M.Sc. PATHOLOGY

G. CRADDOCK McMILLAN

THE EFFECT OF ALLOXAN DIABETES MELLITUS ON EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS IN THE RABBIT.

September, 1946.

THE EFFECT OF DIABETES MELLITUS ON EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS IN THE RABBIT

INTRODUCTION

As social and medical progress have gradually emancipated mankind from many of the more acute and fatal diseases, so have the diseases associated with old and middle age come into prominence. Among these latter is arteriosclerosis - a disease which, with its sequelae, has assumed major importance as a cause of death and physical incapacity. Study of the disease has yielded an immense amount of information but, up to the present, little understanding of its fundamental properties, and nothing of value for its prevention or cure. Any study which may elucidate the pathogenesis of arteriosclerosis is, therefore, of value; and it is to this end that the present experiment was designed.

The basis of this experiment is found in the common clinical impression that persons suffering from diabetes mellitus are especially prone to arteriosclerosis. Obviously an experimental study of the association of these two diseases might elicit important information, but unfortunately it is only within the past few years that such an experiment has become technically feasible. While a method has been available for causing a disease closely resembling human arteriosclerosis in the rabbit for many years, the technical difficulty of inducing diabetes in these animals made a combined study impossible. On the other hand, animals which could be rendered diabetic with relative ease were refractory to the production of a suitable type of arteriosclerosis. Recently, a simple method of rendering rabbits diabetic has made the associative experiment

TABLE OF CONTENTS

possible.

Even a casual study of the problem of human arteriosclerosis discloses a fundamental confusion of thought that pervades the entire subject. It is not, for example, completely agreed that arteriosclerosis is a disease rather than a physiological manifestation of senescence. Nor is the morphology of the changes observed, agreed upon with unanimity. The etiology is deeply confused and the pathogenesis debatable. Because of this perplexing state of the fundamental concepts of arteriosclerosis, it is proposed to consider its various aspects at some length - less in conformity with tradition than of necessity - in establishing a theoretical basis for experimental study and for the maintenance of human and experimental analogy.

PART I

THE HISTORY OF ARTERIOSCLEROSIS[1]

The history of arteriosclerosis deserves some brief mention, not only because of its inherent interest, but also because it traces the development of the concept that it is truly a disease, and is therefore subject to prevention and cure.

Paleopathology has disclosed that the ancients suffered from typical arteriosclerosis, however, there is no indication that they displayed any interest in the disease. Indeed, it is not until the period of Vesalius and Fallopius, and the anatomists who followed that the lesions of arteriosclerosis were described in human material. By 1600 "ossification" of the arteries was generally known and was generally accepted as a natural phenomenon of advancing years. It was Giovanni Morgagni who, in 1761, first gave extensive consideration to the lesions of arteriosclerosis and to their sequelae. In the nineteenth century Bichat applied a histological approach to the problem and somewhat later (1815) Joseph Hodgson came to the conclusion - on somewhat uncertain grounds - that arteriosclerosis was a disease and not simply a sign of the advance of life. The honor of coining the term 'arteriosclerosis' belongs to Jean Frederic Martin Lobstein (1829-33).

At the middle of the nineteenth century the father of modern pathology, Karl Rokitansky, felt that arteriosclerosis could not be attributed to old age alone although old age obviously predisposed to the disease. He suggested further that the condition was due to a deposition of some material on the intimal surface from the blood mass. Virchow, too, considered the lesion as a disease and modified Rokitansky's views by stating that the changes were of

an imbibition into the intima of some substance from the blood.

From the last quarter of the nineteenth century to the present a great amount of thought and investigation have been expended on the lesions comprising arteriosclerosis, and it is this period that has come to consider arteriosclerosis as a disease entity subject to definition, description and experimental study. The names of the most prominent pathologists in this field include those of Thoma, Jores, Marchand - who coined the term atherosclerosis - Klotz, Aschoff and Anitschkow.

THE DEFINITION OF ARTERIOSCLEROSIS

The definition of arteriosclerosis is not an easy matter. Indeed, as late as 1933 Ludwig Aschoff[2] states in rather nebulous terms "To sum up, we understand by arteriosclerosis a chronic disturbance of the vessels which manifests itself by deposits of the most varied kinds in the vascular walls and which becomes irreversible on reaching its climax in vessels impaired by changes attending the process of aging with resulting deformation of the lumen and brittleness of the vascular walls". Such a definition is broad enough to include almost any type of vascular pathology including forms such as syphilitic aortitis, thromboangiitis obliterans and others. It also allows venous and lymphatic as well as arterial vasculature to be included in the terms of reference. And, it is inclusive of the various arterial lesions produced by experimental methods. Bell[3] limits the definition somewhat, preferring a definition by exclusion. He states "All forms of arterial disease except those that are frankly inflammatory in character are commonly called arteriosclerosis". This definition has the advantage of excluding bacterial, spirocaetal, mycotic and allergic forms, of arteritis but, consequently, is not entirely compatible to those

persons who maintain an infectious or allergic etiology for the disease. Furthermore, analagous pathology in non-arterial vasculature is also excluded. Moschcowitz[4] speaks of "a progressive and irreversible affection in which hyperplasia of one or more coats is a primary reaction, with deposition of collagenous, lipoid, hyaline and calcium as a secondary reaction, the totality of both components resulting in thickening, dilatation, deformity and loss of elasticity of the walls.

Boyd[5] refuses to recognize the "omnibus" term arteriosclerosis, substituting for it the three arterial diseases, i.e. atherosclerosis, Monckeberg's medial sclerosis and diffuse hyperplastic sclerosis, that are most commonly included under the term. Hueper[6] too, in his extensive recent review of arteriosclerosis, avoids defining his subject matter, referring merely to "the degenerative and sclerosing arterial diseases known under the collective term of arteriosclerosis". It is apparent from his work, however, that he has a catholic approach to the subject. Winternitz[7] speaks of the "constellation of processes" that the term arteriosclerosis represents, but does not propose a suitable definition. Page[8] begins a discussion of arteriosclerosis and lipoid metabolism with the words: "The problem of arteriosclerosis is the nub of the problem of cardio-vascular disease. Despite this, its cause, its classification, its reproduction and its cure are not surely known".

Indeed, the number of competent authors who propose more or less limited definitions of the disease, and those who avoid any definite formalization of the terms of reference of arteriosclerosis may be quoted almost ad infinitum.

This state of affairs, arising as it does from a lack of knowledge of the etiology and an uncertainty concerning the limits

of the morphology of the disease, is not as distressing as it may appear at first sight, for there is a wide range of general agreement over which a satisfactory definition can be given. The definition in this case must of course contain no statement as to etiology, being of a morphological nature only. It is, in fact, nothing more than a detailed description of what Boyd describes as atherosclerosis; it is the variety of arteriosclerosis that is of particular interest to our present purpose and which will be described later.

In selecting this definition of arteriosclerosis, I do so merely because it is pertinent to the theme of this paper, and I do not propose it as a true and complete definition of the pathological complex known as arteriosclerosis. Actually, I believe that, for the present, no better definition can be formulated than that quoted from Aschoff at the beginning of this section.

THE ETIOLOGY OF ARTERIOSCLEROSIS

As the difficulty of definition of arteriosclerosis can be resolved by selecting one relevant to the experimental work in progress, so, too, can the question of etiology be somewhat clarified in the same manner.

The complexity of the problem of etiology of arteriosclerosis is well exemplified by a table constructed by Hueper[9] and reproduced in full below.

TABLE I - CLASSIFICATION OF ETIOLOGIC FACTORS OF SPONTANEOUS AND EXPERIMENTAL ARTERIOSCLEROSIS.

I - Vasculotonic Agents.

1. Hypotonic agents causing stagnant anoxemia and increased permeability of the relaxed vascular walls through an excessive slowing of the blood flow.

(a) Endogenous agents: Histamine, acetycholine (orthostatic vasomotor insufficiency, hypo-adrenalism, hypothyroidism, hypopituitarism).

(b) Exogenous agents: Nitrates and nitritis, cyanides, carbon monoxide, barbiturates, reduced atmospheric oxygen pressure, arsenic, mercury, manganese, traumatic shock.

2. Hypertonic agents causing constrictory ischemic anoxemia of the vascular tissues by a compression of the vasa vasorum and by reduced vascular permeability of the contracted vascular wall hindering the movement of tissue fluids and the action of diffusion processes.

(a) Endogenous agents: Adrenalin, adrenal cortical hormone, posterior pituitary hormone, thyroid hormone, parathyroid hormone, angiotonine, tyrosine, tyramine, guanidine.

(b) Exogenous agents: Suprarenine, ephedrine and derivatives, ergotine, hydrastine, digitalis, glucosides, nicotine, S-methyl isothiourea, vitamin D, calcium salts, acidosis and hypercalcemia producing chemicals (ammonium chloride, ammonium hydroxide, calcium phosphate, etc.), cold, vibration, barium chloride, solarisation, chemo-allergies, psychic strain, trauma, iodine, uranium, mercury bichloride, cutting of depressor nerves, desoxycorticosterone, aromatic aldehydes.

II - Intravascular Hydrostatic Pressure.

1. Increased hydrostatic pressure (local or general) causing an ischemic anoxemia by compression of the vasa vasorum against the inelastic adventitia and a mechanical overextension of the contractile and elastic elements in the vascular wall.

(a) Endogenous mechanisms: Local, congenital, cardiac and

vascular abnormalities of the pulmonary circulation (open foramen ovale, septum defects, transposition of large vessels, open ductus arteriosus, mitral stenosis, congenital hypoplasia of the small pulmonary arteries or of pulmonary veins). coarctation of aorta, sites of arterial bifurcations. General: Plethora.

(b) Exogenous mechanisms: Local; excessive physical labor, traumatic arteriovenous aneurysm, pulmonary fibrosis of pneumoconiotic or infectious origin, pulmonary emphysema, pulmonary bilharziasis, abnormal static condition (gravity forces in posture (standing), centrifugal and accelerating forces in flying), chronic pulmonary oedema in oxygen poisoning, consumption of excessive amounts of liquids and general circulatory failure.

2. Decreased hydrostatic pressure (local or general) causing an ischemic anoxemia of the vascular walls by reduction or cessation of blood supply.

(a) Endogenous mechanisms: Disuse and senile involution by changes in normal circulation (umbilical artery, omphalomesenteric and hypogastric arteries, ductus arteriosus, uterine and ovarian arteries), splenic arterioles.

(b) Exogenous mechanisms: Proximal and distal parts of ligated arteries, distal part of artery in arteriovenous aneurysm, arteries located in scar tissue (radiodermatitis, floor of chronic gastric ulcer, perinephritic tissue), distal part of arteries with proximal spastic contractions (renal arterioles in nephrosclerosis), scleroderma.

III - Colloidal Plasmatic Disturbances Resulting In The Formation

III - Of Films And Precipitates On The Intima And Causing Thereby An Impairment Of The Exchange Of Gases And Nutritive Substances Across The Interface Between Blood and Intima As Well As Decrease Of The Permeability Of The Vascular Wall.

1. Lipoidal plasmatic disturbances.
   (a) Endogenous disturbances: Hyperlipoidemia in diabetes mellitus, hypothyroidism, essential xanthomatosis, glycogenosis, pregnancy, Gaucher's disease, psoriasis.
   (b) Exogenous disturbances: Hyperlipoidemia with excessive dietary lipoid intake, lipoid nephrosis, starvation, carbon disulphide poisoning, goitrogenic substances (sulphaguanidine, thiouria derivatives, thiocyanates), saponin, loss of blood.
2. Carbohydrate plasmatic disturbances.
   (a) Endogenous disturbances: Glycogenosis.
   (b) Exogenous disturbances: Polyvinylosis, methyl cellulosis, pectinosis, arabinosis.
3. Proteinic Plasmatic Disturbances.
   (a) Endogenous disturbances: Amyloidosis, hyperglobulinemia.
   (b) Exogenous disturbances: Allergic hyperglobulinemia, experimental proteinoses (gelatine, ovalbumin, serum azoproteins).

IV - Hematic Anoxemic Agents: Producers of inert haemoglobin derivatives (carbon monoxide hemoglobin, sulph-hemoglobin, etc.) (carbon monoxide, sulphonamides, nitritis) and disturbances in the oxygen-carbon dioxide balance (reduced atmospheric oxygen pressure, oxygen poisoning (hyperoxemic hyperoxidosis)).

The fact that the above table is not necessarily complete, that we may question the validity of the major part of its content and that the classification is theoretical, need not concern us here.

It merely serves to show the many etiological agents that have been incriminated in the development of degenerative and sclerotic arterial lesions.

In as much as we are primarily concerned with a somewhat limited definition of arteriosclerosis, with a specific experimental method of simulating this type of arteriosclerosis and with the effect of the metabolic disorder known as diabetes mellitus upon it, we can disregard the major part of the list of etiological agents given by Hueper. Suffice it to point out that the agents in which we are tentatively interested are found in this table. These comprise: acidosis, consumption of excessive amounts of liquids, hyperlipaemia in diabetes mellitus, hyperlipoidemia with excessive dietary lipoid intake and carbohydrate plasmatic disturbances. In fact, our attention will be concentrated largely on the last three of these in this experiment.

THE MORPHOLOGY AND PATHOGENESIS OF HUMAN ARTERIOSCLEROSIS IN THE AORTA(10)

In order to define more closely the sense in which the term arteriosclerosis is used in this paper, it is necessary to give a brief description of its morphology. Such a description serves the further purpose of allowing a comparison of experimental findings with the human disease.

The earliest lesions have not been described and at present exist only as hypothetical postulates. The earliest lesions that are described are found in children and may tentatively be classed as the precursors of definite arteriosclerosis. These lesions consist of small fatty flecks or streaks. They are yellowish in color, round or oval in shape, and project above the normal intimal surface.

They are sometimes quite elongate, arranged in rows or confluent. They are located throughout the length of the aorta, being most common in the valves of the left side of the heart and at the base of the aorta in the sinuses of Valsalva or just superior to them. Here their orientation tends to be transverse to the length of the aorta. These fatty lesions are also common along the posterior wall of the descending aorta in its thoracic portion, where they tend to form longitudinally orientated streaks not connected with the mouths of the intercostal arteries. They often are present also in the abdominal aorta and in the large branches of the aorta itself.

Microscopically, the affected areas show a slight intimal swelling as if the tissues were oedematous. The intimal cells, both fibrocytic and macrophagic, show a high content of finely divided lipoid, much of which is cholesterol. Whether or not lipoid is to be found in the lesion before it can be identified within the intimal cells is debatable. In any case, as the lesion advances both intra- and extracellular lipoid are found, the latter somewhat concentrated along the elastic fibrils of the intima. A slight lymphocytic infiltration and a minimal proliferation of fibrous connective tissue cells are noted about the lesion.

At this phase of the lesion opinion is widely split as to its further development. There are several schools of thought on the matter: Zinserling[11] holds that the process is irreversible and progresses to frank arteriosclerosis: Virchow and, more recently, Sanders[12] believed that these lesions bear no actual or causal relationship to arteriosclerosis. Klotz and Manning[13], Leary[14] and others, on the other hand take the intermediate view that some of these fatty lesions heal with complete restitution while others, particularly in adolescence and later ages progress to well developed

atherosclerosis and arteriosclerosis. Whatever may be the final decision on this extremely important point, it is my impression that this latter hypothesis is the most widely accepted and most acceptable. It will be assumed for the present.

The further course of the lesions is agreed upon. The lesions present a varied morphology, one type merging imperceptibly with another. It, therefore, appears reasonable to assume that they constitute various stages of the same process.

Grossly, the early lesions resemble those described above, but they become larger, more conspicuous and tend to confluence with the formation of plaques. Further development converts the inner surface of the lesion into a pearly grey, fibrous area while the fatty core of the lesion undergoes degeneration and necrosis forming a true atheroma. The lesion may now stabilize by the formation of a calcareous plaque in relation to the fatty and necrotic centre, and fibrous tissue, or it may undergo further degeneration and necrosis towards the vessel lumen with ultimate rupture, extrusion of part of the fatty pultaceous content and the formation of an atheromatous ulcer. The base of the ulcer then becomes coated with thrombus material.

