54, 60). Gore's study (54) produced hypercholesterolemia in the rabbits, by use of a diet, and later injecting thromboplastin to induce thrombosis. The pulmonary vessels show a marked inflammatory response and then an xanthomatous reaction characterized by the accumulation of large numbers of foam cells. Pulmonary hypertension which is a primary cause of pulmonary atherosclerosis in man (137) was not present in Gore's animals.

Bacteroides is known to produce venous thrombophlebitis, which would start an inflammatory process. The high level of lipids still present in the animals blood could easily start lipid deposition. These venous lesions may be over old sites of infection and inflammation since inflammation and thrombophlebitis was demonstrated in these animals.

As previously mentioned the rabbits still demonstrate an elevated cholesterol level for several months after cessation of the cholesterol diet. This is due to the

Cholesterol that is being cleared from the tissue stores (43, 44). In the rabbit as well as humans it has been found that fever, infection and endotoxin can also elevate the total serum lipids (33, 47, 83, 99). Foldvari (41) found that Salmonella endotoxin will not cause an increase in total cholesterol levels in cholesterol fed animals, but an elevation of 230% is still seen in the total triglycerides of these animals after endotoxin injection. In view of this, the repeated bacteremias and infection that the rabbits from this study were subjected to, may have helped to keep the lipids at a peak level so that deposition in an injured vessel could be achieved.

In the results were included a group of 4 animals which were designated as having questionable lesions. The lesions that were questioned as abnormal were in an area of thickened vessel wall with increased mucosubstances present on Movat's stain. Some researchers feel that s thickened intima will capture lipids more readily. Wagenvort (137) presents a fibrosed area in a pulmonary vessel of a five year old boy with a heart defect and hypertension. He feels this is a possible inital stage of an atheroma. Other studies have looked at the coronary arteries of children and young adults and they found areas of intimal thickening and atheromas over sites of old infection. Saphir (114) reviewed cases of soldiers, 18 to 29 years of age, who had died of sudden coronary heart disease. In 13

cases of severe sclerosis of the cardiac arteries, 10 had evidence of an old inflammatory process, often rheumatic heart disease. Personen (103) looked at 175 autopsies of infants and children, 0-15 years of age, who had coronary defects as well as infections such as septicemia, pneumonia and viral infections. He found an association between a thickened coronary artery wall and infection. Since a thickened intima captures more lipid than a thin one, the fatty infiltration of the intima in infants especially in infection may predispose to atherosclerosis when considered over a long period of time. Therefore an increase in endothelial permeability due to inflammation may lead to increased lipid entry and atherosclerosis (106).

Klotz in 1910 (113) concluded that infection and bacterial intoxication cause intimal hyperplasia. Perhaps these thickened areas in the rabbits vessels are sites for future lipid deposition. The repeated bacteremia as well as the inflammation and thrombosis that these animals were subjected to could have caused an intimal thickening. Hypertension could have helped to aggrevate the pulmonary atherosclerosis although it is unknown if these animals had hypertension. It is not unlikely however since 75% of the cholesterol fed, infected animals had emphysema which can cause hypertension particularly with pulmonary infection of a chronic nature.

No correlation could be seen between the degree of

pulmonary artery atherosclerosis in the animals to that of the pulmonary veins. In one instance the arteries were graded as having 70% atherosclerosis with venous lesions present, while in another animal venous lesions were seen with only 25% artery involvement. This maybe due to the lack of reliable grading systems for smaller caliber vessels and the evaluation of only one section of lung from any animal.

Adrenal Pathology

The adrenal glands in the rabbits were the only other areas of notable pathology. There is a definite difference in the morphology of the glands between the cholesterol fed, infected animals (group I) and the non-cholesterol fed, infected animals (group III). This difference is less distinct between the cholesterol fed, non-infected animals (group II) and the infected, cholesterol fed animals (group I). Both of these cholesterol fed groups showed the typical cholesterol deposition which involves the cortex.

The adrenals of the cholesterol fed, infected animals (group I) showed more and larger areas of cholesterol deposition and a larger number of focal necrotic areas with scattered chronic inflammatory cells. Four of the animals in group I had focal calcium deposits. Since no cultures of the adrenals were taken it is impossible to say if the

changes were due to a direct infection of the adrenals by the Bacteroides. Stress may have played a role in the changes seen in the adrenals. Stress, in the form of trauma or illness is known to stimulate adrenocorticotropic hormone (ACTH) excretion which in turn causes enlargement of the adrenal and increased formation of hormones (98). ACTH acts primarly on the zona fasiculata and reticularis. Adrenals under constent stress will begin to show scattered inflammatory cells while severe stress such as septicemia or burns can cause focal necrosis and hemorrhage (118). Calcification of the adrenal in the human is seen from hemorrhage due to hypocorticalism, infection or external trauma (128).

On the basis of these facts it is difficult to ascertain the primary cause of the adrenal pathology. It is evident that the chronic bacteremia with B. fragilis enhanced the changes seen in the gland, but there is no way of determining if the calcium deposits were old foci of infection. The 4 rabbits with calcium deposits all showed the most severe atherosclerosis and pulmonary infection. The pulmonary infection with bacteremia might predispose to adrenal infection but the persistent bacteremia would also put the animals under a constent stress. The changes in the adrenals may therefore be attributed to the chronic stress of the hypercholesterolemia and the infection, or to direct infection of the gland.

CONCLUSION AND SUMMARY

Pre-existing atherosclerotic lesions in rabbits enhanced an infection with the anaerobic bacteria, Bacteroides fragilis. Atherosclerosis can setup sites of hypoxia that are then more easily seeded by an anaerobe. A pulmonary infection was established in this study by initiating a chronic bacteremia with the anaerobe.

If this model for anaerobic infection can be better controlled to yield a higher number of animals with a reproducable disease it maybe useful for drug studies. In this particular study the antibiotic doxycycline was used only minimally so that any conclusions as to its effectiveness cannot be made. However, detectable drug levels can be achieved with the I V form of the drug. If the MIC of the infecting organism is known this antibiotic could be of value in treating the disease.

In some instances the infectious process played a role in producing atheromas in the pulmonary veins. The chronic pulmonary infection along with the hyperlipidemia produced vascular damage which predisposed to atheromatous changes in the pulmonary vessels. A correlation between the infection and the increase in atherosclerosis could not be found in any other organs outside the lung. It appears that factors such as infection and injury act to initiate atherosclerosis and then a self-perpetuating cycle occurs.

In this case the atherosclerosis must come first to predispose the animal to infection. Bacteroides in rabbits is apparently of low virulence and a difficult infection to initiate.

This process is seen in humans, particularly in elderly patients who usually exhibit more advanced atherosclerosis. With this in mind, this model may be useful in studying the pathogenesis of atherosclerosis as well as studying the infections initiated by the atherosclerotic changes.

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147. Yoshikawa, T., A. Chow and L. Guze. 1974. Bacteroidaceae Bacteremia with disseminated intravascular coagulation. Amer. J. of Med. 56: 725-728.

Table 1

Incidence of Bacteroidaceae, Clostridia and Peptostreptococci in Blood Cultures in Several Hospitals

Period	No. Positive Blood Cultures*	Bacteroidaceae No. (%)	Clostridia No. (%)	Peptostreptococci No. (%)	References
1958-65	308	8 (2.6)	4 (1.3)	4 (1.3)	Watt & Okubadejo (1967)
1951-67	1,429	12 (0.8)	19 (1.3)	0 (0)	Dalton & Allison (1967)
1960-67	403	4 (0.9)	3 (0.7)	0 (0)	Crowley (1970)
1960	120	4 (3.3)	2 (1.7)	0 (0)	Sonnenwirth (1973)
1970	303	23(7.6)	4 (1.3)	2 (0.7)	Sonnenwirth (1973)
1968-69	3,103	265 (8.5)	44 (1.4)	27 (0.8)	Washington (1971)
1971	847	92 (10.8)	7 (0.8)	9 (1.0)	Washington (1972)

* Those yielding only S. epidermidis or Corynebacterium, Propionibacterium or Bacillus spp. are omitted

** Table taken from: Sonnenwirth, A. 1974. Incidence of intestinal anaerobes in blood cultures. p. 163. In: A. Balws, R. DeHaan, V. Dowell and L. Guze (eds), Anaerobic Bacteria: Role in Disease. C. C. Thomas Publ., Springfield, Ill.