The location of well developed and distorting arteriosclerotic lesions is somewhat different from that of the fatty flecks and streaks, for while they are found again throughout the length of the aorta and in its branches, the most severe and fully developed lesions occur in the abdominal aorta and iliac arteries. The thoracic aorta may be markedly involved, but is usually less so than the abdominal part and here, the involvement is most severe along the posterior wall around or in the vicinity of the orifices of the intercostal arteries. The base of the aorta usually shows relatively

minor involvement.

Microscopically, the lesions differ from fatty flecks and streaks, not only in a simple quantitative manner, but also in the fact that fibrosis becomes the dominant feature. Associated with this fibrosis is a peculiar granular, basophilic necrosis of the fibrillated collagen. The fat containing cells also undergo necrosis and liberate their lipoid content. As in any necrotic and fat containing lesion more or less calcium and even bone formation may be seen. Associated with the lesion are chronic inflammatory cells, an excess vascularity and often frank haemorrhage. Necrosis and scarification may extend deep into the media below the fibrous and atheromatous plaque.

In brief then, we have described a type of arteriosclerosis in man that is a progressive, fatty, necrotic, reactive, fibrotic and sclerotic lesion, and further, we have assumed that its precursor is the lesion of simple fatty streaking. If this assumption is permitted, an assumption which cannot be proven or disproven at present, we are then in a position to describe the lesions of experimental cholesterol arteriosclerosis in the rabbit and to compare them with human arteriosclerosis.

EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS IN THE RABBIT : MORPHOLOGY.

Although there is general agreement among the workers who have produced arteriosclerosis in the rabbit, by feeding cholesterol, concerning its morphology and pathogenesis, certain variations in experimental methods and perhaps animal strains have led to minor variations in the published descriptions of the resulting lesions. The following description is taken largely from the work of

Anitschkow[15,16], Leary[14,17,18,19], and Duff[20,21].

The gross lesions appear first as minute, slightly raised, round or oval, yellowish-white specks that shine through the intimal aortic surface. The first area to be affected is just distal to the aortic ring and about the branch vessels of the arch. The lesions increase in size, become more circumscribed and sharply demarcated, and may attain 1 or 2 millimeters in diameter. At the same time new lesions appear in the thoracic aorta showing some slight tendency to localize on the posterior wall about and between the ostia of the intercostal arteries. The proximal part of the abdominal aorta, about its main branches is also frequently subject to the development of lesions. As the lesions progress there is a tendency to confluence so that plaques and longitudinally orientated streaks are formed, while the intima becomes coarsely roughened and nodular, and the vessel may be subject to irregular dilatation and even aneurysm formation. There is a concomitant loss of vascular elasticity. As the disease advances, lesions appear in the various branch arteries and in the pulmonary artery, however the cerebral and retinal arteries remain free from change.

Microscopically, intimal changes may be detected as much as a month before grossly visible lesions are found. The subendothelial ground substance becomes slightly swollen and after a time fine droplets of fatty material are deposited in the affected area. This change is followed by the accumulation of many or few fat filled macrophages which are morphologically identical with those found in human arteriosclerosis. In this focus of swollen ground substance, fat deposition and foamy phagocytes, stellate cells of the fibroblastic type appear in considerable numbers, and the lesion reaches an appreciable size. As the lesion progresses the foam cells

in the deepest part of the plaque undergo necrosis and release their lipoid content forming an atheromatous "abscess". At the same time fibroblastic activity is accelerated and a relatively thick fibrous layer is formed beneath the intima. The necrotic material tends to become calcified and the calcification may extend to the media which has by this time been affected by a process of muscle and elastic tissue destruction, lipoid deposition, foam cell infiltration and fibrosis.

An additional medial change may occur independently of overlying intimal atherosclerosis. This alteration begins as a focal necrosis in the inner third of the media, the affected area being very pale staining, and containing a flocculent ground substance and cell fragments. The larger areas then develop droplets of lipoid but only the occasional small phagocytic mononuclear cell is ever found.

If the experimental animal is discontinued from cholesterol feeding after a time period when well developed arteriosclerotic lesions may be assumed to have developed, then the lesions slowly regress. The fatty materials in the plaques lessen in quantity, particularly in the region of the aortic arch. The superficial layers of the lesion heal by the formation of fibrous tissue with abundant collagen and elastic fibrils. The smallest lesions presumably heal with complete restitution but the smallest ones which remain consist of fibrous connective tissue with a minute amount of intra- or extracellular lipoid. The larger lesions retain a necrotic lipoid-rich base, while calcification is a frequent occurrence.

SPONTANEOUS ARTERIOSCLEROTIC LESIONS IN THE RABBIT, AND THE SPECIFICITY OF CHOLESTEROL INDUCED EXPERIMENTAL ARTERIOSCLEROSIS

In the above morphological description it has been tacitly

assumed that while the lesions were indubitably associated with a diet rich in cholesterol, this unusual amount of cholesterol was in fact the cause of the lesions. Before proceeding to examine the morphological relationships that may exist between human arteriosclerosis and experimental cholesterol arteriosclerosis in the rabbit, it is well to establish the etiological status of cholesterol relative to the associated lesions.

Fox[22] has studied the occurrence of spontaneous vascular lesions in many species of wild, captive and domestic animals. Considering the rabbit, he states that the incidence of spontaneous aortic arteriosclerosis in ordinary rabbit stock has an incidence of 0 to 34 per cent. The lesions appear either as a focal, whitish, granularity of the intima or as cup-like depressions overlain by a smooth intima. The lesion commonly affects the mediastinal aorta.

Microscopically, the lesions are fundamentally medial in location. Areas of muscle cells in the middle or inner thirds of the media become necrotic and disappear, while the elastica collapses together in rather straight parallel rows. The formation of clear calcium plates is the end result. Secondarily, the intima shows a swelling of the ground substance and a fibrillar thickening of the intima in which fine elastic fibrils are found. There may be superadded a minimal deposition of lipoid material and occasional calcium strips. Obviously, the lesion differs grossly and microscopically from those induced by cholesterol feeding. The literature contains at least two reports of arteriosclerotic lesions in rabbits that apparently occurred spontaneously and that were identical with those produced experimentally. Nuzum et al[23] found the lesion in 6 of 190 rabbits while Ophuls[24] found it in one rabbit just received into his laboratory. These rabbits were subject to a variety of experimental and unknown factors and, therefore, the reported findings

lose much of their significance. Indeed, when one considers the many thousands of rabbits that have been studied with particular reference to their vasculature without the finding of this type of lesion, it becomes evident that the two reports referred to above were, in all probability, not concerned with true spontaneous lesions. They do indicate, nevertheless, that such spontaneous lesions might, unfortunately, confuse an experiment of the present type. In the presence of an adequate control series, however, the probability of such an occurrence becomes vanishingly small, and it may be assumed that no lesions resembling experimental cholesterol arteriosclerosis can occur spontaneously. Nevertheless, in selected, in-bred stock, it is possible to obtain strains that show a high and severe incidence of inherited atherosclerosis (Greene)[25].

Of the many experimental methods[9] now known whereby some form of arteriosclerosis may be produced, none give lesions that are more than superficially comparable with the type of lesion in which we are interested except cholesterol feeding. It is, therefore, apparent that this lesion does not exist unless cholesterol is fed in large amounts. It has not been shown whether cholesterol feeding without a concomitant cholesterolemia can produce the lesions, but all available evidence is against such a hypothesis. Bailey[26] fed minute amounts of cholesterol without obtaining lesions. Lesions apparently do not occur until various of the parenchymatous organs have become loaded with free or esterified cholesterol. We may conclude, therefore, that cholesterol feeding and presumably a cholesterolemia are, if not the cause of cholesterol arteriosclerosis in the rabbit, at least the sine qua non of the lesion.

THE DISSIMILARITIES OF HUMAN ARTERIOSCLEROSIS WITH EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS IN THE RABBIT: THE ANALOGY OF THE TWO DISEASES.

While it is a relatively simple matter to compare the morphological similarities and dissimilarities of human and experimental cholesterol arteriosclerosis, a comparison on any other basis is exceedingly difficult. In a latter part of this paper a consideration of cholesterolemia and of diabetes mellitus in relation to the human and animal lesions will be given, but for the present it is well to confine ourselves to the morphological changes observed.

Duff[20,21] has made the most rigorous study of this aspect of the subject. He finds, in contra-distinction to Anitschkow[16] and to Leary[18,19], that the differences observed are of sufficient magnitude to require careful consideration and factual explanation rather than casual elimination. The most obvious difference lies in the fact that the production of the experimental lesions requires first that the reticulo-endothelial system and various interstitial tissues be almost saturated with cholesterol-rich lipoid material. No such lipoid depositions are found in ordinary human arteriosclerosis. A second gross difference is apparent in the distribution of the arterial lesions. In the rabbit these are found in the aorta, its major and secondary branch arteries and in the pulmonary arteries; the cerebral and retinal arteries are exempt. Moreover, the most severely affected areas are found in the mediastinal aorta. In man, on the other hand, while the aorta and its branches are the main site of affection the abdominal portion of the aorta shows the most advanced and severe lesions. The pulmonary arteries are not affected unless there is an associated and presumably pre-existent hyper-

tension of the pulmonary circulation. Contrarily, the cerebral and retinal vessels are often affected. A third difference is found in the microscopic medial lesions which, as described above, occur in the experimental animals in the absence of overlying intimal change.

It is apparent that the similarities between the two types of lesions are three. The first, is the gross and microscopic morphology of the intimal lesions. The second is the tendency of these lesions to localize about the ostia of branch arteries. The third is the microscopic and gross appearance of the mature and regressing lesions. Of the earliest lesions nothing can be said because there is no agreement as to what the earliest human lesions are.

I do not propose to enter the argument that is current concerning the degree of parallelism that exists between the experimental and the human diseases. In the comparison of any human and the corresponding experimental diseases the question of analogy presents more or less difficulty. In the present case the difficulty is compounded by the uncertainty that pervades the entire field of the pathology of arteriosclerosis. A consideration of the equivalence of the two diseases, therefore, becomes a matter of speculation based upon experience, observation and training. There are those who hold that none of the differences is of an essential nature (Anitschkow)[16] and who maintain that an absolute identity between the natural and experimental lesions is too exacting a requirement (Leary)[18]. Others[21] maintain that the similarities are great but that the differences are worthy of consideration in any analogy that is attempted.

It suffices our present purpose to emphasize that the similarities of the human and experimental cholesterol lesions are sufficiently striking to suggest that there are some common factor

or factors operating in their etiology and pathogenesis. If such be the case, then these factors deserve to be elucidated and to have their relative importance gaged.

PART I

SUMMARY

For the purposes of the present experiment it was found necessary to denote the term arteriosclerosis by a morphological account of the changes commonly found in that variety of arteriosclerosis known as atherosclerosis. The connotation was strictly limited to this morphologic definition, and the two terms were made synonymous. The experimental lesion was defined morphologically in a similar manner, separated from spontaneous arterial lesions, and compared with the human disease. As a result of this comparison, the experimental lesion was found to be similar but not identical with the human lesion. The etiology of both human and experimental lesions was considered, and it was found that cholesterol feeding was a sine qua non for the production of the disease in rabbits. No etiological conclusions by analogy or otherwise were attempted with reference to the human disease.

PART II THE RELATIONSHIP OF DIABETES MELLITUS AND ARTERIOSCLEROSIS IN MAN.

Having established the terms of reference and the limitations of human and experimental cholesterol arteriosclerosis, we may now examine the second component of this study, namely, the correlation between diabetes mellitus and arteriosclerosis. Again, the type of arteriosclerosis concerned is of the atheromatous variety[27,28], and again, cholesterol is an etiological agent of interest - this time with particular reference to the human lesion. Before proceeding further, it is well to examine the statement "correlation between diabetes mellitus and arteriosclerosis". This statement implies that the arteriosclerosis is secondary, in part at least, to the diabetes. Such may not be the case, and Moschcowitz[29] would reverse the statement so that diabetes is secondary to arteriosclerosis, and, many years ago Naunyn[90] suggested that, while the connection between arteriosclerosis and diabetes was unknown, perhaps the arteriosclerosis caused functional disturbances in the pancreas. Indeed, this appears to be true in a minority of cases,[28] but the etiology of diabetes is so obscure from the pathological point of view[28], that little is gained by attempting to assign a single etiology to it. It suffices to point out that diabetes is a sporadic disease with fairly well demarcated genetic tendencies[30], that it occurs at all age periods[31] and is subject to remission in rare cases. Arteriosclerosis is, undoubtedly, a hereditary disease of universal incidence after the age of 60 years[32], but it is not subject to simple genetic formulation, and it does not appear to be capable of more than transitory, partial healing. The occurrence of diabetes mellitus in young individuals in the absence of demonstrable arteriosclerosis, and the occurrence of advanced arterio-

sclerosis in persons without detectable diabetes makes the hypothesis that diabetes is secondary to arteriosclerosis a difficult one to sustain. However, in those persons who suffer from both afflictions the hypothesis is still possible. Nevertheless, the common observation that diabetes precedes arteriosclerosis in many cases, militates against it. It is almost unanimously maintained that the atherosclerosis associated with diabetes is largely secondary to it.

The demonstration that diabetes leads to an excessive and premature degree of atherosclerosis depends, in the main, on findings in the peripheral and coronary arteries. The fact that the discussion in Part I was limited, largely, to the aorta does not raise a relevant objection, for it is largely a matter of convenience which determines whether the main or the lesser vasculature is considered; the lesions appear to be essentially the same in either case. It is true that diabetics are particularly prone to sequelae secondary to peripheral or coronary arteriosclerosis, as will be shown, but this appears to be an artefact of observation rather than a real difference in the type of lesions found in diabetic and non-diabetic arteriosclerosis. The possibility that such a conclusion is not warranted should be borne in mind, however, for the lesions may in fact possess superficial morphological differences, and may well possess fundamental etiological dissimilarities.

Warren[28] states that the arteriosclerosis in the diabetic is fundamentally and predominantly an atherosclerotic lesion, so much so that he believes that it is possible to tell in some cases at least if a peripheral artery was obtained from a diabetic person. Lisa et al[33] on the other hand, after careful examination of legs

amputated for vascular disease in both diabetics and non-diabetics were unable to tell which was which from an examination of the arteries. Buerger[44] also states that the lesions are essentially similar. At the present time the opinion of Warren is dominant, and it is generally held that diabetic arteries emphasize the atherosclerotic component more than do non-diabetic arteriosclerotic arteries.

Statistics dealing with the incidence and degree of atherosclerosis and sequelae in diabetic persons are somewhat difficult to interpret because of a fundamental lack of adequate control material from non-diabetic persons. The best control studies appear to be those of Willius et al[32] and of Ophuls[34,35]. It is obvious, however, from the material to be presented that diabetics do suffer an increased incidence of the disease.