Table 2

Experimental Groups of Rabbits
and Their Treatment

Group	Treatment	Animal's #
Ia	Cholesterol-fed; *Bacteroides* injections	2-5 9 & 10 Total# = 6
Ib	Cholesterol-fed; *Bacteroides* injections	11-20 Total# = 10
II	Cholesterol-fed; no *Bacteroides* injections	1* & 8* 21-23 Total# = 5
III	Normal diet; *Bacteroides* injections	24-28 Total# = 5

* Rabbit 1 & 8 were used as drug controls for doxycycline investigations.

Table 3

Ingredients of Normal Diet
(Purina Lab Chow 5301)

alfalfa meal
ground yellow corn
wheat midlings
soybean meal
cane molasses
vitamin B12 supplement
calcium pantothenate
vitamin A Supplement
D activated animal sterol
niacin
choline chloride
riboflavin supplement
methionine hydroxy analogue calcium
defluorinated phosphate
iodized salt
iron sulfate
manganous oxide
copper sulfate
cobalt carbonate
zinc oxide

Criteria for Evaluation of Atherosclerotic Lesions of the Aorta

Table 4

Weighting for Extent of Disease*

Group	Area involved %	Approximate mean area %	Weighting
0	0-5	2.5	1
A	6-15	10.0	4
B	16-33	25.0	10
C	34-50	40.0	16
D	51-100	75.0	30

* Taken From: Gore, I and C. Tejada. 1957. The Quantitative appraisal of Atherosclerosis. Amer. J. of Path. 33: 875-885.

Criteria for Evaluation of Atherosclerotic Lesions of the Aorta

Table 5

Types of Atherosclerotic Lesions*

Grade I:	Lipid streaks, spots, and patches. These are very superficial, thin, yellow, with endothelial accumulations which may just perceptibly elevate the intimal surface. Small, punctate, discrete, yellow, pure lipid, nodular elevations in the ascending aorta are included in this category.
Grade II:	Elevated, smoothly surfaced, fibrous plaques of variable lipid content. The pearly plaque is the type lesion of this category but others are yellow and distinguishable from grade I only by the associated presence of sclerosis.
Grade III:	Plaques with ulceration, necrosis, or hemorrhage.
Grade IV:	Calcified plaques.

* Taken from: Gore, I. and C. Tejada. 1957. The Quantitative appraisal of atherosclerosis. Amer. J. of Path. 33: 875-885.

Criteria for Evaluation of Atherosclerotic Lesions of the Aorta

Table 6

Weighting for Grade of Lesions

Grade	Weight
I	1
II	10
III & IV	100

* Taken from: Gore, I. and C. Tejada. 1957. The Quantitative appraisal of atherosclerosis. Amer. J. of Path. 33: 875-885.

Table 7

Atherosclerotic Grading of Rabbits Aortas and Extent of Aortic Valve Involvement

Rabbit's#	Profile	Index	Valve Involvement
Group Ia			
2	B(3,6,0,1)m'	2.43	** "
3	O(10,0,0,0)	0.03	*
4	B(7,3,0,0)	1.23	*
5	A(5,5,0,0)	0.73	**
9	O(6,4,0,0)	0.15	-
10	B(6,3,0,1)m	1.53	*
Group Ib			
11	B(8,2,0,0)	0.93	*
12	A(8,2,0,0)	0.37	*
13	O(10,0,0,0)	0.03	*
14	C(9,1,0,0)	1.01	*
15	B(9,1,0,0)	0.63	*
16	A(7,3,0,0)	0.49	*
17	B(5,5,0,0)	1.83	*
18	B(9,1,0,0)	0.63	*
19	O(10,0,0,0)	0.03	-
20	A(6,4,0,0)	0.61	*
Group II			
1	B(5,5,0,0)	1.83	*
8	none	-	-
21	O(10,0,0,0)	0.03	-
22	B(10,0,0,0)	0.33	*
23	A(10,0,0,0)	0.13	*

' Calcium was only seen microscopically and therefore only given a weight of 10.

" A grading scale of * to **** is used; one * meaning the least involvement and four * the most severely involved. - means there is no atheroma involvement of the valve.

Table 8

Extent of Pulmonary Artery Atherosclerosis and the Presence or Absence of Atheromas in the Pulmonary Veins of the Rabbits

Rabbit's#	Pulmonary artery	Pulmonary vein
Group I	%	
2	20	*''
3	50	-
4	25	*
5	50	*
9	25	-
10	20	-'''
11	20	?
12	20	?
13	40	-
14	70	*
15	20	*
16	40	-
17	70	?
18	20	?
19	20	-
20	50	-
		Total# 5/16 31%
Group II		
1	25	-
8	none	-
21	20	-
22	25	-
23	30	-
Group III		
24	-	-
25	-	-
26	-	-
27	-	-
28	-	-

' Indicates the lesion is present.
'' Indicates the lesion is not present.
''' Indicates there were areas of thickening and fibrosis but no true foam cell lesions.

Table 9

Atherosclerosis in the Coronary Vessels;
the Endocardial and Epicardial Branches
and the Papillary Branches

Rabbit's#	Endocardial & Epicardial Branches	Papillary Branches
Group I		
2	*'	*"
3	-	-
4	-	-
5	-	-
9	-	-
10	-	-
11	-	*
12	*	-
13	-	-
14	-	-
15	-	*
16	-	*
17	*	-
18	*	-
19	*	*
20	*	-
Group II		
1	-	*
8	-	-
21	*	-
22	-	-
23	-	*

Total# with coronary vessel involvement:
12/21 = 57%

' Indicates the lesion is present
" Indicates the lesion is not present

Table 10

Zone Diameter and Concentration of Doxycycline at Timed Intervals - Rabbits 1 and 8

Rabbit 1

Time	Zone dia.	Concentration*
	mm	ug
½h	18.4	5.8
1h	17.3	4.1
2h	16.4	3.1
4h	14.0	1.5
8h	--	1

Rabbit 8

Time	Zone dia.	Concentration*
	mm	ug
½h	18.0	5.1
1h	16.8	3.5
2h	15.6	2.3
4h	13.4	1.0
8h	--	1

* Values for concentrations of doxycycline extrapolated from curve of fig 15.

Table 11

Rabbits Receiving Doxycycline, Type Received and Route of Administration

Rabbit's#	Type		Amount	
	I V	Oral	I V	Oral
2	I V	oral	20mg	150mg
3	--	oral	--	150mg
4	--	oral	--	150mg
5	--	oral	--	150mg
9	--	oral	--	150mg
10	--	oral	--	150mg
17	I V	--	16mg	--
1*	IV	oral	19mg	150mg
8*	I V	oral	17mg	150mg

* 1 and 8 are drug controls. They received no Bacteroides injections.

Table 12

Days After Termination of Diet until Death;
Total Number of Bacteroides injections
of Group I

Rabbit's # Group I	Days since termination of diet	# injections of Bacteroides*
2	99	11
3	100	13
4	101	13
5	72	8
9	73	8
10	44	3
11	68	15
12	79	15
13	71	15
14	77	15
15	72	15
16	69	15
17	71	12
18	30	8
19	78	15
20	72	15

* Dose 2.0×10^9 organisms in 1 ml of sterile saline.

Table 13

Rabbits from Group I* with Positive Cultures and their Location

Rabbit's#	#cultures taken	#positive	Location
4	5	1	spleen
9	5	1	spleen
11	4	1	blood
12	4	1	blood
18	4	4	blood, lung liver, spleen

Total # positive = 5/16 = 31%

* Group I contained 16 animals all fed the cholesterol diet and who received the Bacteroides injections.

Table 14

Types of Lung Pathology Seen in all Rabbit Groups with Numbers and Percentages of Animals Involved

Rabbit's #	Pnuemonia	Pleurisy	Bronchitis	Emphysema	Abscess	Thrombo-phlebitis	Arteritis
Group Ia							
2	-'	-	*	*	-	-	-
3	-	*	-	*	-	-	-
4	*"	-	-	-	-	-	-
5	-	-	*	*	-	-	-
9	-	*	*	*	-	-	-
10	*	-	*	*	-	-	-
Total Ia	2/6	2/6	4/6	5/6	0	0	0
%	33%	33%	66%	83%	0%	0%	0%
Group Ib							
11	*	*	-	*	-	-	-
12	*	*	-	-	*	*	*
13	*	*	-	*	-	-	-
14	-	-	*	*	*	*	*
15	*	*	*	*	-	-	-
16	-	*	-	-	-	-	-
17	-	-	*	*	-	-	-
18	*	-	*	-	-	*	-
19	*	*	*	*	-	-	-
20	*	*	*	*	-	-	*
Total Ib	7/10	7/10	6/10	7/10	2/10	3/10	3/10
%	70%	70%	60%	70%	20%	30%	30%
Total of Ia & Ib	9/16	9/16	10/16	12/16	2/16	3/16	3/16
% of Ia & Ib	56%	56%	63%	75%	13%	19%	19%
Group II							
1	-	-	-	-	-	-	-
8	-	*	-	-	-	-	*
21	-	-	*	*	-	-	-
22	*	*	-	*	-	-	-
23	-	-	-	*	-	-	-
Total II	1/5	2/5	1/5	3/5	0	0	1/5
%	20%	40%	20%	60%	0%	0%	20%
Group III							
24	*	-	*	-	-	-	-
25	*	-	-	-	-	-	-
26	-	-	-	-	-	-	-
27	*	-	*	-	-	-	-
28	-	*	*	*	-	-	-
Total III	3/5	1/5	3/5	1/5	0	0	0
%	60%	20%	60%	20%	0%	0%	0%

' indicates the process or lesion is not present
" indicates the process or lesion is present

Table 15

Types of Adrenal Lesions Found
in all Rabbit Groups

Rabbit's#	Cholesterol Deposits	Necrosis	Inflammation	Calcium
Group I				
2	*'	*	*	*"
3	*	*	*	-
4	*	*	*	-
5	*	*	*	*
9	*	-	-	-
10	*	*	*	*
11	*	*	*	-
12	*	-	-	-
13	*	*	*	-
14	*	*	*	-
15	*	*	*	-
16	*	*	*	-
17	*	*	*	-
18	*	*	*	*
19	*	-	-	-
20	*	*	*	-
Group II				
1	*	-	-	-
8	*	-	-	-
21	*	-	-	-
22	*	-	-	-
23	*	-	-	-
Group III				
24	-	-	*	-
25	-	-	*	-
26	-	-	*	-
27	-	-	*	-
28	-	-	*	-

' Indicates the lesion is present
" Indicates the lesion is not present

Figure 1

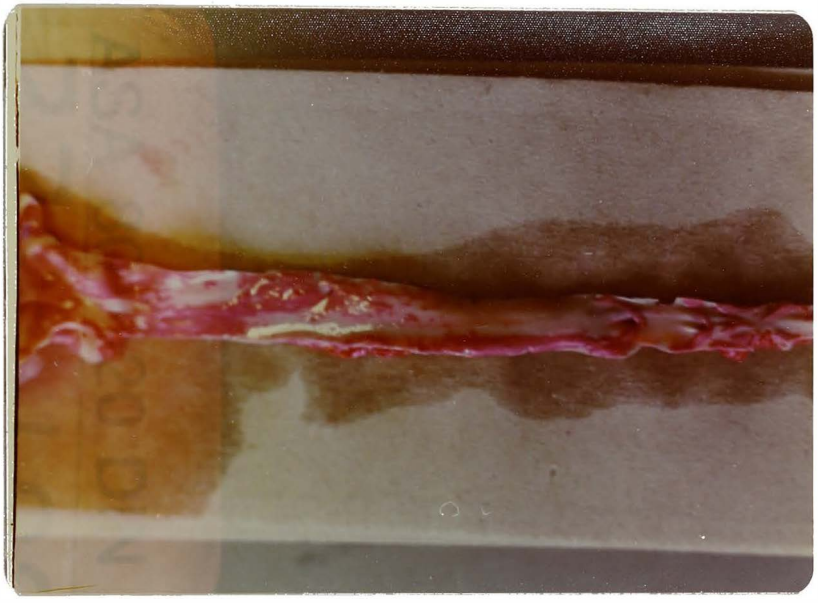

Fig 1 - Aorta. Stained with Sudan IV to intensify the plaques and facilatate grading. The red areas are the atheromas.

Figure 2

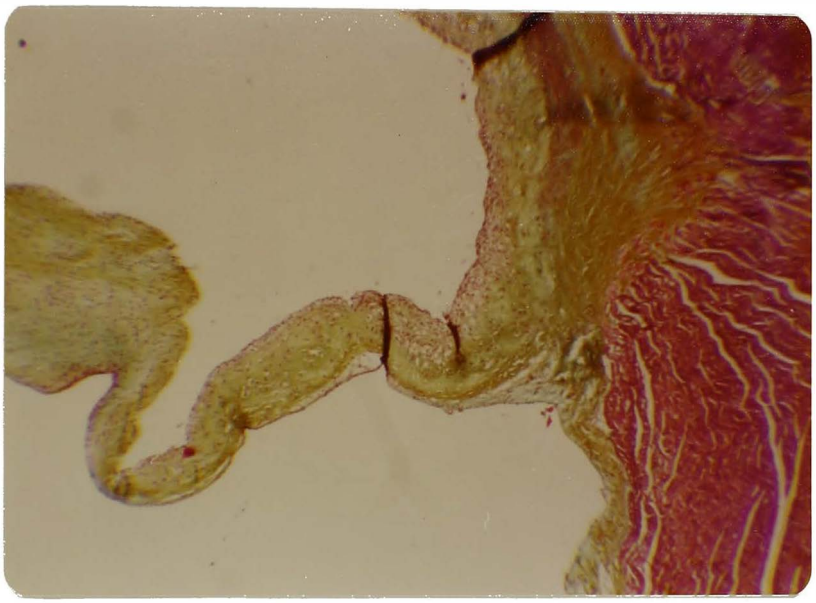

Fig 2 - Aortic Valve. Atheromatous lesions seen at base of valve. Movat's Stain. Original magnification- 60x

Figure 3

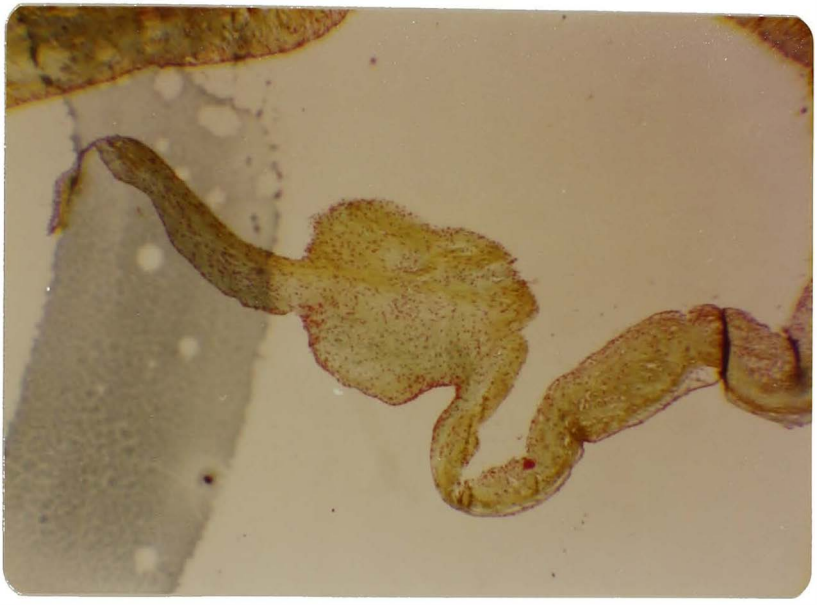

Fig 3 - Aortic Valve. View of atherosclerotic involvement of valve leaflet. Movat's stain. Magnification- 60x