Apparently an association between diabetes and angina pectoris was first noted in 1864[36] and 1883[36]. Blotner[36] analyzed 77 fatal cases of diabetes with a control group of 450 non-diabetics of ages 40 to 80 years. He found coronary sclerosis in 45% of the diabetics, all over 34 years, and similar sclerosis in only 21% of the control group. Death was attributable to the heart in 43% of the diabetics. Root et al[37] from a study of 349 diabetic autopsies over the years 1921 to 1939 with a control group of 3400 autopsies from the years 1925 to 1939 found coronary occlusion in 32% of the diabetics of all ages and in only 6% of the non-diabetics. Coronary narrowing without occlusion was present in 19% of the diabetics and 14% of the non-diabetics. Only 49% of the diabetics showed no significant coronary artery sclerosis while 80% of the non-diabetics were in this class. These figures are somewhat biased by the fact that the control group contained an excessive number of

individuals below the age of 40 years. However, a breakdown of the figures into age groups maintains approximately the same ratios. He found further, that up to the age of 60 years, five times as many males as females suffer coronary occlusion in the non-diabetic group, while among diabetics the ratio was about 1:1 (the sex incidence of diabetes is 4.6:5.2, male:female[31]). Moreover, in the group which did not develop significant sclerosis, a short duration or a mild form of diabetes was a crucial determinant. Lisa et al[38], from autopsy studies on 193 diabetics and 2092 non-diabetics all over the age of 40 years, found coronary artery sclerosis in 70% of diabetics and 60% of non-diabetics; of these, sclerosis was moderate or severe respectively in 35% and 65% of the diabetics and in 48% and 52% of the non-diabetics; and of these, myocardial infarction was present in 30% of the diabetics and in 22% of the non-diabetics. A study of age groups showed an increase of moderate sclerosis in diabetics over non-diabetics for the 5th decade, which was supplanted by an increase in severe sclerosis in diabetics over non-diabetics from the 6th to 9th decades. It is interesting to note that the age period did not alter the incidence of myocardial infarction. Enklewitz[39] found coronary thrombosis twice as frequent in diabetics as in non-diabetics. There were 92 diabetic autopsies in his series. Nathanson[40] autopsied 100 diabetics and had a control group of 249 autopsies of persons over 50 years of age. 52.7% of diabetics of 50 or more years of age had severe coronary sclerosis, while only 8.2% of the control group had comparable lesions. In 113 cases of coronary disease the sex ratio was 3:1 male:female, whereas, in the cases of his diabetic series it was 1.8:1. Wilder[41] in 49 autopsies on diabetics in the period 1919 to 1925 found advanced coronary

sclerosis in 34%. Root[42] found coronary sclerosis in 60% of 55 diabetics at autopsy. Levine and Brown[43] reported glycosuria in 24% of 145 cases of clinical coronary thrombosis, a figure which included some transient glycosuriae. They state "....it has been striking to find so many diabetics in this group, and we feel that among the distinct disease entities that are etiologically related to coronary thrombosis, diabetes is second in importance only to a previously existing hypertension. The average age of the diabetic group was 58.1 years, of the series 57.8 years. Nor was the prognosis altered.....this leads one to feel that it (diabetes) had no causitive influence in the disease of the coronary arteries but merely indicated the type of person who had a vulnerable vascular system".

Peripheral arteriosclerosis as indicated by pathological examination, X-ray or the signs and symptoms of local circulatory failure has been studied by many authors. Lisa and co-workers[33] examined 55 diabetic and 51 non-diabetic legs amputated for vascular disease during the years 1930 to 1940. Gangrene was present in 39 diabetic legs and in 31 non-diabetic ones. Assuming an incidence of diabetes of 1% of the general population, it is apparent that there is a marked incidence of peripheral vascular disease among the diabetic group. Eisile[45] studied 73 juvenile diabetics who had had the disease for 20 or more years. 30% showed X-ray evidence of arteriosclerosis of the legs at an average age of 29 years, and one half of these had hypertension. Dry and Hines[46] found 230 diabetics among a group of 7073 diabetic patients, who had clinical evidence of occlusive peripheral vascular disease. Their non-diabetic control group consisted of 219 such patients among a group of 197,894. 60% of this latter group had blood sugar studies performed on them.

Their age groups began at the 4th decade. The authors found that the absolute incidence of the lesion in the diabetic group was very much higher in every decade, and that the ratio for the entire series was 11:1, diabetic:non-diabetic. The lesion occurred a decade earlier among the diabetic group. The sex ratio was 7:1, male:female for the non-diabetics and 2:1 among the diabetics, while occlusive vascular disease was 80 times as frequent among the women of the diabetic group as it was among those of the non-diabetic series. Kramer[47] analyzed 58 cases of diabetic gangrene occurring among a group of 1008 diabetics, and reviewed some of the relevant literature. His findings are shown in Table 2.

TABLE 2

INCIDENCE OF DIABETIC GANGRENE

Reported By	Hospital	Years	No. of Diabetics	No. with Gangrene	% Gangrene
Blotner and Fitz	Peter Bent Brigham	1913-1925	969	69	7.0
McKittrick and Root	New England Deaconess	1923-1928	4066	163	4.08
Wendt and Peck	Grace Hosp.	1919-1929	1073	113	10.7
Futcher	Johns Hopkins	1889-1925	1025	49	4.5
Coller and Marsh	Univ. Hosp. Michigan	1921-1925	650	20	3.2
Lemann	New Orleans	1921-1926	471	25	5.3
Paullin (by Lemann)	Georgia Charity, New Orleans	1921-26	560	15	2.65
		1921-26	439	79	18.0
Lemann	Touro, New Orl.	1921-1926	201	20	10.0
	Boston City	1921-1926	967	109	11.2
	Mass. General	1921-1926	600	58	9.0
	Montr. General	1921-26	1016	36	3.5
Kramer		1920-1930	1008	58	5.75
			13045	814	6.2

In addition Kramer found 28 threatened and 89 potential cases of gangrene among his 1008 diabetic cases. Morrison and Bogan[48] studied the arteries of the legs in 324 diabetics by X-ray. The ages of the patients were between 2 and 81 years. They found that calcification of the leg vessels to be rare in normal persons of less than 40 years of age, while for the age period 40 to 50 years, it was 36%. Among the corresponding diabetic group it was 63%. There were 22 cases of diabetic gangrene, all over 40 years of age, and 21% of the diabetic group showed advanced calcification, again all being more than 40 years old. From a study of the duration of the diabetes they concluded that the incidence of vascular calcification increases with the patient's age and with the duration of his diabetes, and that the severity of the lesion increases in a corresponding manner. Theybelieve, therefore, that diabetes is an etiological factor in the production of peripheral vascular calcification. Wilder[41] found that gangrene was responsible for 14 of 81 diabetic deaths in the age period of 39 to 75 years. Shepardson[49] studied the leg vessels by X-ray in a group of 50 diabetics who were less than 40 years of age and who had had diabetes for more than 5 years. 36% showed visible calcification. This author found that the clinical severity of the diabetes was of no importance in the development of calcification, and considered duration important only because a certain time must elapse before the effects of the diabetes are manifest as vascular calcification. Two patients of 11 and 13 years of age showed calcification in his series. White and Hunt[50] found that 9 of 48 diabetics of more than 4 years duration and less than 21 years of age had calcification of leg arteries as determined by X-ray. Letulle and co-authors[51] found calcified peripheral arteries in 55 of 71 diabetic patients. Collens and

Wilensky[52] in a loosely written report on various types of peripheral vascular disease found that 48 of 124 cases occurred in diabetics and concluded that "while sclerotic changes occur almost as frequently in non-diabetic as in diabetic persons, the vulnerability of diabetic tissues to infection results in thrombotic processes that subsequently terminate in death of tissues." Mandelberg and Sheinfeld[53] reported 128 cases of major amputation of gangrenous legs in diabetics, finding that 62.5% occurred in females, and that 5 cases occurred in the age period 40 to 49 years.

Information concerning the aortae of diabetic persons is both morphologic and chemical. Lehnherr[54] analyzed 25 aortae from non-diabetics, 25 from diabetics and 6 children's aortae. He found that the process of atheromatosis was accompanied by definite changes in the lipid deposit, lipid allocation and calcium and phosphorous deposition which were similar in the diabetic and non-diabetic aortae. The diabetic aortae differed only in having exaggerated lipid changes and more calcification. The aortae of diabetics of middle and later life showed changes comparable with those in the aortae of non-diabetics of an older age period. There was also an increased deposit of calcium and phosphorus in diabetic aortae that contained the same amount of cholesterol as did non-diabetic aortae. Lisa et al[38] found that aortic sclerosis was not significantly increased in 193 diabetics. However, when the sclerosis was selected on the basis of a classification of moderate and severe, it was found that less diabetics than non-diabetics had moderate sclerosis while 63% of diabetics had severe sclerosis against 45% of the non-diabetics. Warren[28] reports an incidence of arteriosclerosis of 85.2% in 264 diabetics of all ages at autopsy, while among 108 diabetics of less 27 than 51 years of age he found

65.7% to have arteriosclerosis. Rabinowitch[55] using a combined method[56] for the clinical establishment of arteriosclerosis found that 54.7% of 243 diabetics of less than 51 years of age had arteriosclerosis, and of 81 diabetics of this age group who had had diabetes for more than 5 years, 85.1% suffered from clinically detectable arteriosclerosis. Wilder[41] reports arteriosclerosis of grade 2 or more (on a scale of 4) in 41 of 51 autopsies on diabetic individuals. Page and Warren[57] report arteriosclerosis in 7 of 11 young diabetics, ages 12 to 33 years, at autopsy. A review by the author[58] of 92 autopsies on diabetics at this Institute showed 32 of 33 males to have grade 2 or more arteriosclerosis of the aorta (on a scale of 4) and 56 of 59 females to be similarly affected. The average age of this group at death was 61.5 years, the age periods varying from the 3rd to the 9th decades.

The number of reports which indicate that there is an association between diabetes mellitus and arteriosclerosis may be multiplied many times, but enough have been reviewed here to indicate that such an association does exist. The reported studies indicate that the diabetic is subject to an earlier onset and a more severe or rapidly progressive form of arteriosclerosis than is the non-diabetic. The morphology of the diabetic type of arteriosclerosis appears to emphasize the atheromatous and calcific aspects of the lesion, and such a morphological variation apparently finds chemical confirmation. The etiology of the arteriosclerosis remains obscure however.

EXPERIMENTAL DIABETES AND ARTERIOSCLEROSIS

The experimental study of diabetes mellitus[59] has produced little of value with reference to the vascular changes which may occur, for the majority of writers are interested in the

metabolic aspects of the disease or in the pathology of the pancreas, and seldom mention the vascular system in their reports[60]. Dragstedt[61], in the course of studies on lipocaic found an incidence of intimal atherosclerosis in the abdominal and thoracic aorta of 15% of 160 totally depancreatized dogs. Arteriosclerosis of this type and location is very rare in domestic dogs[22]; Dragstedt found only 5 instances of arteriosclerosis in a group of 400 control animals in his laboratory, and in these cases the lesions were small and were confined to the ascending aortic arch. It is to be noted that the depancreatized animals all had normal or reduced total blood lipids, and that they all received diets containing 20 to 40% of fat. Six other dogs maintained on low fat diets with lipocaic supplements failed to show arteriosclerosis. Both groups of animals were maintained on less than 30 units of insulin daily. In a group of 21 partially depancreatized dogs, which required up to 150 units of insulin daily and which had a mild lipaemia without evidence of lipocaic deficiency, atherosclerosis was found only once. Studying the effect of lipocaic on cholesterol induced arteriosclerosis in the rabbit Dragstedt[61] gave 77 cholesterol fed animals up to one-half the daily dose of lipocaic required by totally depancreatized dogs. The deposition of fat and cholesterol in the liver was inhibited, but there was no appreciable effect on either the induced hypercholesterinemia or the arteriosclerosis. Fisher[62] reported aortic atherosclerosis in one of six totally depancreatized dogs maintained on insulin, suggesting that the lesion might be due to the diabetic process, or to some toxic action of the insulin, or to some toxic substance in the insulin solution. Duff, Wilson and McMillan[63] in a study that is still in progress, have indicated that alloxan diabetes in the rabbit may occasionally result in

grossly visible atherosclerosis.

It is apparent that no basic conclusions can be drawn from experimental animal work with reference to the association of diabetes and arteriosclerosis. However, the observations of Dragstedt and those made at this Institute indicate that such an association exists.

CHOLESTEROL, DIABETES MELLITUS AND ARTERIOSCLEROSIS

The presence of large amounts of cholesterol in atheromatous lesions[82,83] has led to many investigations into the behavior of the blood cholesterol and other lipids in diabetes, arteriosclerosis and other diseases. The fundamental metabolic behavior of cholesterol in health and disease is almost unknown[64,65]. It appears to be related to fat metabolism and transport, and to be formed from very short chain molecules[66]. In all diseases in which it is elevated, excepting only diabetes mellitus and perhaps arteriosclerosis itself, there is no good evidence that the incidence and degree of arteriosclerosis are abnormal. Studies[63,67] of experimental alloxan diabetes have shown that temporary, intermittent and recurring lipaemia and cholesterolaemia often occur, but as yet, induced arterial lesions are rare in this condition.

The earliest observation that connected a disordered fat metabolism with diabetes was the finding of lipaemia at a time when blood letting was a common practice. About 100 years later it was found that the blood cholesterol was also elevated. In 1916 Bloor[68] studied 38 diabetics and found that while the individual blood lipids retained their interrelations, all of the lipoids were increased. Denis[69] found a cholesterolemia in diabetes only, and not in other conditions. Oser and Karr[70] found cholesterolemia in diabetes, and found that insulin prevented marked aberrations

from normal. Various authors[27,50,55,71,72,73,74,75,76,77,78,79,80] have studied adult or child groups of diabetics and have reported more or less elevated serum cholesterol levels. In general, they find that the cholesterol level is not correlated with the blood sugar level, that it is highest in uncontrolled and acidotic diabetic states, that it is a measure of the clinical severity of the disease, that it is of prognostic value, and that a low fat diet and adequate diabetic control tend to keep the lipids within normal limits. Various authors differ in the significance which they attribute to cholesterolemia in diabetics with arteriosclerosis. Perhaps the most accurate, and one of the few reports that are statistically analyzed is that of Rabinowitch[55]. He found that 187 untreated, fully established diabetics had an average cholesterol of 242 mgms.%. A similar group of 163 very early diabetics had an average level of 212 mgms.%, and a group of 128 potential diabetics had an average cholesterol of 166 mgms.%. The differences in these average figures is significant. There was no significant difference in cholesterol levels of 300 random cases of diabetes with reference to the presence or absence of arteriosclerosis. However, because these cases included factors other than diabetes and arteriosclerosis which might influence the cholesterol values, a further group of 167 diabetics, in whom no disturbances other than diabetes and cardio-vascular disease were present, were studied. In this group, the 94 patients who were under 50 years of age showed a significantly higher cholesterol level when arteriosclerosis was present than when it was absent. When this group of 167 patients was further biased by excluding those who had hypertension, the same relationship was found, the age group above 50 years showing no significant difference in average cholesterol levels in the presence or absence

of arteriosclerosis, and the age group under 50 years showing a significantly increased cholesterol level in the presence of arteriosclerosis. His data indicates further that the duration of the diabetic state was not an important factor in the development of arteriosclerosis; that poor control of the diabetes appeared to cause arteriosclerosis, and that the "high-carbohydrate-low-fat diet" delayed the development of cardio-vascular disease.

The reported analyses of blood cholesterol levels in persons with arteriosclerosis in the absence of diabetes, are so contrary as to be beyond interpretation. Kountz et al[8] in discussing these results attribute this confusion to the unreliability of techniques for estimating cholesterol; to failure to control the experimental conditions; and to other unknown or unrecognized factors. They studied 212 patients of 40 to 85 years of age, finding that elderly female patients had a higher average blood cholesterol level than elderly males, but that the males had a higher incidence and earlier onset of atherosclerosis than the females. Peripheral atherosclerosis was more common among elderly patients with low blood cholesterol values than among those with hypercholesterolemia.

It would appear, therefore, that diabetics suffer from a cholesterolemia, and that, in a highly selected group, there is a positive correlation between hypercholesterolemia and arteriosclerosis. Furthermore, there is a positive correlation between the degree of control of the diabetic state, the degree of hypercholesterolemia and the degree of arteriosclerosis. Nothing can be concluded about a possible association between blood cholesterol levels and simple arteriosclerosis as yet.

PART II

SUMMARY

Ample evidence is at hand to show that diabetics suffer an increased incidence and an earlier onset of arteriosclerosis and its sequelae. The types of arteriosclerosis affecting diabetic and non-diabetic persons appear to have essentially the same morphological and chemical properties, although superficial differences may exist. An experimental demonstration that diabetes may cause arteriosclerosis is indicated. The relationship between hypercholesterolemia, diabetes and diabetic arteriosclerosis has been shown to be a real one, but an association between cholesterolemia and simple arteriosclerosis has not been established in humans. It is apparent, therefore, that a common etiological factor in the two types of arteriosclerosis is neither established nor indicated, although it may, in fact, exist. The problem, however, is clarified to the degree that one is justified in seeking the significance of the relationship between hypercholesterolemia, diabetes mellitus and atherosclerosis by experimental procedures.

PART III REPORT OF EXPERIMENT

The experiment reported here is in process at the time of writing. The present statement must, therefore, be in the nature of a progress report. On the basis of the findings presented in Parts I and II, the experiment was designed to show if the existence of alloxan diabetes in the rabbit would modify the establishment, degree or type of atherosclerosis induced by cholesterol feeding. Two of the metabolic variants introduced by this double experimental procedure, namely the blood sugar values and the serum cholesterol values, were followed quantitatively in order to provide some basis of reference for any morphological deviations that might appear in the gross or microscopic forms of the arteriosclerosis.

MATERIALS AND METHODS

ANIMALS - The 40 animals reported on were domestic white rabbits of both sexes. These animals were purchased on the open market, and were, as far as could be determined, in normal health. The weights of the animals varied from about 1 to 2.5 kgms. when they were received, and they were, with one exception, immature.

MAINTENANCE OF ANIMALS - The rabbits were received in groups of about 8 to 12 and were kept, as far as possible, in individual metal cages. During the immediate period after they were rendered diabetic they were kept in large metabolism cages. Food consisted of Purina Rabbit Chow. Both food and water were given ad lib.

OBTAINING BLOOD SAMPLES - The rabbits were bled from a transverse razor cut through the central vessels of the dorsal surface of the ear. The ear was prepared by shaving and by cleaning with 80% alcohol. If vasodilatation were desired, this was obtained by the application of moist heat or xylol to the ventral aspect of the pinna. Subsequent cuts were made about 2 mms. progressively proximal

to the initial one. About 2 cc. of blood was allowed to drip off the tip of the ear into a centrifuge tube at each bleeding, and a further 0.1 cc. of blood was pipetted from the immediate location of the ear cut. All blood samples were obtained in the morning, and all, except those of the immediate post-alloxan period, were taken after the animal had been without food for 12 to 16 hours.

Animals were allowed 7 to 14 days to acclimatize themselves to the animal room before blood samples were taken. Two "normal" fasting determinations were then made on different days, at 3 to 10 day intervals. The day following the second determination, alloxan was administered to about half of the group, and two or three days later a non-fasting blood sample was taken on the treated rabbits. The untreated rabbits were bled at the same time. Subsequent samples were taken from treated and untreated control animals at intervals of 1 to 2 weeks, always in the fasting state and at the same time.

ALLOXAN ADMINISTRATION - Alloxan (Eastman Kodak Co.) was freshly prepared as a 5% solution in distilled water[84,59]. It was administered to the largest animals of a group at a dosage level of 200 mgms. per kilo. of body weight, and was injected into the marginal ear vein over a period of 20 to 60 seconds. The drug was given in the morning in the non-fasting state. Such treatment caused diabetes.

POST-ALLOXAN THERAPY - In order to obtain a practicable survival rate, protamine zinc insulin (Toronto) and glucose were administered to the treated animals. Six hours after the injection of alloxan, each animal received 2 units of insulin subcutaneously in the ear, and 10 to 20 cc. of 20 to 10% glucose in physiological saline or water intravenously. This therapy was repeated after a further six

hours. Thereafter, for a period of 5 to 14 days, each animal received about 10 cc. of 20% glucose and 1 to 4 units of protamine zinc insulin daily. At least one non-fasting blood sample was taken during this period. After the permanent cessation of this therapy a period of three days was allowed to elapse before routine fasting blood samples were taken. Therapy was varied in accordance with the clinical condition of the animal; some received crystalline insulin (Toronto), physiological saline and oral or subcutaneous glucose as indicated.

URINE SUGAR AND URINE ACETONE - These were determined ante- and post-mortem by means of dry powdered reagents (Galactest, and Acetone Test; Denver Chemical Mfg. Co. Inc. New York) in a casual manner, and were used as a guide to therapy or in determining the cause of death.

CHOLESTEROL FEEDING - Cholesterol was fed in two forms to two different groups of animals, and was fed in equal amounts to the diabetic and non-diabetic animals of each group. The first method consisted in feeding the animals 0.5 gms. of pure cholesterin (Merck) in No. 00 gelatin capsules (Parke, Davis Co.)[85]. For the first few feedings the capsules were coated with molasses, but thereafter they were merely placed in the animals' mouths. Because the desired degree of cholesterolemia was not obtained by this method, it was changed to feeding cholesterol in oil by stomach tube. The cholesterol was dissolved in the quantity of 33 gms. in 1 litre of sun flower seed oil (National Drug Co.)[15,16] by heating to 60°C. The solution was then allowed to assume room temperature, and was found to contain a moderate amount of anhydrous cholesterol crystalline precipitate. The mixture was vigorously agitated before administration. The dose of cholesterol in oil was 0.25 gms. of

cholesterol, or about 8 cc. of the mixture given through an empty stomach tube. A soft rubber 14 F. urethral catheter was employed as a stomach tube. The rabbit was placed in a confining box in the upright position, a perforated wooden mouth gag inserted and the tube passed into the stomach. On withdrawal, the tube was thoroughly cleaned before re-use. The rabbits were maintained in an upright position for about 1 minute after the feeding, and were in this position for from 2 to 5 minutes on each occasion.

With both types of feeding the cholesterol was given once daily for about 5 days of the week, and the feeding was continued over a total period of 90 days from the beginning of this treatment. Cholesterol feeding was not begun until at least 45 days had elapsed after the administration, of alloxan in order to avoid, as far as possible, the post-alloxan hypercholesterinemia.

AUTOPSIES AND METHODS OF KILLING - Those animals which died or were accidentally killed were autopsied as soon as possible. If immediate autopsy were not possible, the animal was placed in a refrigeration unit at about 40°F. Animals that completed their experimental course were killed by the injection of nembutal or of 30 cc. of air into the marginal ear vein, and were examined at once.

TISSUES AND MICROSCOPIC PREPARATIONS - All tissues were examined, and all except skin, skeletal muscle, bone and marrow, prostate, bladder, uterus and tubes, intestine, mesentery, parathyroids and lymph nodes were fixed in 10% formol-saline. The pituitary and pancreas were fixed in Helly's fluid. The heart, aorta and iliac arteries were removed in toto, opened and attached to cardboard strips with gauze. They were then fixed in the opened state in 10% formol-saline and stained with Scharlach Red. Appropriate frozen sections were then examined microscopically. The aorta and hearts were stained with

haematoxylin-eosin, haematoxylin van Giesen and Verhoeff's elastic tissue stains. Pancreases were stained with Gmori's granule stain, haematoxylin van Giesen and haematoxylin-eosin.

ESTIMATION OF BLOOD SUGAR - The micromethod used has been in use in this department for several years[86]. Determinations are made on 0.1 cc. of blood; color is developed by an iron-gum Ghatti reagent, and is determined in a Luxor photo-electric colorimeter. The method is a modification of Folin's micro-technique, and seems to be accurate, as judged by repeated and intermittent standardization curves, and duplicate determinations to about plus or minus 5%. Blood sugars were determined within 2 to 4 hours of obtaining the sample. No standards were determined with each group of samples, but each group contained samples assumed to be normal.

ESTIMATION OF SERUM CHOLESTEROL - Free and total serum cholesterol were determined by the micromethod of Sperry[87]. The following modifications were necessarily adopted: the determinations were made on twice the usual amounts of extract; the color developing reagents were added from burettes rather than from pipettes; the centrifuge was operated at 1800 r.p.m. with a large 8 unit head. The method was found to be satisfactory as judged by repeated standardization curves except for values of less than 20 mgms.% and for estimating the very small quantities of cholesterol often in normal rabbit serum it was judged to be most inexact.

Samples were analyzed within 2 weeks after being obtained, and were kept tightly stoppered in the ice box until analysis. In order to obtain as much serum as possible from a given sample of blood, the fresh blood was allowed to clot and stand at room temperature for 12 to 24 hours before the serum was separated. Occasional duplicate determinations gave a satisfactory degree of similarity in

the results obtained unless these were below 10 mgms.%. For this and previously stated reasons the occasional value of free cholesterol below 6 mgms.% or of total cholesterol below 10 mgms.% were arbitrarily assumed to be 6 and 10 mgms.% respectively, and were so recorded. Ester cholesterol and ester cholesterol per cent of total cholesterol were determined arithmetically.

LIPAEMIA - Lipaemia was determined by inspection within about 1 hour of obtaining a sample and it was graded on an arbitrary scale of 0 to 4. Some lipaemic blood smears were stained for fat.

CONTROL - The fundamental control animals for this experiment, i.e. normal and diabetic rabbits subject to the same chemical determinations, are provided by a concurrent experiment by Dr. D. C. Wilson in this Institute. The intra-experimental control is found in corresponding groups of diabetic and non-diabetic rabbits subjected to approximately similar conditions, procedures and determinations. It was intended initially to provide a strict sex and weight pair control, but experimental conditions soon made it apparent that this was not feasible, and it was abandoned in favor of grouped observations. No attempt was made to control the large number of other variables which might occur.

CREDITS - Technical assistance in calibrating micropipettes, which could not be purchased, was given by Mr. Nye, the Institute's Chief Technician, and he is also responsible for the staining and preparation of microscopic material. Chemical procedures were selected by Dr. D. C. Wilson, and were in use at the time this experiment was undertaken. Routine chemistry was done largely by Miss C. McGuire. Specially designed rabbit boxes and mouth gags were constructed by Mr. J. Giroux of the Institute workshop. Dr. Kenneth Evelyn solved several difficulties that occurred with the photo-

electric colorimeter. I am particularly indebted to Dr. D. C. Wilson who, working on a concurrent and very similar experiment, was a constant consultant. It was with him that the basic and highly valuable therapeutic method of obtaining animal survival during the acutely diabetic period was developed. And it was on the basis of his previous year's experience in this field that many procedures were established. Photographic and copy work were done by Mr. H. Coletta, the Institute's photographer.

The experiment was conceived and directed by Dr. G. Lyman Duff.

RESULTS

Alloxan was administered to 22 animals, causing diabetes in all but one (#39). A group of 18 animals were not given alloxan, and all of these maintained normal blood sugar values. Cholesterol was fed in pure form to 2 diabetics and 3 non-diabetics (Nos. 1, 4, 5, 7 and 8) for a period of 90 days, causing typical mild cholesterol feeding hypercholesteremia in 4 (Nos. 1, 5, 7 and 8) and typical atherosclerosis in 3 (Nos. 5, 7 and 8). An oil solution of cholesterol was fed to 3 diabetic and two non-diabetic animals (Nos. 35, 38, 39, 40, 41) for a period of 90 days without the development of cholesterol feeding hypercholesterinemia or the development of atherosclerosis except in one animal (#39, resistant to alloxan; cholesterol levels normal throughout) that showed microscopic minimal atherosclerosis in the aorta and coronary arteries. The remainder of the animals died before the necessary experimental procedure could be completely finished, or they were transferred to other experiments.

The following blood chemistry values were obtained: (Protocols, statistical data[88,89,91], figures, charts and photomicrographs are included in the appendix).

BLOOD CHEMISTRY MEAN VALUES

	Normal Mean	Post-alloxan under therapy Mean	Post-alloxan Free From Therapy Mean	Post-alloxan With Cholesterol feeding Mean	Non-Diab. With Cholesterol feeding Mean
Blood Sugar mgms.%	103.5	336	378.3	508	101.3
Free Cholesterol mgms.%	11.5	-	48	-	-
Total Cholesterol mgm.%	35.7	-	83	-	-
Ester Cholesterol mgm.%	24.8	-	35	-	-
Ester Cholesterol % of total	63.6	-	51.7	-	-

Analysis of these values shows that there was a significant[+] rise in blood sugar after the administration of alloxan in the alloxan treated group as a whole. The post-alloxan blood sugar values while under the influence of protamine zinc insulin and glucose therapy did not differ significantly from later values free from such therapy. These former values included 5 animals that showed no hyperglycaemia during therapy, and 16 animals whose group mean blood sugar value was 408.3 mgms.% while under therapy. One animal that was resistant to alloxan (39) was excluded from the calculations related to post-alloxan blood sugar values free from therapy in an attempt to minimize any difference in the values obtained while the animals were subject to and free from therapy. Cholesterol values obtained during the period of therapy were not suitable for statistical analysis.

However, during the post-alloxan period free from therapy, cholesterol values were found to be significantly altered. Again, 9 of the 17 animals analyzed here did not develop a post-alloxan hypercholesterinemia during the therapy free period, but they were included in order to reduce any difference that might be found. The mean free serum cholesterol of the group was significantly increased relative to normal, as was the total serum cholesterol value. However, the ester value was not significantly altered, while the ester per cent of total cholesterol value was significantly decreased.

The cholesterol values obtained during the period of cholesterol feeding are not properly subject to analysis and can

[+]The standard of reference for significance is assumed to be a probability of 0.05 or less.

only be studied as individual observations. The same factors render the blood sugar values similarly unsuitable, but the necessary number of animals for such an analysis is so closely approached, and the values obtained are so highly significant that it is reasonable to state that during the period of cholesterol feeding (pure and in oil solution) there is a highly significant increase in blood sugar levels among diabetic animals while there is no increase among non-diabetic animals.

Other observations that may be noted in passing are the occurrence of lipaemia in the diabetic animals, and an almost universal tendency to weight loss in the diabetic group. The usual alloxan diabetic phenomena of polydipsia, polyuria, glycosuria, initial and terminal acidosis, and insulin hyper-reactivity were also observed, but were not accurately studied.

The relationship found between blood total cholesterol levels and the occurrence of arterial lesions is shown in the table below. In this table the cholesterol level is expressed as a factor which represents the number of days of hypercholesterolemia above the normal total cholesterol mean level plus three times its standard deviation. The crude factor merely expresses an area of exposure above an arbitrary threshold. It includes both diabetic and feeding hypercholesterolemia without distinction, but it contains no time relationships relevant to the time of hypercholesterinemia and the time of the observation of arterial lesions. An examination of data on this latter point shows that the data is insufficient to be intelligible. For the same reasons, observations on free, ester and ester per cent of total cholesterol are not presented although they are highly pertinent.

TOTAL CHOLESTEROL LEVEL IN RELATION TO ARTERIAL LESIONS

Number	Alloxan	Total Cholesterol Factor Mgm - days	Arterial Lesions of Aorta & Heart		
			Atherosclerosis Intimal	Medial	Spontaneous Arteriosclerosis
26	+	10,040	+	+	-
7	-	5,760	+	-	-
8	-	4,980	+	-	-
4	-	4,470	+	+	-
40	+	2,520	+	-	-
5	-	1,860	+	-	+
37	-	690	-	-	+
24	+	620	-	-	-
35	+	420	-	-	+
1	+	96	-	-	-
42	-	6	-	-	-
39	Resistant	0	+	-	+
2	+	0	-	-	+
3	+	0	-	-	+
18	+	0	-	-	-
21	+	0	-	-	+
23	+	0	-	-	-
27	+	0	-	-	-
32	+	0	-	-	+
34	+	0	-	-	+
41	+	0	-	-	+
19	-	0	-	-	-
20	-	0	-	-	-
22	-	0	-	-	+
25	-	0	-	-	+
28	-	0	-	-	+
38	-	0	-	-	+

Examination of the above table shows that all animals exposed to more than 1,859 mgm-days of total cholesterol showed intimal atherosclerosis. In one case (#40) the lesion was an incidental observation and, therefore, could not be proven to contain lipids. It is also to be noted that one animal (#39) showed intimal atherosclerosis in the absence of a hypercholesterolemia. Again the lesions were an incidental finding and could not be proven to contain lipid. This animal was fed cholesterol in oil for 90 days and was resistant to alloxan. The highest cholesterol factor observed (#26) occurred after alloxan injection in the absence of cholesterol feeding. Gross and microscopic fat containing intimal and superficial medial lesions were found in this animal's aorta. One animal showed a fat containing medial lesion induced by cholesterol feeding (#4). Spontaneous medial disease appears to be random in its incidence in this series. The data is insufficient to attempt any grading of the severity of the lesions observed. Application of the Chi^2 test to the presence or absence of atherosclerosis in the presence or absence of a cholesterol factor indicates that the significance of the results is doubtful. However, if the factor is set at above and below 1,500 mgm-days, then the presence of atherosclerosis with a factor of more than 1,500 mgm-days becomes highly significant.

Pathologically, observation of the aorta, heart and coronary arteries, and of the pancreas confirms the findings of others in early cholesterol feeding induced atherosclerosis of the arteries[17,20,21] and of the earlier changes induced in the pancreas by alloxan[59]. These changes including, as they do, minimal lipoid deposits in the cellular and interstitial elements of the arterial intima and interstitial elements of the aortic media; and the necrosis of beta cells, loss and atrophy of pancreatic islets, predominance of islet alpha

cells, and hydropic degeneration of islet cells in the pancreas will not be considered further.