Figure 4

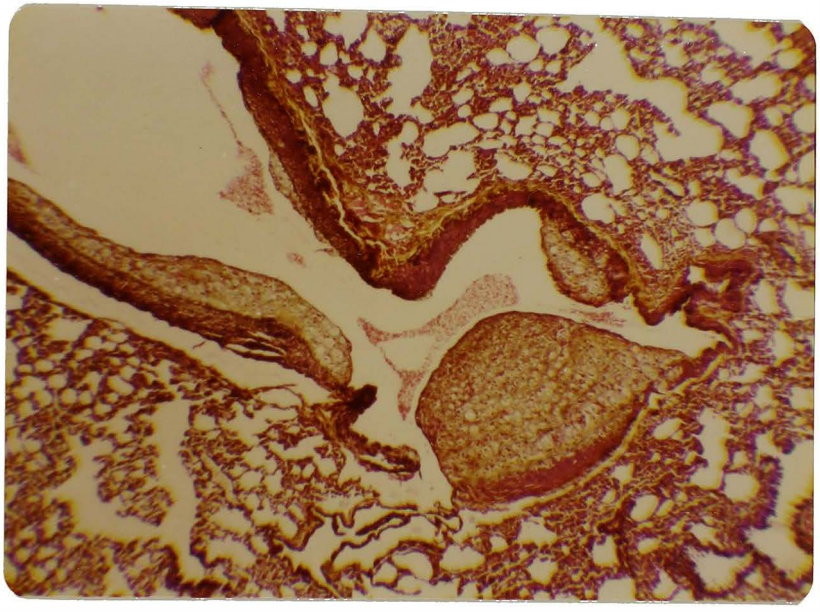

Fig 4 - Pulmonary artery. Typical foam cell lesions are seen. Movat's stain. Original magnification 60x

Figure 5

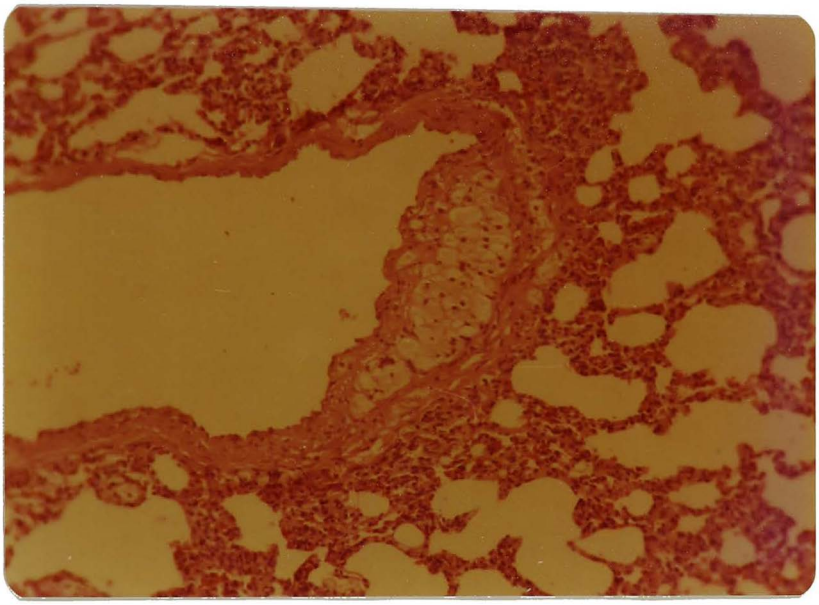

Fig 5 - Pulmonary Vein. Foam cell lesion in the lung. Hematoxylin and Eosin stain. Original magnification 150x

Figure 6

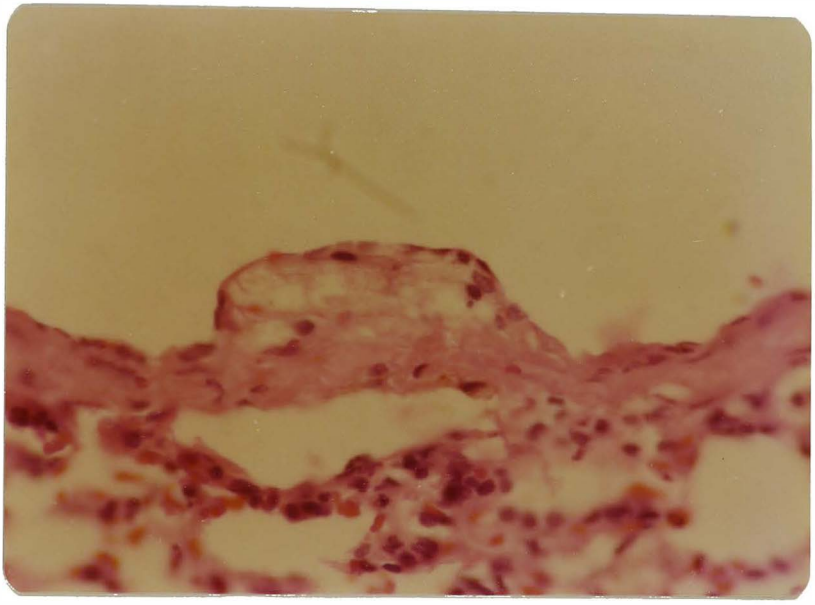

Fig 6 - Pulmonary Vein. Small foam cell lesion in lung. Hematoxylin and eosin stain. Original maginification 600x

Figure 7

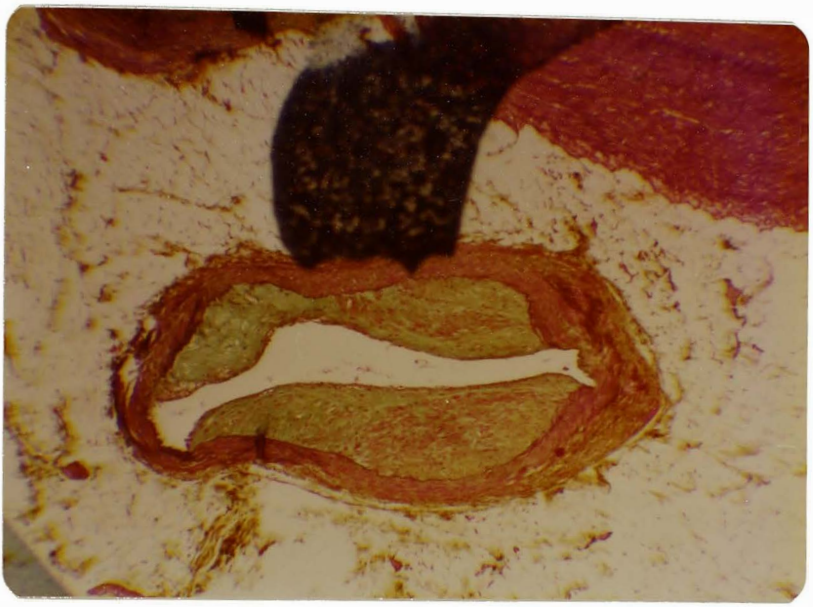

Fig 7 - Coronary Artery. Atherosclerotic plaque in large epicardial artery. Movat's stain. Original magnification 60x

Figure 8

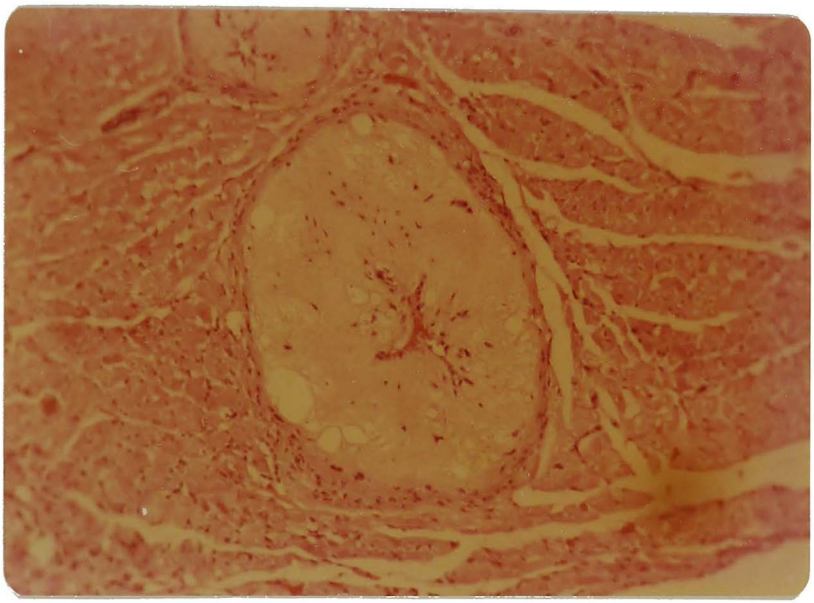

Fig 8 - Coronary Vessel. A vessel of the papillary muscle which is nearly occluded by lipid deposition. Hematoxylin and eosin stain. Original magnification 150x.

Figure 9

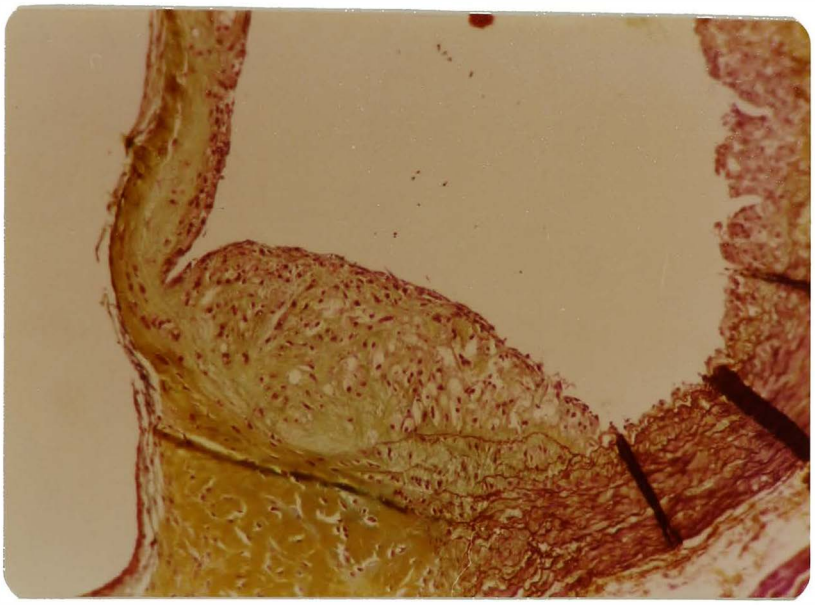

Fig 9 - Aorta. A foam cell lesion near and extending onto a valve. Movat's stain. Original magnification 60x.

Figure 10

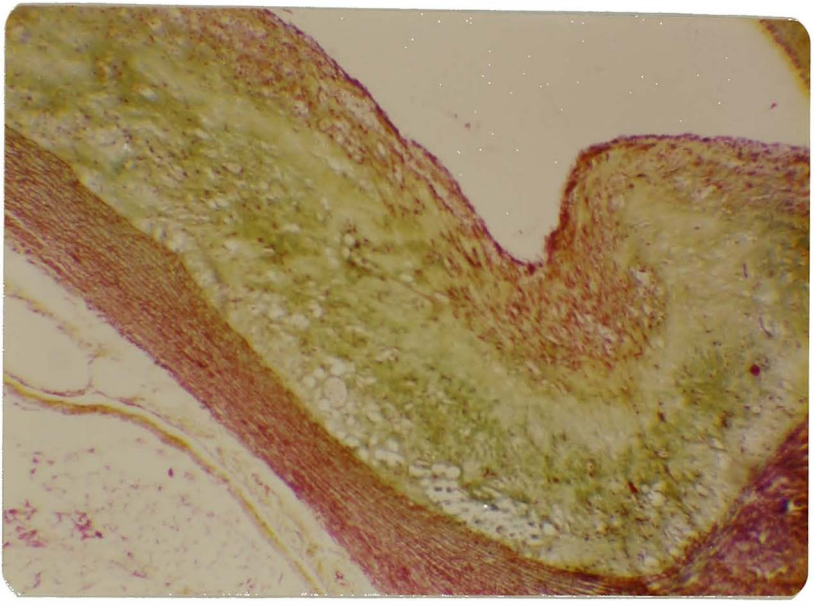

Fig 10 - Aorta. A large atheroma with a fibrous capsule over an area of foam cells and lipid material. Movat's stain. Orignial magnification 60x.

Figure 11

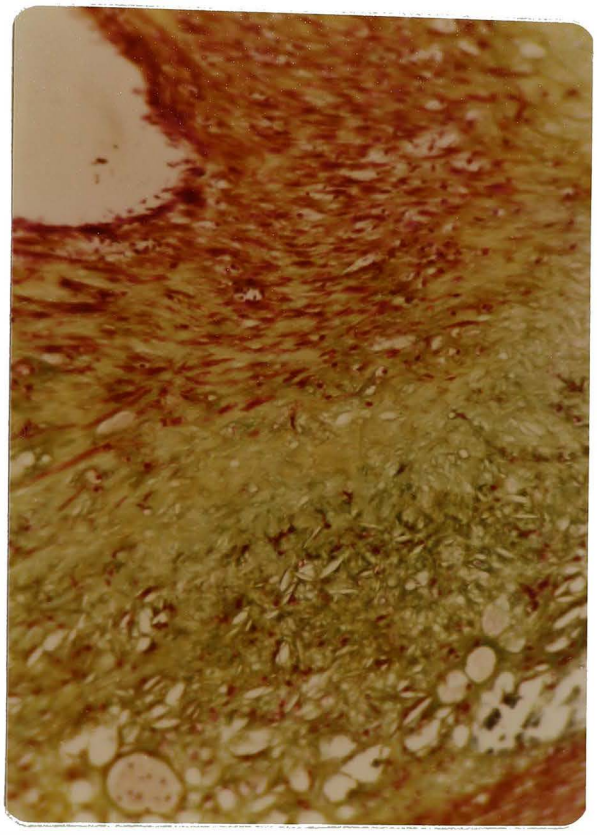

Fig 11 - Aorta. A higher magnification of fig 10 to show the fibroblasts and the underlying area of necrosis and calcium clefts. Movat's stain. Original magnification 150x.

Figure 12

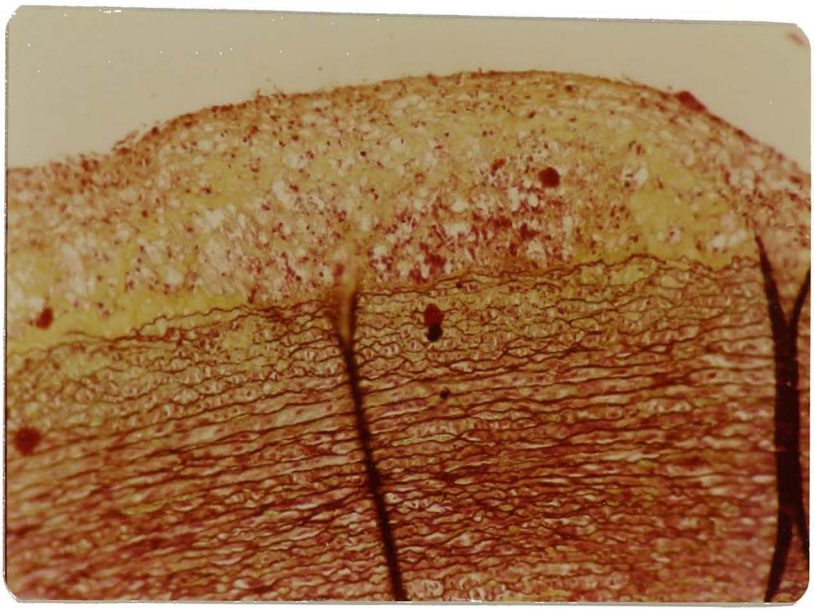

Fig 12 - Aorta. An atheroma with a "sterile abscess" and gruel formation due to the death and necrosis of foam cells at the bottom of the plaque. Movat's stain. Original magnification 60x.