However, two new morphological observations have been made and will be reported here.

Animal #26 developed a hypercholesterinemia after the injection of alloxan. Associated was a lipaemia so marked that the serum solidified at refrigerator temperature. The hypercholesterolemia reached its peak 16 days after alloxan was given and 24 days before death. By extrapolation it is estimated that the cholesterol values would have returned to normal 40 or more days after the alloxan was given. The animal was diabetic and died in coma. The cholesterol values were the highest that have been reported, reaching 860 mgms.% total serum cholesterol and 480 mgms.% free serum cholesterol.

Grossly, when the aorta was stained with Sudan, minute slightly raised round spots were seen in the intima. They were quite numerous, concentrating particularly in the ascending aortic arch with lesser numbers down to the mid-abdominal portion of the vessel. There was no tendency to localize about branch arteries, nor was there any confluence of lesions. Spontaneous arteriosclerosis was absent.

Microscopically, lipoid was seen in intra- and extracellular foci in both the intima and related superficial media. The fat containing cells were similar to those found in cholesterol feeding atherosclerosis. Elastic tissue - H.V.G. stain showed a small lesion consisting of vacuolated foamy cells bulging like a disc in the intima and in continuity with a small number of similar cells in the underlying media through a minute perforation in the internal elastic membrane. The lesion had an appearance suggesting that it

had arisen in the media and flowed into the intima. In some other areas the media appeared to show a similar lesion without involvement of the internal elastic membrane or intima.

A striking new pancreatic lesion was found in many diabetic pancreases. The following table shows the occurrence of this lesion which consists in a hydropic degeneration of the islet-duct ductules. The table also shows the time relationships between the occurrence of the lesion and the administration of alloxan. Cholesterol or oil feeding are not shown to affect its development. The lesion apparently does not occur in the absence of diabetes, but occurs with and without the presence of a hyperlipaemia and/or hypercholesterinemia. The critical time period for its development appears to lie between 45 and 70 days.

HYDROPIC DEGENERATION OF PANCREATIC DUCTULES

Number	Days after Alloxan	Hydropic Degeneration Of Ductules	Remarks
1	135	+	Severe diabetes.
4	135	+	Diabetes, hyperlipaemia, hypercholesterinemia.
35	133	+	Severe diabetes, hyperlipaemia.
39	133	Normal	Resistant to alloxan.
40	133	+	Diabetes, hyperlipaemia, hypercholesterinemia.
41	133	+	Severe diabetes.
18	70	+	Diabetes.
34	67	Normal	Diabetes.
21	59	+	Diabetes.
23	54	Normal	Diabetes, slight lipaemia.
2	45	+	Diabetes, hyperlipaemia.
26	40	-	Lyzed and necrotic
32	12	Normal	Diabetes.
24	10	Normal	Diabetes, hyperlipaemia, hypercholesterinemia.
27	2	Normal	--

The remainder of the animals, 12 in all, did not receive alloxan and did not show hydropic ductule changes.

Grossly, no abnormality was noted, but microscopically, the lesion appeared as an extensive and severe hydropic degeneration of the ductule epithelial cells. The cells often showed only a cytoplasmic membrane and a nucleus, and no visible cytoplasm. The ductules were thus brought into unexpected prominence. Often the hydropic cells had the conformation of islets and it became impossible to tell whether they were ductule or islet cells. However, gradations of hydropic degeneration of islets could be traced to areas of this type, suggesting that some of them were islets. On the other hand, some similar areas showed a definite lumen, implying that they were ductules. In as much as the ductules normally connect with the islets[92] such a mixture of conflicting appearances was probably to be expected, and a review of the sections indicated that it was morphologically impossible to tell in many cases whether a hydropic area was derived from ductule or islet tissue.

CONCLUSIONS AND DISCUSSION

It is obvious that no data sufficient to warrant any conclusions within the title of this thesis are available at the time of writing.

The various technical difficulties of the experiment have been mastered however, and the normal and diabetic metabolism concerned have been largely established. At present, a 50% animal survival rate to the completion of the experimental procedures may be confidently expected. The metabolic findings are not unusual, and their discussion is not within the scope of this paper. One interesting abnormality was a significant increase in blood sugar levels among diabetic animals during the period of cholesterol feeding. Suitable control data to establish the reason or reasons for this apparent aberration are not available as yet. The experiment continues.

In reference to the histopathology observed in the arterial vasculature[20,21] and the pancreas[59], the observations of others were confirmed. The fatty intimal lesion occurring in the aorta of an animal with a severe hyperlipaemia and hypercholesterolemia in the absence of cholesterol feeding is quite unique. I am informed by Dr. G. L. Duff that he has observed a lesion in a human cerebral artery that was somewhat similar in appearance. Until the lesion is confirmed - which associated experimentation in this Institute may do within a few months - it is not profitable to discuss it further. It suffices to point out that the data suggests that the lesion was caused by an associated post-alloxan disturbance in metabolism, and a disturbance in lipid metabolism in particular.

The hydropic degeneration of pancreatic ductules has been reported in anterior pituitary diabetes in dogs[59], but has not been reported as a sequelae of alloxan diabetes. Only highly theoretical interpretations of its significance are possible. Perhaps the most facile explanation would be that the islet and ductule tissue are both embryologically and functionally related, and that, in an attempt to compensate for the loss of islet tissue, the ductules so alter their metabolism that they become degenerate. There are many objections to such an interpretation: the possible influence of the pituitary is not considered, the lesion is not reported in diabetic human pancreases, and so forth. Until more is known about alloxan diabetes in general, speculation about the lesion is not fully warranted.

SUMMARY

An analysis of the morphology of human atherosclerosis and of experimental cholesterol arteriosclerosis in the rabbit indicates that the human and the artificial lesions approximate each other closely. This morphological similarity suggests that some identical etiological factor or factors may be common to the two lesions, but such commutual agents have not been proven, as yet. It has also been shown that similar atheromatous vascular lesions are more frequent and severe in persons who suffer from diabetes mellitus associated with hyperlipaemia and hypercholesterinemia. Thus, cholesterol appears as an element in human atherosclerotic lesions, as an abnormal factor in diabetic individuals suffering from an excessive expression of atherosclerosis, and as a mediator in experimental atherosclerosis. Nevertheless, present knowledge is found to be quite unable to assess the etiological importance of cholesterol in relation to atherosclerosis.

An experimental study of the effect of pre-existing alloxan diabetes mellitus on the morphological expression of atherosclerosis induced in the rabbit by cholesterol feeding has been undertaken in order to apportion the relative significance of cholesterol in the three examples of arteriosclerosis, and in order to clarify the relationship of diabetes mellitus per se to atherosclerosis.

The experimental report, which comprises an analysis of the vascular and pancreatic lesions and the metabolic behavior of 40 rabbits has, at the time of writing, produced little information relative to the experimental problem defined. However, incidental observations have been made both in the aorta and in the pancreas that are of interest and importance.

It has been shown that the metabolic disturbances which

occur in the early post-alloxan diabetic period may, if the resultant hypercholesterinemia and hyperlipaemia are severe enough, be associated with an atheromatous, lipid-rich aortic lesion of unique appearance. It has also been proven that a post-alloxan diabetic status of more than two months duration is almost invariably associated with a heretofore undescribed hydropic degeneration of the ductules of the pancreas of the rabbit.

While no relevant conclusions or even hypotheses may be drawn justifiably from the data that is available at present, nevertheless the uncompleted experiment has been shown to be theoretically valid, technically practicable and productive of new, factual information.

BIBLIOGRAPHY

1. Long, Esmond R. — The Development of Our Knowledge of Arteriosclerosis. Arteriosclerosis: A Survey of the Problem. Cowdry, E.V. MacMillan Co. 1933. Ch. 1. pp. 19-50.

2. Aschoff, Ludwig — Introduction: Arteriosclerosis: A Survey of the Problem. Cowdry, E.V. MacMillan Co. 1933. pp. 1-18.

3. Bell, E.T. — A Text-book of Pathology. Lea and Febiger. 1941. pp. 556.

4. Moschcowitz, Eli — Arteriosclerosis. Jour. Mount Sinai Hosp. 1946, 12:1003.

5. Boyd, William — A Text-book of Pathology. Lea and Febiger. 1939. Ed. 3. pp. 437-444.

6. Hueper, W.C. — Arteriosclerosis: A General Review. Arch. Path. 1944, 38:162-181, 245-285, 350-364. 1945, 39:51-65, 117-131, 187-216.

7. Winternitz, M.C., Thomas, R.M., and Lecompte, P.M. — The Biology of Arteriosclerosis. Introduction. XVII - XXIII. Charles C. Thomas, Springfield, 1938.

8. Page, I.H. — Arteriosclerosis and Lipid Metabolism. Biological Symposia. Cattell, Jaques. Ageing and Degenerative Diseases. Vol. XI. Moore, R.A. The Jaques Cattell Press. Lancaster, Pa. 1945. pp. 43-73.

9. Hueper, W.C. — The Relation Between Etiology and Morphology in Degenerative and Sclerosing Vascular Diseases. Biological Symposia. Cattell, Jaques. Ageing and Degenerative Diseases. Vol. XI. Moore, R.A. The Jaques Cattell Press. Lancaster, Pa. 1945. pp. 1-42.

10. Ophüls, W. — The Pathogenesis of Arteriosclerosis. Arteriosclerosis: A Survey of the Problem. Cowdry, E.V. MacMillan Co. 1933. Ch. 9. pp. 249-270.

11. Zinserling, W.D. — Untersuchungen über Atherosclerose. 1. Über die Aortenverfettung bei Kindern. Virchows Arch. P. path. Anat. 1925, 255:676.

12. Sanders, W.E. Atherosclerosis with Special Reference to Physiologic Development and Pathologic Changes in Intima. Am.J.M.Sc. 1911, 142:727.

13. Klotz, O. and Manning, M.F. Fatty Streaks in the Intima of Arteries. Jr. Path. and Bact. 1911-12, 16:211.

14. Leary, T. Atherosclerosis: Special Consideration of Aortic Lesions. Arch. Path. 1936, 21:419.

15. Anitschkow, N. and Chalatow, S. Über experimentelle Cholesterinsteatose. Centralbl. f. allg. Path. u. path. Anat. 1913, 24:1.

16. Anitschkow, N. Experimental Arteriosclerosis in Animals. Arteriosclerosis: A Survey of the Problem. Cowdry,E.V. MacMillan Co. 1933. Ch. 10. pp. 271-322.

17. Leary, T. Experimental Atherosclerosis in the Rabbit Compared with Human (Coronary) Atherosclerosis Arch. Path. 1934, 17:453.

18. Leary, T. Atherosclerosis: Etiology. Arch. Path. 1936, 21:459.

19. Leary, T. Genesis of Arteriosclerosis. Arch. Path. 1941, 32:507.

20. Duff, G.L. The Nature of Experimental Cholesterol Arteriosclerosis in the Rabbit. Arch. Path. 1936, 22:161.

21. Duff, G.L. Experimental Cholesterol Arteriosclerosis and its Relationship to Human Arteriosclerosis. Arch. Path. 1935, 20:81-123, 259-304.

22. Fox, H. Arteriosclerosis in Lower Mammals and Birds: its Relation to the Disease in Man. Arteriosclerosis: A Survey of the Problem. Cowdry, E.V. MacMillan Co. 1933. Ch. 6. pp. 153-193.

23. Nuzum, F.R., Elliot, A.H., Richards, D.E. and Priest, B.V. The Occurrence and Nature of Spontaneous Arteriosclerosis and Nephritis in the Rabbit. Arch. Path. 1930, 10:697. (Quoted by Duff, reference 21)

24. Ophüls, W. Spontaneous Arteriosclerosis of the Aorta (Atheroma) in a Rabbit. J.A.M.A. 1907, 48:326. (Quoted by Duff, reference 21)

25. Greene, H.S.N. Personal Communication.

26. Bailey, C.H. Atheroma and Other Lesions Produced in Rabbits. J.Exper.Med. 1916, 23:69.

27. Labbé, M., and Heitz, J. De la Cholestérinémie chez les Sujets affectés d'Artérites Oblitérantes. Ann. de méd. 1925, 18:108.

28. Warren, S. The Pathology of Diabetes Mellitus. Lea and Febiger. 1938, 2nd Ed.

29. Moschcowitz, Eli. Vascular Sclerosis: With Special Reference to Arteriosclerosis. Oxford University Press, 1942. Ch. 12. pp. 125-128.

30. Snyder, L.H. Medical Genetics. Duke University Press. 1941, Ch. 7, pp. 76-78.

31. Pincus, G., Joslin, E.P., and White, P. The Age Incidence Relations in Diabetes Mellitus. Am.J.Med.Sc. 1934, 188:116.

32. Willius, F.A., Smith, H.L., and Sprague, P.H. A Study of Coronary and Aortic Sclerosis: Incidence and Degree in 5060 Consecutive Post Mortem Examinations. Proc. Staff Meet. Mayo Clin. 1933, 8:140.

33. Lisa, J.R., Magiday, M., and Hart, J.F. Peripheral Arteriosclerosis in the Diabetic and the Non-diabetic. J.A.M.A. 1942, 118:1353.

34. Ophüls, W. Arteriosclerosis, Cardio-vascular Disease and their Relation to Infectious Disease. Stanford University Publications. University Series: Medical Science. 1921-1926, 1:1.

35. Ophüls, W. A Statistical Survey of 3000 Autopsies. Stanford University Publications. University Series: Medical Science. 1926, 1:125. pp. 242-269.

36. Blotner, H. Coronary Disease in Diabetes Mellitus. New England J. Med. 1930, 203:709.

37. Root, H.F., Bland, E.F., Gordon, W.H., and White, P.D. Coronary Atherosclerosis in Diabetes Mellitus. J.A.M.A. 1939, 113:27.

38. Lisa, J.R., Magiday, M., Galloway, I. and Hart, J.F. Arteriosclerosis with Diabetes Mellitus. J.A.M.A. 1942, 120:192.

39. Enklewitz, M. Diabetes and Coronary Thrombosis Am. Heart Jour. 1934, 9:386.

40. Nathanson, M.H. Coronary Disease in 100 Autopsied Diabetics. Am. J. Med. Sc. 1932, 183:495.

41. Wilder, R.M. Necropsy Findings in Diabetes. Southern Med. J. 1926, 19:241.

42. Root, H.F. Arteriosclerosis in the Legs and Heart in Diabetes. New York State J. Med. 1928, 28:1287.

43. Levine, S.A., and Brown, C.L. Coronary Thrombosis. Medicine. 1929, 8:253.

44. Buerger, L. The Circulatory Disturbances of the Extremities. W.B.Saunders Co. 1924. pp. 427-431.

45. Eisile, H.E. The Juvenile Diabetic Patient Surviving Twenty Years. J.A.M.A. 1942, 120:188.

46. Dry, T.J., and Hines, E.A. The Role of Diabetes in the Development of Degenerative Vascular Disease.

47. Kramer, D.W. Diabetic Gangrene: Incidence and Pathogenesis: Analysis of 58 Cases among 1008 Diabetics.

48. Morrison, L.B., and Bogan, I.K. Calcification of Vessels in Diabetes. J.A.M.A. 1929, 92:1424.

49. Shepardson, H.C. Arteriosclerosis in the Young Diabetic Patient. Arch. Int. Med. 1930, 45:674.

50. White, P., and Hunt, H. Cholesterol of the Blood of Diabetic Children. New England J. Med. 1930,202:607.

51. Letulle, M., Labbé, M., and Heitz, J. De la Calcification des Artéres chez les Diabétiques. Arch. d. mal du coeur. 1927, 20:577.

52. Collins, W.S., and Wilensky, N.D. Intermittent Venous Occlusion in Treatment of Peripheral Vascular Disorders. J.A.M.A. 1937, 109:2125.

53. Mandelberg, A., and Sheinfeld, W. Diabetic Amputations: Amputation of Lower Extremity in Diabetics - Analysis of 128 Cases. Am. Jour. Surg. 1946, 71:70.