Figure 13

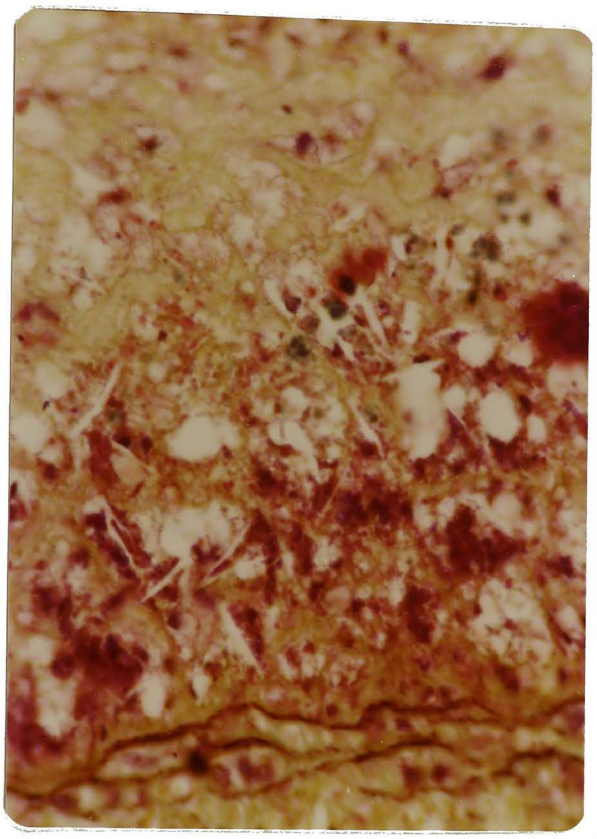

Fig 13 - Aorta. Higher magnification of fig 12 to show area of necrosis and cholesterol clefts. Movat's stain. Original magnification 150x.

Figure 14

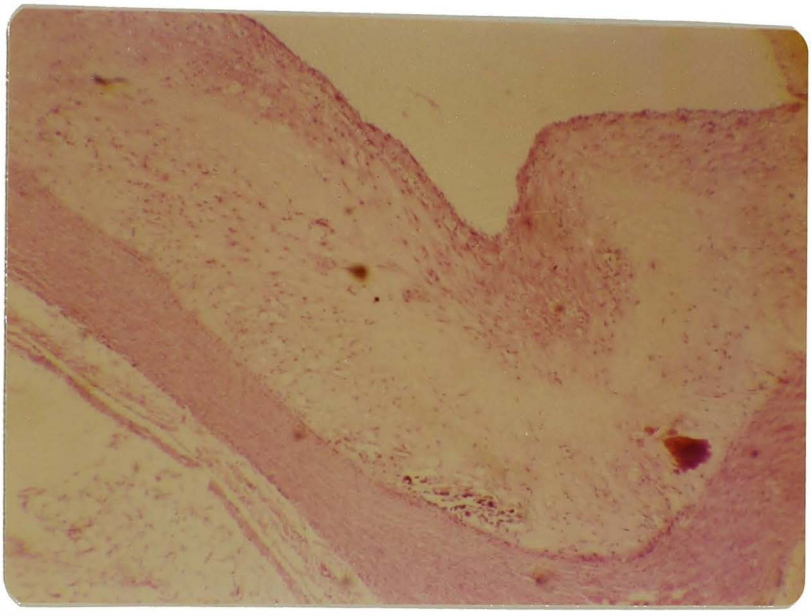

Fig 14 - Aorta. Calcium formation seen at bottom of a fibrous plaque. Hematoxylin and Eosin stain. Original magnification 60x.

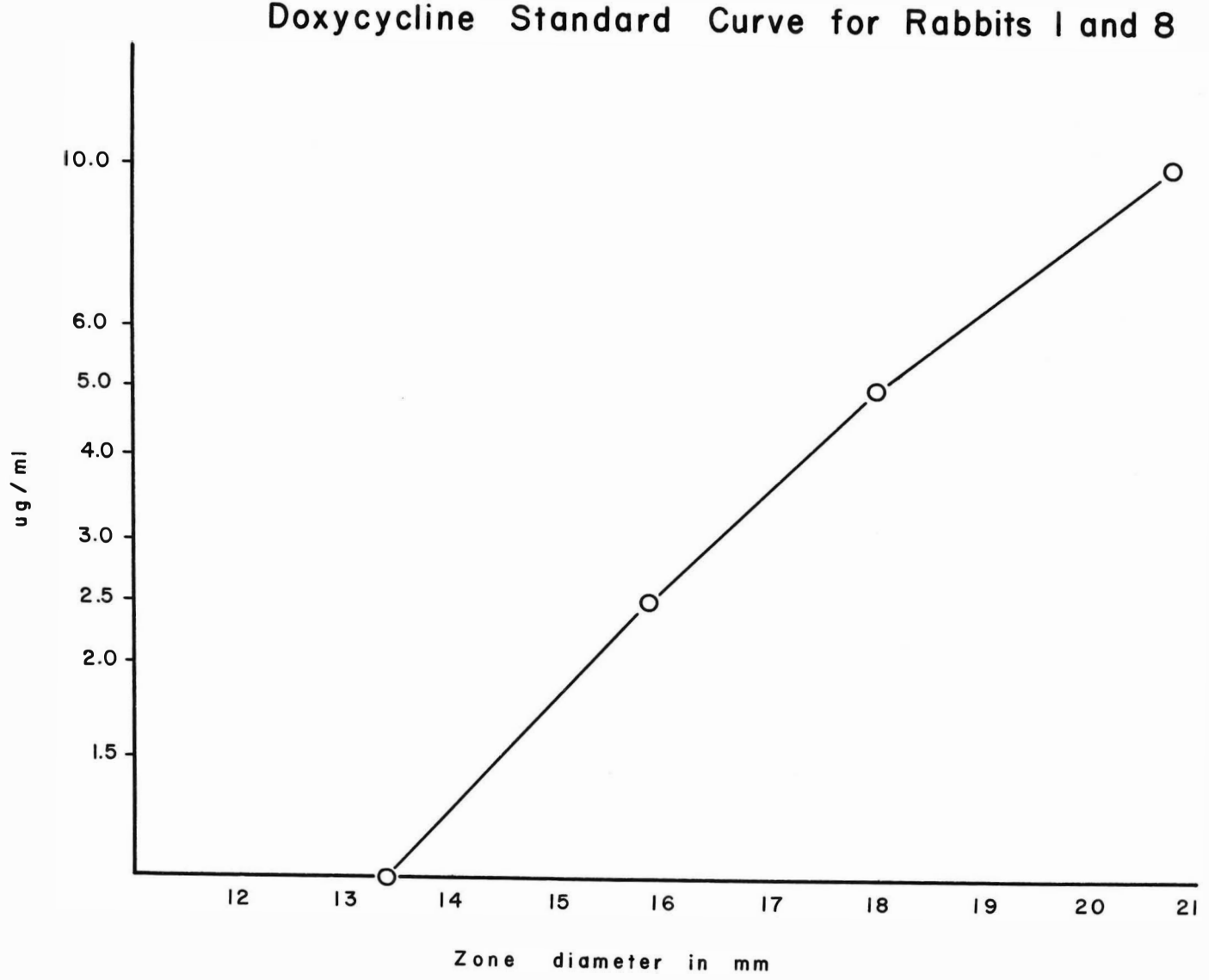
Doxycycline Standard Curve for Rabbits I and 8
10.0
6.0
5.0
4.0
3.0
2.5
2.0
1.5
ug / ml
12
13
14
15
16
17
18
19
20
21
Zone diameter in mm

Figure 16

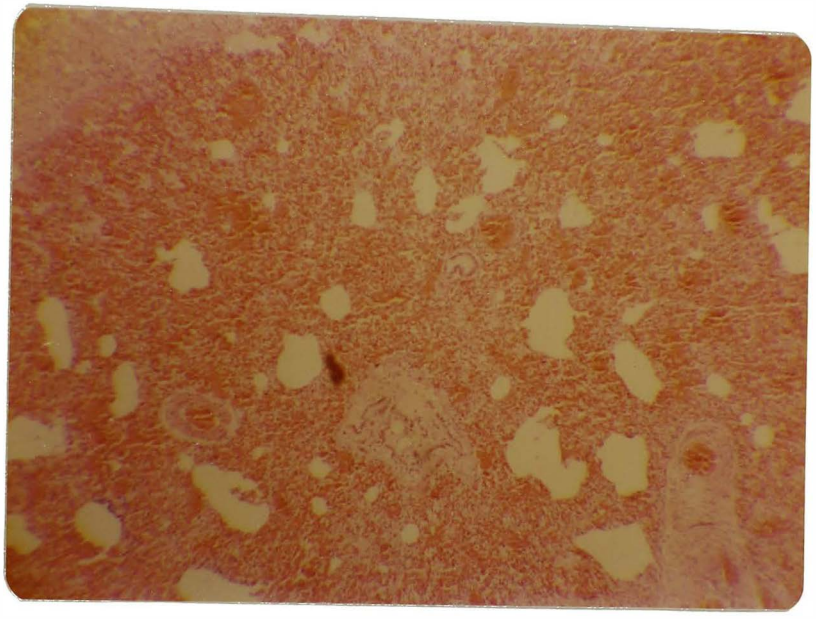

Fig 16 - Lungs. The pneumonic process seen in rabbit 18. Note hemorrhage. Hematoxylin and Eosin stain. Original magnification 60x.

Figure 17

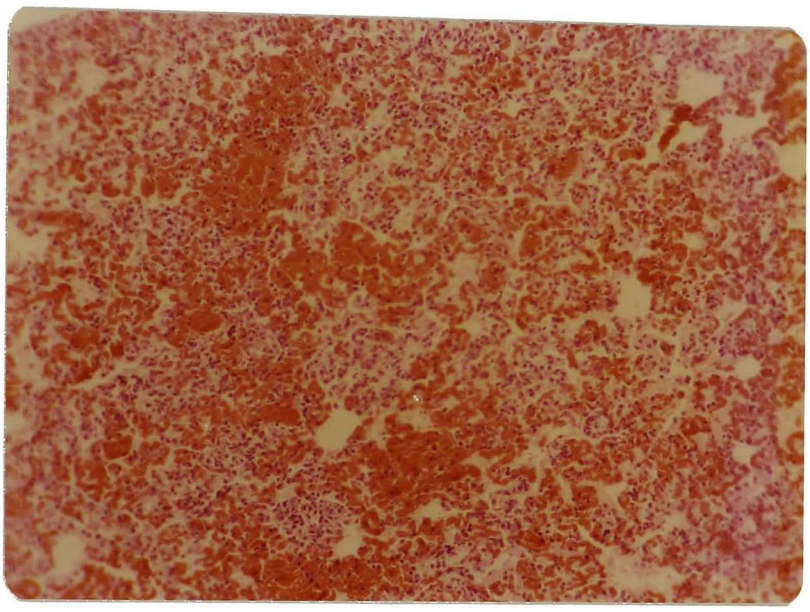

Fig 17 - Lungs. A higher magnification of fig 16 to show mononuclear cell response and hemorrhage. Hematoxylin and Eosin stain. Original magnification 150x.

Figure 18

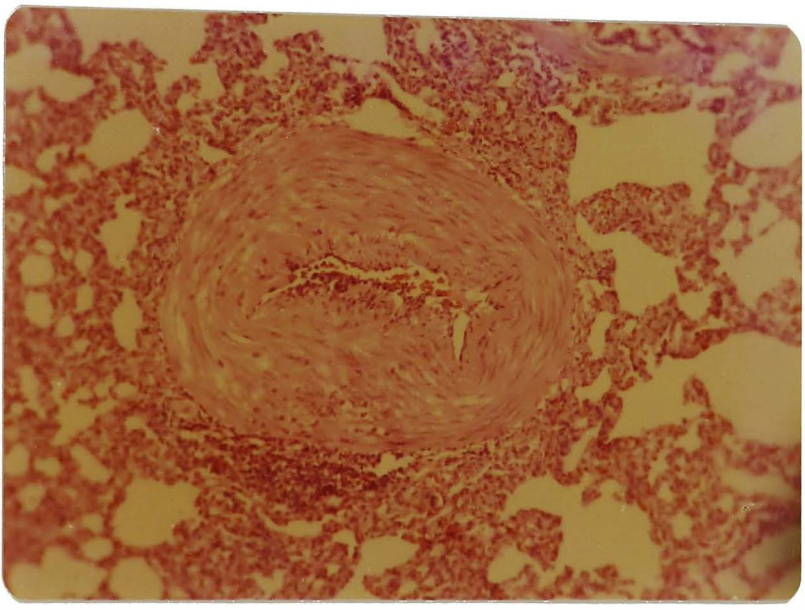

Fig 18 - Pulmonary Artery. Arteritis with inflammatory cells through the entire vessel wall. Hematoxylin and Eosin stain. Original magnification 60x.

Figure 19

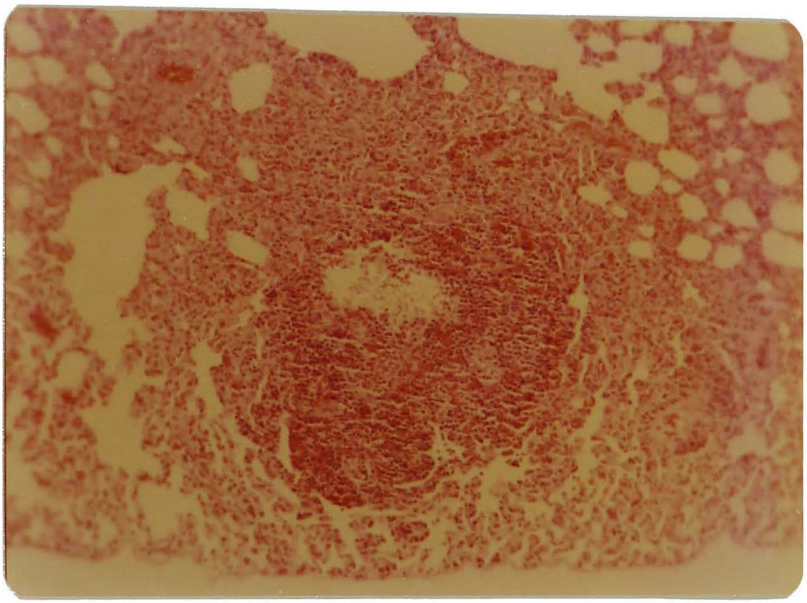

Fig 19 - Lung. A pulmonary abscess from rabbit 14. Hematoxylin and Eosin stain. Original magnification 150x.

Figure 20

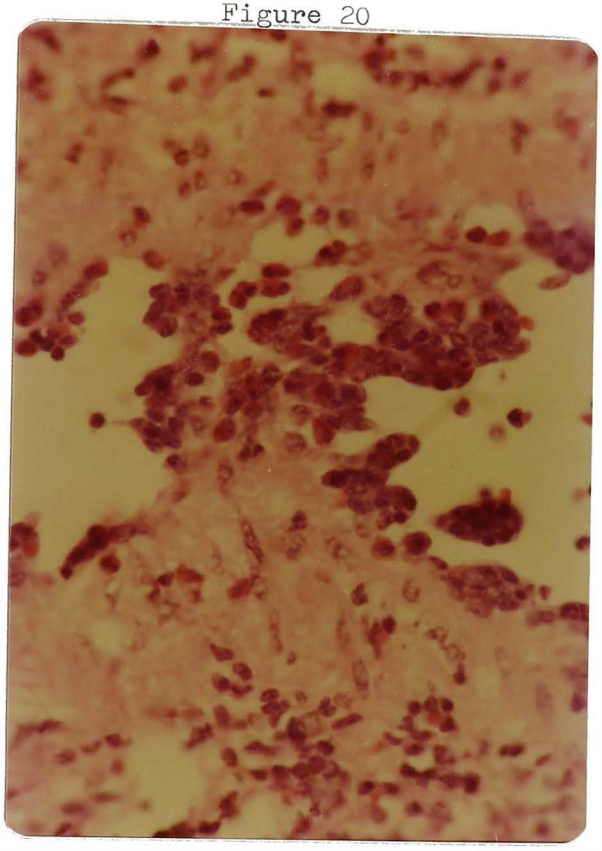

Fig 20 - Lung. Thrombophlebitis seen in the cholesterol-fed-infected animals. Hematoxylin and Eosin stain. Original magnification 150x.