54. Lehnherr, E.R. Arteriosclerosis and Diabetes Mellitus. New England J. Med. 1933, 208:1307.

55. Rabinowitch, I.M. Arteriosclerosis in Diabetes. Ann. Int. Med. 1935, 8:1436.

56. Rabinowitch, I.M., Richie, W.L., and McKee, S.H. A Statistical Evaluation of Different Methods for the Detection of Arteriosclerosis in Diabetes Mellitus. Ann.Int.Med. 1934, 7:1478.

57. Page, I.H., and Warren, S. The Pathology of Diabetes in Young Adults. New England J. Med. 1929, 200:766.

58. McMillan, G.C. Unpublished.

59. Duff, G.L. The Pathology of the Pancreas in Experimental Diabetes Mellitus. Am.J.Med.Sc. 1945, 210:381.

60. Lukens, F.D.W., and Dohan, F.C. Experimental Pituitary Diabetes of Five Years' Duration with Glomerulosclerosis. Arch. Path. 1946, 41:19.

61. Dragstedt, L.R. The Role of the Pancreas in Arteriosclerosis. Biological Symposia. Cattell, Jaques. Ageing and Degenerative Diseases. Vol. XI Moore, R.A. The Jaques Cattell Press. Lancaster, Pa. 1945, pp. 118-132.

62. Fisher, N.F. Attempts to Maintain the Life of Totally Depancreatectomized Dogs Indefinitely by Insulin. Am.J.Physiol. 1923, 67:634.

63. Duff, G.L., Wilson, D.C., and McMillan, G.C. Unpublished.

64. Bills, C.E. Physiology of the Sterols, Including Vitamin D. Physiol. Rev. 1935, 15:1.

65. Weinhouse, S. The Blood Cholesterol. Arch. Path. 1943, 35:438.

66. Rittenberg, D., and Schoenheimer, R. Deuterium as an Indicator in the Study of Intermediary Metabolism. J.Biol.Chem. 1937, 121:235.

67. Kendall, F.E., Meyer, W., Lewis, L., and Victor, J. Alloxan Diabetes in Rabbits. Production of Hypercholesterolemia, Hyperlipaemia and Adrenal Cortical Lesions. Proc.Soc.Exper.Biol. and Med. 1945, 60:190.

68. Bloor, W.R. The Lipoids ("fat") of the Blood in Diabetes. Jour. Biol. Chem. 1916, 26:417.

69. Denis, W. Cholesterol in Human Blood Under Pathological Conditions. Jour.Biol.Chem. 1917,29:93.

70. Oser, B.L., and Karr, W.G. The Lipoid Partition of the Blood in Health and Disease. Arch.Int.Med. 1925, 36:507.

71. Rabinowitch, I.M. The Cholesterol Content of Blood Plasma in Diabetes Mellitus. Arch.Int.Med. 1929, 43:363.

72. Rabinowitch, I.M. The Cholesterol Content of Blood Plasma in Juvenile Diabetes. Arch.Int.Med. 1929, 43:373.

73. Rabinowitch, I.M. The Cholesterol Content of the Blood Plasma as an Index of Progress in Insulin Treated Diabetics. Canad. M. A.J. 1927,17:171.

74. Joslin, E.P., Bloor, W.R., and Grey, H. The Blood Lipoids in Diabetes. J.A.M.A. 1917, 69:375.

75. Joslin, E.P., Root, H.F., White, P., and Marble, A. The Treatment of Diabetes Mellitus. Lea and Febiger Co. 1940, 7th Ed. pp.141 ff.

76. Remond and Rouzard Sur la Valeur Prognostique de la Cholesterinémie dans le diabète. Bull. de l'Acad. de Med. 1923, 89:60.

77. Grey, H. Lipoids in 1000 Diabetic Bloods. Am. J. Med. Sc. 1924, 168:35.

78. Labbé, M., and Lenfantin, H. Les Lésions Artérielles des Diabétiques Décelées par la Radiographie. Bull. et mém. Soc. méd. d. hôp. de Paris. 1924, 48:522.

79. Boyd, G.L. Blood Cholesterol in Diabetic Children. Am. J. Dis. Child. 1929, 38:490.

80. Hunt, M. Cholesterol in the Blood of Diabetics Treated at the New England Deaconess Hospital. New England J. Med. 1929, 201:659.

81. Kountz, W.B., Sonnenberg, A., Hofstatter, L., and Wolff, G. Blood Cholesterol in Elderly Patients. Biological Symposia. Cattell, Jaques. Ageing and Degenerative Diseases. Vol. XI Moore, R.A. The Jaques Cattell Press. Lancaster, Pa. 1945. pp. 79-86.

82. Zeek, P.M. A Chemical Analysis of Atherosclerotic Lesions in Human Aortas. Am.J.Path. 1936, 12:115.

83. Wells, H.Gideon. The Chemistry of Arteriosclerosis. Arteriosclerosis: A Survey of the Problem.. Cowdry,E.V. MacMillan Co. 1933. Ch. 11. pp. 323-353.

84. Dunn, J.S., Kirkpatrick, J., McLetchie, N.G.B., and Telfer, S.V. Necrosis of the Islets of Langerhans Produced Experimentally. J.Path. and Bact. 1943, 55:245.

85. Pollak, O.J. A New Method of Feeding Cholesterol to Animals. Arch. Path. 1944, 37:337.

86. Duff, G.L., and Starr, H. Experimental Alloxan Diabetes in Hooded Rats. Proc.Soc.Exper.Biol. and Med. 1944, 57:280.

87. Sperry, W.M. Micromethod for Determination of Total and Free Cholesterol. Am.J.Clin.Path.Tech.Supp. 1938, 2:91.

88. Paterson, D.D. Statistical Technique in Agricultural Research. McGraw-Hill Book Co. Incl. 1939.

89. Broom, M.E. Educational Statistics. American Book Co. 1936.

90. Naunyn, B. Der Diabetes Melitus, pg. 84. Band 7, Theil 6, Specielle Pathologie und Therapie; Nothnagel, H. Alfred Holder, Wein 1898.

91. Mainland, D. Statistical Methods in Medical Research. National Research Council of Canada. (Unpublished)

92. Bensley, R.R. Studies on the Pancreas of the Guinea Pig. Am.J.Anat. 1911, 12:297.

APPENDIX

STATISTICAL FACTORS

NORMAL VALUES

1. Normal fasting blood sugar factors (grouped observations on 40 rabbits, at least two determinations being made on each animal)

Number of determinations, N = 148

Sum of determinations, Ey = 15318

Mean value, $M = \frac{Ey}{N} = 103.5$ mgms.%.

Degrees of freedom, N - 1 = 147

Sum of deviations from mean squared $E(y - M)^2 = 27058$

Standard deviation of mean, S.D $\sqrt{\frac{E(y - M)^2}{N - 1}} = 13.6$

Standard error of mean, S.E $\frac{S.D}{\sqrt{N}} = 1.11$

Standard error of standard deviation, S.E S.D $= \frac{0.7071.S.D}{N} = 0.77$

Median, $Md = L + \frac{(\frac{N}{2} - Sb)}{fm} \times i = 103.2$ mgms.%

where mid-class is 100 to 109; L is the lower limit of the mid-class and = 100; N = 148; Sb is the number of items below mid-class and = 63; fm is the frequency of the mid-class and = 34; i is the class interval and = 10.

Standard error of median, S.E $Md = \frac{1.253.S.D}{N} = 1.4$

2. Normal free serum cholesterol factors (grouped observations on 40 rabbits, at least two observations being made on each animal)

Number of determinations = 143

Mean = 11.5 mgms.%

Standard deviation of mean = 5.4

Standard error of mean = 0.45

Median = 12.2 mgms.%

Standard error of median = 0.56

3. Normal total serum cholesterol factors (as above)

Number of determinations = 143

Mean = 35.7 mgms.%

Standard deviation of mean = 19.6

Standard error of mean = 1.6

Median = 32.3 mgms.%

Standard error of median = 2.0

4. Normal ester serum cholesterol factors (as above)

Number of determinations = 143

Mean = 24.8 mgms.%

Standard deviation of mean = 16.7

Standard error of mean = 1.4

Median = 20.6 mgms.%

Standard error of median = 1.7

5. Normal ester cholesterol per cent of total cholesterol (as above)

Number of determinations = 143

Mean = 63.6%

Standard deviation of mean = 15.5

Standard error of mean = 1.3

Median = 67.4%

Standard error of median = 1.6

EXPERIMENTAL VALUES

1. Blood sugar factors 2 or 3 days after alloxan administration and during insulin and glucose therapy (based on single observations on 21 animals including a level of 134 mgms.% in rabbit #39 that was resistant to alloxan)

Number of determinations = 21

Mean = 336 mgms.%

Standard deviation of mean = 165

Standard error of mean = 36

1a. Five selected blood sugar values lying below 135 mgms.% from the above group including #39.

Number of determinations = 5

Mean = 103.8 mgms.%

Standard deviation of mean = 21.5

Standard error of mean = 8.9

1b. The remaining 16 blood sugar values from the group above.

Number of determinations = 16

Mean = 408.3 mgms.%

Standard deviation of mean = 113

Standard error of mean = 28

2. Post-alloxan blood sugar factors free from therapy (based on grouped observations on 20 rabbits: #39 is excluded)

Number of determinations = 61

Mean = 378.3 mgms.%

Standard deviation of mean = 63.5

Standard error of mean = 8.1

3. Post-alloxan free serum cholesterol factors free from therapy (based on observations on 17 animals: #39 is excluded)*

Number of determinations = 55

Mean = 48 mgms.%

*(The above calculations include 9 animals that did not show a hypercholesterinemia during the therapy free period)

Standard deviation of mean = 83

Standard error of mean = 11.4

4. Post-alloxan total serum cholesterol factors free from therapy (as above)

Number of determinations = 55

Mean = 83 mgms.%

Standard deviation of mean = 128

Standard error of mean = 17.6

5. Post-alloxan ester serum cholesterol factors free from therapy (as above)

Number of determinations = 55

Mean = 35 mgms.%

Standard deviation of mean = 52.7

Standard error of mean = 7

6. Post-alloxan ester cholesterol per cent of total serum cholesterol free from therapy (as above)

Number of determinations = 55

Mean = 51.7%

Standard deviation of mean = 21.2

Standard error of mean = 3

7. Normal blood sugar factors during the period of cholesterol feeding (based on grouped observations on 5 animals over a period of 90 days, including #39)

Number of determinations = 38

Mean = 101.3 mgms.%

Standard deviation of mean = 14.8

Standard error of mean = 2.4

7a. Post-alloxan (diabetic) blood sugar factors during the period of cholesterol feeding (based on grouped observations on 5 animals over a period of 90 days)

Number of determinations = 37

Mean = 508 mgms.%

Standard deviation of mean = 82

Standard error of mean = 13.5

8. Data regarding variations in cholesterol values during the period of insulin therapy and during the period of cholesterol feeding are not suitable for analysis.

PROTOCOLS

Female PROTOCOL #E.1 Series 1.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.336	111	20	68	48	71	-
10	2.338	97	12	56	44	79	-
12	Alloxan, Glucose, Protamine Zinc Insulin						
14	2.610	83	16	64	48	75	-
19	2.737	440	6	40	36	90	-
28	2.624	284	16	56	40	71	-
33	2.620	340	12	56	44	78	-
41	2.538	423	16	44	28	63	-
47	2.483	362	12	28	16	55	-
55	2.449	390	19	52	33	63	-
56	Pure Cholesterol Feeding						
69	2.352	560	24	48	24	50	-
75	2.410	512	14	46	32	69	-
83	2.254	518	28	72	44	61	-
91	2.220	466	6	32	26	84	-
103	2.221	486	16	28	12	43	-
117	2.240	548	8	24	16	66	-
131	2.110	540	13	44	31	70	-
147	1.980	635	36	128	92	71	-

Course: Not remarkable. Received 76 pure cholesterol feedings, 0.5 gms. per dose in 90 days.

Cholesterol factor: 96 mgm-days of total cholesterol above M + 3.S.D

Death: Killed by air embolism on completion of experiment.

Autopsy: Wasting of tissues. Aorta not remarkable.

Microscopic: Pancreas - Loss and atrophy of islets. Hydropic degeneration of islets. Hydropic degeneration of ductules.
Aorta - Normal.
Heart and coronary vessels - Normal.

Female PROTOCOL #E.2 Series 1.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	1.690	110	20	48	28	58	-
10	2.137	97	12	28	16	57	-
12	Alloxan, Glucose, Protamine Zinc Insulin						
14	2.323	224	20	56	36	64	1
19	2.388	318	8	36	28	78	2
28	2.201	300	232	256	24	0	4
33	2.056	336	152	240	88	37	4
41	2.040	462	132	156	24	15	3
47	1.970	371	20	40	20	50	1
55	1.895	456	49	78	29	37	-
56	Pure Cholesterol Feeding						
58	Died						

Course: Not remarkable. Received two 0.5 gm. doses of pure cholesterol

Cholesterol factor: 0 mgm-days of total cholesterol above M + 3.S.D

Death: Choked to death on food pellet.

Autopsy: Moderate wasting. Bilateral lung abscesses in hilar regions. Spontaneous arteriosclerosis of ascending aortic arch.

Microscopic: Pancreas - Loss and atrophy of islets. Loss of beta cells. Hydropic degeneration of islets. Hydropic degeneration of ductules.
Aorta - Normal.
Heart and coronary vessels - Normal.

Male PROTOCOL #E.3 Series 1.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.473	114	12	32	20	62	-
10	2.500	84	12	36	24	67	-
12	Alloxan, Glucose, Protamine Zinc Insulin						
14	2.732	188	12	40	28	70	
19	2.744	412	6	32	26	81	
27	Died						

Course: Stopped eating on 24th day. Given insulin and glucose until death.

Cholesterol factor: 0 mgm-days of total cholesterol above M + 3.S.D

Death: Diabetic coma.

Autopsy: Cloudy swelling of heart, liver and kidneys. Hypertrophy of pyloric ring with stenosis. Stomach full after 3 days of fasting. Aorta not remarkable.

Microscopic: Pancreas - Not obtained.
Aorta - Normal.
Heart and coronary vessels - Fibrosis of myocardium. Spontaneous arteriosclerosis of coronary artery.

Male PROTOCOL #E.4 Series 1.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.881	110	24	40	16	40	-
10	3.180	123	12	36	24	66	-
12	Alloxan, Glucose, Protamine Zinc Insulin						
14	3.269	366	24	56	32	57	1
19	3.270	304	12	48	36	75	2
28	3.200	275	232	296	64	21	4
33	3.200	302	208	296	88	30	4
41	3.183	426	156	176	20	11	2
47	3.127	398	88	112	24	21	1
55	3.150	424	23	52	29	56	1
56	Pure Cholesterol Feeding						
69	3.075	470	68	112	44	40	1
75	2.995	444	24	47	23	49	1
83	2.998	502	56	90	34	38	1
91	3.045	460	58	100	42	42	1
103	2.978	386	66	140	74	53	2
117	2.960	474	39	70	31	44	1
131	2.680	464	22	81	59	72	-
147	2.640	554	51	116	65	56	-

Course: Not remarkable. Received 76 0.5 gm. doses of pure cholesterol in 90 days.

Cholesterol factor: 4470 mgm-days of total cholesterol above M + 3.S.D

Death: Killed with intravenous nembutal.

Autopsy: Very little body fat. Aorta showed a most minimal atherosclerosis of arch.

Microscopic: Pancreas - Loss and atrophy of islets. Loss of beta cells. Hydropic degeneration of islets. Hydropic degeneration of ductules.
Aorta - Medial lipidosis.
Heart and coronary vessels - Normal.

Female PROTOCOL #C.5 Series 1.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4.
0	2.268	89	32	72	40	55	-
10	2.320	91	24	112	88	78	-
14	2.507	88	28	96	68	71	-
19	2.614	94	28	96	68	71	-
28	2.970	86	24	68	44	65	-
33	3.120	106	16	64	48	75	-
41	3.385	115	20	32	12	37	-
47	3.400	87	12	28	16	57	-
55	3.540	94	14	32	18	56	-
56	Pure Cholesterol Feeding						
69	3.710	120	22	68	46	68	-
75	3.800	73	13	41	28	68	-
83	3.910	85	12	44	32	73	-
91	3.955	89	17	66	49	74	-
103	4.060	78	21	44	23	52	-
117	4.130	107	27	98	71	72	-
131	4.140	102	38	156	118	75	-
147	4.060	116	57	212	155	73	-

Course: Treated with 0.5% silver nitrate and 10% argyrol for purulent conjunctivitis from 47 to 75th days. Treated with Lethane for ear mites on 103rd day. Received 76 0.5 gm. doses of cholesterol in 90 days.