Figure 21

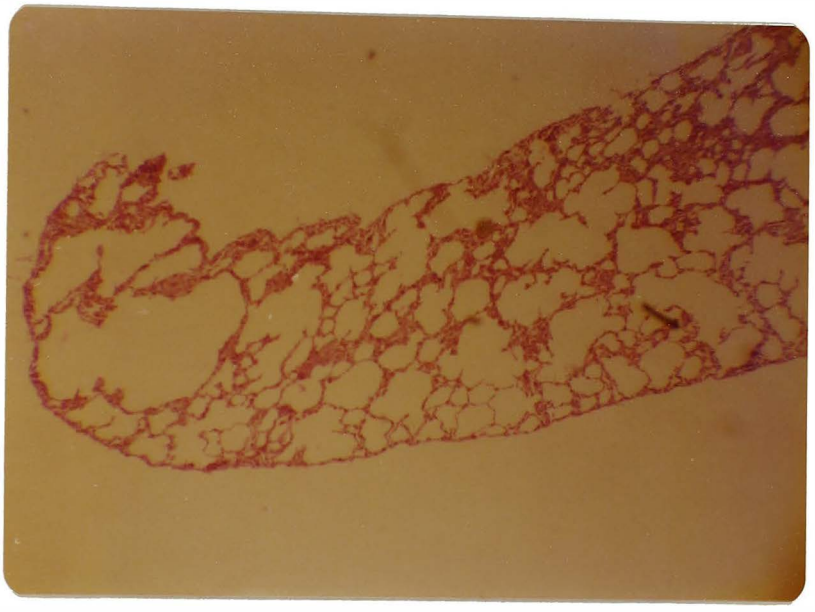

Fig 21 - Lung. Emphysema with the typical clubbing of the alveoli. Hematoxylin and Eosin stain. Original magnification 60x.

Figure 22

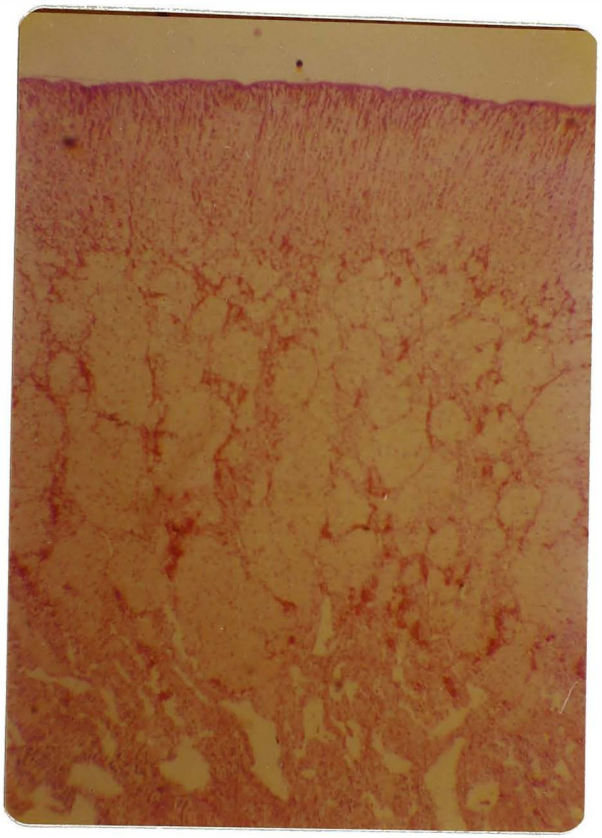

Fig 22 - Adrenal. Type of pathology seen in a majority of the cholesterol-fed-infected animals with cholesterol deposition and necrosis of the cortical cells. Hematoxylin and Eosin stain. Original magnification 60x.

Figure 23

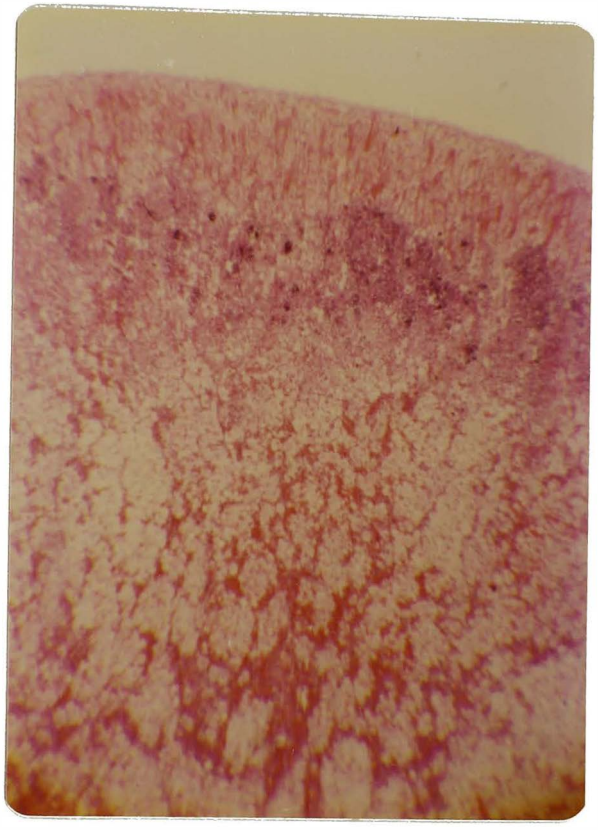

Fig 23 - Adrenal. From rabbit 18, who died during the second series of Bacteroides injections. Note the hyperemia, calcium deposits and inflammation. Hematoxylin and Eosin stain Original magnification 60x.

Figure 24

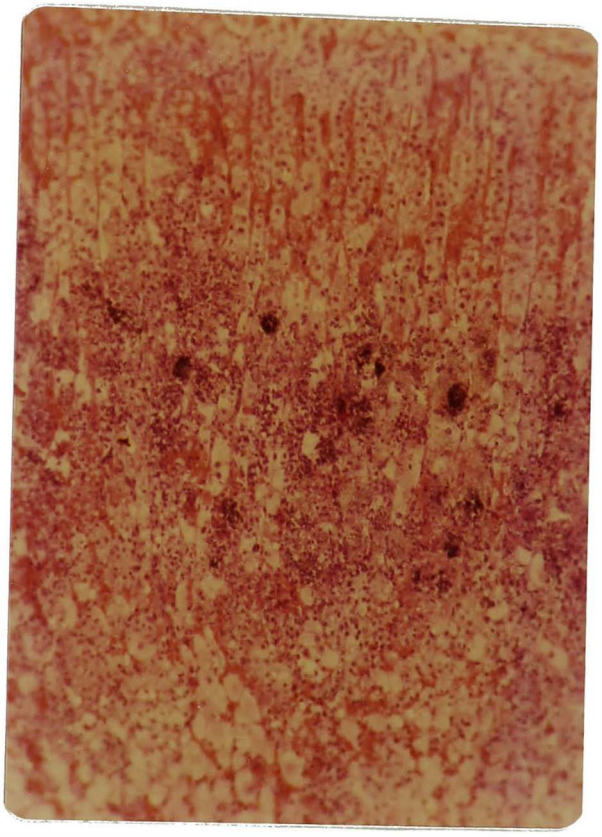

Fig 24 - Adrenal cortex. A higher magnification of fig 23 to show the calcium deposition and inflammatory response. Hematoxylin and Eosin stain. Original magnification 150x.