Cholesterol factor: 1860 mgm-days of total cholesterol above M + 3.S.D

Death: Killed with intravenous nembutal on completion of experiment.

Autopsy: Generally negative. Aorta showed slight atherosclerosis of thoracic and abdominal portions.

Microscopic: Pancreas - Normal.
Aorta - Intimal atherosclerosis. Spontaneous arteriosclerosis.
Heart and coronary vessels - Normal.

Female PROTOCOL #C.6 Series 1.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.596	95	16	60	44	73	-
10	2.758	85	20	36	16	45	-
14	2.860	100	12	60	48	80	-
19	2.994	115	12	52	40	78	-

Course: Animal transferred to another experiment.

Female PROTOCOL #C.7 Series 1.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Free mgms.%	Cholesterol Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.370	94	16	40	24	60	-
10	2.510	90	16	68	52	76	-
14	2.637	103	16	72	56	78	-
19	2.649	96	16	60	44	73	-
28	2.863	83	16	68	52	76	-
33	2.880	83	16	68	52	76	-
41	2.998	106	20	48	28	58	-
47	3.060	88	12	32	20	62	-
55	3.084	98	14	50	36	72	-
56	Pure Cholesterol Feeding						
69	3.404	96	10	47	37	79	-
75	3.410	74	20	88	68	77	-
83	3.470	83	14	68	54	79	-
91	3.660	75	25	50	25	50	-
103	3.740	109	21	112	91	82	-
117	3.650	103	62	212	150	70	-
131	3.700	110	35	240	205	85	-
147	3.356	119	99	304	205	67	-

Course: Not remarkable. Received 76 0.5 gm. doses of pure cholesterol in 90 days.

Cholesterol factor: 6720 mgm-days of total cholesterol above M + 3.S.D

Death: Killed with intravenous nembutal on completion of experiment.

Autopsy: Abscess of left upper lobe of lung. Aorta showed slight atherosclerosis of thoracic and abdominal parts.

Microscopic: Pancreas - Normal.
Aorta - Intimal atherosclerosis.
Heart and coronary vessels - Normal.

Male

PROTOCOL #C.8

Series 1.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.675	106	20	36	16	44	-
10	2.745	91	8	28	20	72	-
14	2.838	89	8	44	36	82	-
19	2.850	94	8	32	24	75	-
28	2.773	83	12	36	24	67	-
33	2.760	81	12	42	30	71	-
41	2.800	97	6	20	14	70	-
47	2.782	96	12	20	8	40	-
55	2.840	120	26	50	24	48	-
56	Pure Cholesterol Feeding						
69	2.812	112	4	28	24	86	-
75	2.820	78	10	42	32	76	-
83	2.824	88	8	24	16	66	-
91	2.825	86	6	20	14	70	-
103	2.870	84	25	32	7	22	-
117	2.850	126	22	128	106	83	-
131	2.750	104	50	304	254	82	-
147	2.760	104	90	296	206	70	-

Course: Not remarkable. This was a mature animal. Received 76 0.5 gm. doses of pure cholesterol in 90 days.

Cholesterol factor: 4980 mgm-days of total cholesterol above M + 3.S.D

Death: Killed with intravenous nembutal on completion of experiment.

Autopsy: Nodular thickening of skin. Aorta showed slight atherosclerosis of thoracic and abdominal portions.

Microscopic: Pancreas - Normal.
Aorta - Intimal atherosclerosis.
Heart and coronary vessels: Paravascular myocarditis.

Male PROTOCOL #E.18 Series 2.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.010	124	11	28	17	61	-
3	2.206	98	14	28	14	50	-
4	Alloxan, Glucose, Protamine Zinc Insulin						
7	2.125	76	8	28	20	71	-
20	2.205	350	10	44	34	77	-
34	2.350	326	16	44	28	64	-
48	2.510	440	13	42	29	69	-
62	2.510	?	25	56	31	55	-
62	Cholesterol in Oil Feeding						
74	Died						

Course: Received 5 0.25 gm. doses of cholesterol 3.3% in sun flower seed oil.

Cholesterol factor: 0 mgm-days of total cholesterol above M + 3.S.D

Death: From lipoid pneumonia.

Autopsy: Lipoid pneumonia, extensive, bilateral. Aorta normal.

Microscopic: Pancreas - Loss and atrophy of islets. Hydropic degeneration of islets. Hydropic degeneration of ductules.
Aorta - Acute para-aortitis (lipoidal)
Heart and coronary vessels - Paravascular myocarditis.

Male | Protocol #C.19 | Series 2.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Free mgms.%	Cholesterol Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.210	122	7	40	33	82	-
3	2.304	83	10	18	8	45	-
7	2.255	97	6	10	4	40	-
20	2.330	93	6	32	26	82	-
34	2.620	89	8	40	32	80	-
48	3.060	97	17	40	23	57	-
62	2.940	?	11	32	21	66	-
62	Cholesterol in Oil Feeding						
76	2.640	101	8	26	18	69	-
81	Died						

Course: Bilateral ectopion when received. Quickly developed bilateral purulent conjunctivitis. Treated occasionally with 10% Argyrol. Developed persistent snuffles about 62nd day. Received 11 0.25 gm. doses of cholesterol 3.3% in sun flower seed oil.

Cholesterol factor: 0 mgm-days of total cholesterol above M + 3.S.D

Death: Extensive lipoid pneumonia. Injured by passing 16 F. catheter.

Autopsy: Pneumonia, lung abscesses, pleurisy and mediastinitis. Aorta normal.

Microscopic: Pancreas - Normal.
Aorta - Acute para-aortitis
Heart and coronary vessels - Normal.

Female PROTOCOL #C.20 Series 2.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Free mgms.%	Cholesterol Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	1.900	118	10	54	44	81	-
3	2.106	90	15	68	53	78	-
7	2.200	101	9	24	15	62	-
34	2.000	132	18	64	46	72	-

Course: When received this animal had a slight tendency to hold her head tilted to the right. The signs and symptoms rapidly became exaggerated. A tendency to fall to the left and to lie on the left side arose, and then a semi-spastic paralysis and a marked lateral nystagmus developed.

Cholesterol: 0 mgm-days of total cholesterol above M + 3.S.D

Death: Killed by air embolism.

Autopsy: Acute gastritis, pitting of kidneys, grey foci in liver. Brain normal. Aorta normal.

Microscopic: Pancreas - Normal.
Aorta - Normal.
Heart and coronary vessels - Normal.
Brain - Subacute meningitis. Focal granulomatous reaction in cortex.
Liver - Foci of coagulative necrosis.
Kidneys - Minimal pyelonephritic scarring.
Stomach - Acute gastritis.
Other organs normal.

Male PROTOCOL #E.21 Series 2.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.180	128	9	36	27	75	-
3	2.231	83	6	16	10	63	-
4	Alloxan, Glucose, Protamine Zinc Insulin						
7	2.060	118	6	24	18	75	-
20	1.985	372	6	20	14	70	-
34	2.100	342	9	40	31	78	-
48	2.300	412	12	32	20	62	-
62	2.270	?	16	42	26	62	-
62	Cholesterol in Oil Feeding						
63	Died						

Course: Not remarkable.

Cholesterol factor: 0 mgm-days of total cholesterol above M + 3.3.D

Death: Accidental death, killed by a massive intrapulmonary injection of oil.

Autopsy: Oil in lungs, trachea and bronchi. Atelectasis of lungs. Aorta normal.

Microscopic: Pancreas - Loss and atrophy of islets. Hydropic degeneration of islets. Hydropic degeneration of ductules.
Aorta - Normal.
Heart and coronary vessels - Spontaneous arteriosclerosis of coronary arteries.

Male PROTOCOL #C.22 Series 2.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Free mgms.%	Cholesterol Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	1.700	86	6	24	18	75	-
3	1.822	86	7	12	5	42	-
7	1.765	99	8	36	28	78	-
20	2.025	111	6	36	30	83	-
34	2.140	99	8	36	28	78	-
48	2.380	104	9	24	15	62	-
62	2.410	?	14	36	22	61	-
62	Cholesterol in Oil Feeding						
64	Died						

Course: Not remarkable. Received 3 0.25 gm. doses of cholesterol 3.3% in sun flower seed oil.

Cholesterol Factor: 0 mgm-days of total cholesterol above M + 3.S.D

Death: Accidental death. Massive injection of oil into lungs.

Autopsy: Intrapulmonary oil. Atelectasis of lungs. Aorta showed spontaneous arteriosclerosis of ascending arch.

Microscopic: Pancreas - Normal.
Aorta - Normal.
Heart and coronary vessels -.Normal.

Male PROTOCOL #E.23 Series 2.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Free mgms.%	Cholesterol Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.250	112	6	16	10	62	-
3	2.300	87	6	12	6	50	-
4	Alloxan, Glucose, Protamine Zinc Insulin						
7	2.225	356	6	12	6	50	-
20	2.135	372	36	80	44	55	1
34	1.960	358	15	28	13	46	-
48	1.680	404	8	28	20	71	-
58	Died						

Course: Given protamine zinc insulin and glucose on 56th and 57th days.

Cholesterol factor: 0 mgm-days of total cholesterol above M + 3.S.D

Death: In diabetic coma.

Autopsy: Severe emaciation. A few small yellowish foci present in the liver. Aorta normal.

Microscopic: Pancreas - Atrophy of islets. Loss of beta cells.
Aorta - Normal.
Heart and coronary vessels - Normal.

Female PROTOCOL #E.24 Series 2.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Free mgms.%	Cholesterol Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	1.950	103	13	46	33	72	-
3	2.720	90	8	27	19	70	-
4	Alloxan, Glucose, Protamine Zinc Insulin						
7	1.830	410	56	116	60	52	2
14	Died						

Course: Not remarkable.

Cholesterol Factor: 620 mgm-days of total cholesterol above M + 3.S.D

Death: Insulin shock and diabetes.

Autopsy: Wasting of tissues. Aorta normal.

Microscopic: Pancreas - Loss and atrophy of islets. Recent necrosis of islet cells. Loss of beta cells. Hydropic degeneration of islets.
Aorta - Normal.
Heart and coronary vessels - Focal fibrosis of myocardium.

Female PROTOCOL #C.25 Series 2.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.500	102	7	28	21	75	-
3	2.506	82	6	10	4	40	-
7	2.450	110	8	22	14	64	-
20	2.650	105	6	42	36	86	-
34	2.550	97	15	60	45	75	-
48	2.560	98	11	48	37	77	-
62	2.770	?	17	66	49	74	-
62	Cholesterol in Oil Feeding						
66	Died						

Course: Not remarkable. Received 4 0.25 gm. doses of cholesterol 3.3% in sun flower seed oil. Blind in left eye (corneal opacity)

Cholesterol factor: 0 mgm-days of total cholesterol above M + 3.S.D

Death: Lipoid pneumonia. Aorta showed spontaneous arteriosclerotic lesions in ascending arch.

Microscopic: Pancreas - Normal.
Aorta - Normal.
Heart and coronary vessels - Normal.

Female PROTOCOL #E.26 Series 2.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	1.900	117	11	65	54	83	-
3	1.875	96	9	32	23	72	-
4	Alloxan, Glucose, Protamine Zinc Insulin						
7	1.930	108	31	42	11	26	-
20	1.525	374	480	860	380	44	4
34	1.370	338	62	164	102	62	-
42	Died						

Course: Not remarkable. The animal showed the greatest hyperlipaemia obtained in this experiment.

Cholesterol Factor: 10,040 mgm-days of total cholesterol above M + 3.S.D

Death: Diabetic coma.

Autopsy: Wasting of tissues. Large area of white pultaceous material about pancreas. Aorta showed slight intimal atherosclerosis.

Microscopic: Pancreas - Coagulative and caseous necrosis with subacute inflammation.
Aorta - Intimal atherosclerosis. Medial atherosclerosis (superficial) Rupture of internal elastic membrane.
Heart and coronary vessels - Normal.

Female PROTOCOL #E.27 Series 2.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.700	117	8	36	28	78	-
3	2.720	105	9	29	20	69	-
4	Alloxan, Glucose, Protamine Zinc Insulin						
6	Died						

Course: Not remarkable.

Cholesterol Factor: 0 mgm-days of total cholesterol above M +.3.S.D

Death: Insulin shock.

Autopsy: Hyperaemia of viscera. Subpleural petechial haemorrhages. Aorta normal.

Microscopic: Pancreas - Loss and atrophy of islets. Recent necrosis of beta cells.
Aorta - Normal.
Heart and coronary vessels: Subacute interstitial myocarditis.

Female PROTOCOL #C.28 Series 2.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	1.800	99	10	50	40	80	-
3	1.850	82	12	26	14	54	-
7	1.990	107	11	18	7	39	-
20	2.200	110	8	72	64	89	-
34	2.340	102	16	58	42	72	-
48	2.220	111	22	72	50	69	-
62	2.460	?	20	58	38	66	-
62	Cholesterol in Oil Feeding						
63	Died						

Course: Not remarkable. Received 2 0.25 gm. dose of cholesterol 3.3% in sun flower seed oil.

Cholesterol Factor: 0 mgm-days of total cholesterol above M + 3.S.D

Death: Accidental death from massive intrapulmonary injection of oil.

Autopsy: Intrapulmonary oil. Atelectasis of lungs. Aorta normal.

Microscopic: Pancreas - Normal.
Aorta - Normal.
Heart and coronary arteries - Fibrosis of myocardium. Spontaneous arteriosclerosis of coronary arteries.

Male PROTOCOL #E.32 Series 3.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	3.100	112	7	16	9	56	-
6	3.200	110	6	18	12	67	-
7	Alloxan, Glucose, Protamine Zinc Insulin						
9	3.510	534	18	50	32	64	-
19	Died						2

Course: Not remarkable.

Cholesterol Factor: 0 mgm-days of total cholesterol above M + 3.S.D

Death: Diabetic coma.

Autopsy: Not remarkable. Aorta normal.

Microscopic: Pancreas - Loss and atrophy of islets. Loss and necrosis of beta cells.
Aorta - Normal.
Heart and coronary vessels - Spontaneous arteriosclerosis of coronary arteries.

Male PROTOCOL #C.33 Series 3.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.230	104	12	25	13	52	-
6	2.250	111	6	36	30	83	-
7	Died						

Course: Not remarkable.

Cholesterol Factor: 0 mgm-days of total cholesterol above M + 3.S.D

Death: Died from haemorrhage from ear cut.

Autopsy: Not performed.

Male PROTOCOL #E.34 Series 3.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.800	104	9	20	11	55	-
6	2.820	97	6	24	18	75	-
7	Alloxan, Glucose, Protamine Zinc Insulin						
9	3.040	486	10	50	40	80	-
21	2.850	306	7	24	17	71	-
34	2.950	322	?	?	?	?	-
48	2.830	342	15	28	13	46	-
49	Cholesterol in Oil Feeding						
62	2.850	348	24	56	32	57	-
72	2.820	?	16	92	76	82	-

Course: Determinations taken immediately after death on 72nd day. Received 18 0.25 gm. doses of cholesterol in sun flower seed oil.

Cholesterol Factor: 0 mgm-days of total cholesterol above M + 3.S.D

Death: Accidental death caused by catheter.

Autopsy: Bronchopleural fistula and pneumothorax, right. Aorta showed spontaneous arteriosclerosis of ascending aortic arch.

Microscopic: Pancreas - Loss and atrophy of islets. Loss of beta cells. Hydropic degeneration of islets.
Aorta - Spontaneous arteriosclerosis.
Heart and coronary vessels - Focal myocarditis. Subacute endocarditis. Spontaneous arteriosclerosis of coronary arteries.

Male PROTOCOL #35 Series 3.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	3.010	101	16	28	12	43	-
6	3.130	106	11	72	61	85	-
7	Alloxan, Glucose, Protamine Zinc Insulin						
9	3.350	494	25	80	55	69	-
21	3.110	364	38	62	24	39	-
34	3.030	402	10	26	16	62	-
48	2.980	338	8	44	36	82	-
49	Cholesterol in Oil Feeding						
62	2.990	418	42	76	34	45	-
76	2.810	496	50	108	58	54	-
90	2.780	586	42	60	22	37	-
104	2.880	470	48	72	24	33	-
118	2.740	490	62	100	38	38	1
125	2.760	670	160	184	24	13	2
139	2.610	600	130	270	140	52	1

Course: Not remarkable. Received 64 feedings of 0.25 gms. of cholesterol in sun flower seed oil in 90 days.

Cholesterol Factor: 420 mgm-days of total cholesterol above M + 3.S.D

Death: Killed by air embolism on completion of experiment.

Autopsy: Wasting of tissues. Extensive psuedo-tuberculosis of mandible and liver. Spontaneous arteriosclerosis of ascending aortic arch.

Microscopic: Pancreas - Loss and atrophy of islets. Hydropic degeneration of islets. Hydropic degeneration of ductules.
Aorta - Normal.
Heart and coronary vessels - Spontaneous arteriosclerosis of coronary arteries.

Male PROTOCOL #C.37 Series 3.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale in 4
0	1.920	100	7	16	9	56	-
6	1.970	103	6	44	38	86	-
9	2.020	90	10	32	22	69	-
21	2.200	87	10	26	16	62	-
34	2.370	92	8	44	36	82	-
48	2.420	94	6	28	22	78	-
49	Cholesterol in Oil Feeding						
62	2.370	113	34	50	16	32	-
76	2.310	114	40	78	38	49	-
90	1.860	?	96	204	108	53	-

Course: Not remarkable. Determinations of the 90th day are post mortem. Received 30 0.25 gm. doses of cholesterol 3.3% in sun flower seed oil.

Cholesterol Factor: 690 mgm-days of total cholesterol above M + 3.S.D

Death: Natural causes.

Autopsy: Acute purulent bronchitis and confluent bilateral bronchopneumonia. Aorta showed spontaneous arteriosclerosis of ascending arch.

Microscopic: Pancreas - Normal.
Aorta - Spontaneous arteriosclerosis.
Heart and coronary vessels - Normal.

Male PROTOCOL #38 Series 3.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	1.750	117	10	12	2	16	-
6	1.850	113	12	68	56	82	-
9	1.920	109	24	64	40	62	-
21	2.080	101	10	40	30	75	-
34	2.270	91	10	28	18	64	-
48	2.560	105	6	29	23	79	-
49	Cholesterol in Oil Feeding						
62	2.690	112	16	44	28	64	-
76	2.720	105	16	44	28	64	-
90	2.990	106	10	32	22	69	-
104	2.980	112	10	16	6	37	-
118	3.090	119	8	16	8	50	-
125	3.140	113	12	27	15	55	-
139	3.200	102	10	32	22	69	-

Course: Not remarkable. Received 64 feedings of 0.25 gms. of cholesterol in sun flower seed oil in 90 days.

Cholesterol Factor: 0 mgm-days of total cholesterol above M + 3.S.D

Death: Killed by air embolism on completion of experiment.

Autopsy: No pathology.

Microscopic: Pancreas - Normal.
Aorta - Normal.
Heart and coronary vessels - Spontaneous arteriosclerosis of coronary arteries.

Male PROTOCOL #39 Series 3.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.000	122	16	28	12	43	-
6	2.070	114	6	50	44	88	-
7	Alloxan, Glucose, Protamine Zinc Insulin						
9	2.320	134	26	80	54	68	-
21	2.530	98	9	39	30	77	-
34	2.700	84	12	24	12	50	-
48	3.080	111	6	29	23	79	-
49	Cholesterol in Oil Feeding						
62	3.070	97	28	44	16	36	-
76	3.110	109	18	48	30	62	-
90	3.270	106	16	32	16	50	-
104	3.390	103	10	24	14	58	-
118	3.450	107	12	16	4	25	-
125	3.410	126	10	20	10	50	-
139	3.510	110	10	46	36	78	-

Course: Resistant to injection of 200 mgms. of alloxan per kilogram of body weight. Received 64 feedings of 0.25 gms. of cholesterol in sun flower seed oil in 90 days.

Cholesterol Factor: 0 mgm-days of total cholesterol above M + 3.S.D

Death: Killed by air embolism on completion of experiment.

Autopsy: Negative except for spontaneous medial sclerosis of ascending aortic arch.

Microscopic: Pancreas - Normal ?
Aorta - Intimal atherosclerosis. Spontaneous arteriosclerosis.
Heart and coronary vessels - Intimal atherosclerosis.

Male PROTOCOL #40 Series 3.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.110	107	9	28	19	68	-
6	2.200	115	6	44	38	86	-
7	Alloxan, Glucose, Protamine Zinc Insulin						
9	2.390	590	16	64	48	75	-
21	2.090	490	30	108	78	70	1
34	2.160	426	50	116	66	57	2
48	2.120	552	9	28	19	68	-
49	Cholesterol in Oil Feeding						
62	2.150	600	194	220	26	12	3
76	2.050	518	98	124	26	21	2
90	1.960	538	16	30	24	80	1
104	1.920	584	82	88	6	7	1
118	2.070	660	46	50	4	8	2
125	2.050	630	230	244	14	6	4
139	1.890	660	34	104	70	68	-

Course: Not remarkable. Received 64 feedings of 0.25 gms. of cholesterol in sun flower seed oil in 90 days.

Cholesterol factor: 2520 mgm-days of total cholesterol above M + 3.S.D

Death: Killed by air embolism on completion of experiment.

Autopsy: Atrophy of testes and other tissues. Aorta normal.

Microscopic: Pancreas - Loss and atrophy of islets. Hydropic degeneration of islets. Hydropic degeneration of ductules.
Aorta - Normal.
Heart and Coronary Vessels - Intimal atherosclerosis of coronary artery.

Male

PROTOCOL #41

Series 3.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.450	107	15	30	15	50	-
6	2.460	113	9	44	35	80	-
7	Alloxan, Glucose, Protamine Zinc Insulin						
9	2.750	432	19	72	53	74	-
21	2.490	318	7	56	49	87	-
34	2.650	318	6	16	10	62	-
48	2.740	316	6	20	14	70	-
49	Cholesterol in Oil Feeding						
62	2.740	420	14	20	6	30	-
76	2.870	438	16	64	48	75	-
90	2.860	366	16	24	8	33	-
104	2.980	400	9	13	4	31	-
118	2.950	380	9	32	23	72	-
125	2.900	470	20	28	8	29	-
139	2.920	388	12	52	40	77	-

Course: Not remarkable. Received 64 feedings of 0.25 gms. of cholesterol in sun flower seed oil in 90 days.

Cholesterol Factor: 0 mgm-days of total cholesterol above M + 3.S.D

Death: Killed by air embolism on completion of experiment.

Autopsy: Slight atrophy of tissues. Spontaneous medial sclerosis of ascending aortic arch.

Microscopic: Pancreas - Loss and atrophy of islets. Hydropic degeneration of islets. Hydropic degeneration of ductules.
Aorta - Spontaneous arteriosclerosis.
Heart and coronary vessels - Normal.

Male PROTOCOL #C.42 Series 3.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.040	115	14	30	16	53	-
6	1.990	107	11	66	55	83	-
9	2.040	108	16	60	44	73	-
21	2.010	106	10	42	32	76	-
34	2.060	88	6	20	14	70	-
48	2.080	110	6	16	10	63	-
49	Cholesterol in Oil Feeding						
62	2.080	?	45	100	55	55	-
62	Died						

Course: Not remarkable.

Cholesterol Factor: 6 mgm-days of total cholesterol above M + 3.S.D

Death: Laryngeal obstruction after passing catheter.

Autopsy: Bilateral pneumonia and large lung abscesses. Fibrous pleural adhesions. Large mass of thick white purulent material obstructing larynx. Aorta normal.

Microscopic: Pancreas - Autolyzed.
Aorta - Normal.
Heart and coronary vessels - Normal.

Male — PROTOCOL #E.43 — Series 4.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.000	102	10	20	10	50	-
14	2.480	110	6	20	14	70	-
15	Alloxan, Glucose, Protamine Zinc Insulin						
17	2.630	372	9	38	29	76	-
28	2.590	224	8	42	34	81	-
42	2.920	292	9	16	7	44	-
59	2.720	328	6	40	34	85	-
71	2.870	332					-
76	Cholesterol in Oil Feeding						

Course: In progress.

Male — PROTOCOL #C.44 — Series 4.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	1.600	115	10	16	6	38	-
14	1.950	83	10	16	6	37	-
28	2.280	124	10	16	6	38	-
42	2.850	96	26	44	18	41	-
59	3.360	110	10	50	40	80	-

Course: Transferred.

Male — PROTOCOL #C.45 — Series 4.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	1.900	104	6	20	14	70	-
14	1.970	111	10	14	4	28	-
28	2.250	99	10	24	14	58	-
42	2.580	96	9	28	19	68	-
59	3.010	140	6	24	18	75	-
71	3.260	129					-
76	Cholesterol in Oil Feeding						

Course: In progress.

Male

PROTOCOL #C.46

Series 4.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	1.600	103	8	22	14	64	-
14	1.880	102	12	28	16	57	-
28	2.400	93	10	18	8	44	-
42	2.780	93	8	30	22	73	-
59	3.160	142	7	20	13	65	-
71	3.090	119					-
76	Cholesterol in Oil Feeding						

Course: In progress.

Male

PROTOCOL #C.47

Series 4.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	1.430	118	10	24	14	58	-
14	1.650	115	8	20	12	60	-
28	2.160	89	6	20	14	70	-
42	2.600	92	12	28	16	57	-
59	3.070	147	10	26	16	61	-
71	3.110	118					-
76	Cholesterol in Oil Feeding						

Course: In progress.

Male

PROTOCOL #C.48

Series 4.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	1.570	109	10	16	6	37	-
14	1.470	111	10	30	20	67	-
28	1.830	99	6	18	12	67	-
42	1.870	93	18	22	4	18	-
59	2.030	136	6	20	14	70	-
71	2.150	118					-
76	Cholesterol in Oil Feeding						
77	Killed						

Course: Fracture of back on 76th day.
Cholesterol Factor:
Death: Air embolism.
Autopsy: Fracture of lumbar region.
Microscopic:

Male — PROTOCOL #E.49 — Series 4.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.240	116	11	30	119	63	-
14	2.410	121	12	16	4	25	-
15	Alloxan, Glucose, Protamine Zinc Insulin						
17	2.490	426	13	44	31	70	-
28	2.450	410	14	26	12	42	-
42	2.550	404	16	20	4	20	-
59	2.660	484	9	16	7	44	-
71	2.720	520					-
76	Cholesterol in Oil Feeding						

Course: In progress.

Male — PROTOCOL #E.50 — Series 4.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.320	139	7	16	9	56	-
14	2.640	109	8	12	4	33	-
15	Alloxan, Glucose, Protamine Zinc Insulin						
17	2.780	332	20	58	38	66	-
28	2.960	330	6	8	2	25	-
42	3.300	404	9	14	5	36	-
59	3.530	390	6	12	6	50	-

Course: Transferred.

Male — PROTOCOL #E.51 — Series 4.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	2.220	118	6	16	10	63	-
14	2.180	103	6	12	6	50	-
15	Alloxan, Glucose, Protamine Zinc Insulin						
17	2.220	300	6	32	26	70	-
28	2.220	334	16	36	20	56	-
42	2.230	412	104	116	12	10	1
59	2.220	470	72	88	16	18	2
71	2.200	430					-
76	Cholesterol in Oil Feeding						

Course: In progress.

Male

PROTOCOL #E.52

Series 4.

Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	1.830	124	6	12	6	50	-
14	2.220	125	10	42	32	76	-
15	Alloxan, Glucose, Protamine Zinc Insulin						
17	2.260	534	12	44	32	73	-
28	2.310	348	14	48	34	71	-
42	2.330	330	9	20	11	55	-
59	2.520	340	10	20	10	50	-
71	2.530	420					
76	Cholesterol in Oil Feeding						

Course: In progress.

Female

PROTOCOL #E.53

Series 4.

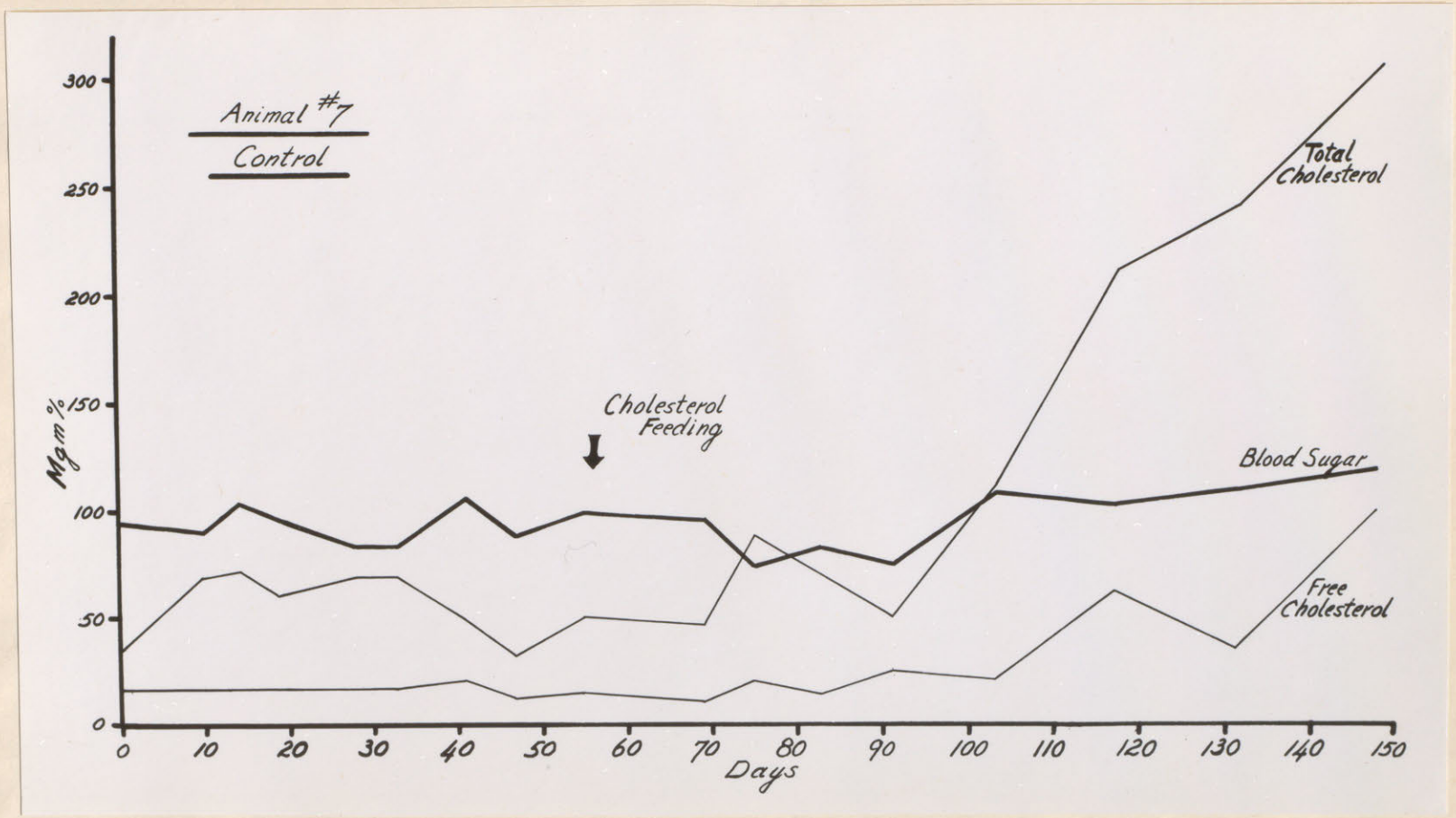
Day of Experiment	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Total mgms.%	Ester mgms.%	Ester %	Lipaemia Scale of 4
0	1.870	135	6	12	6	50	-
14	2.090	116	6	10	4	40	-
15	Alloxan, Glucose, Protamine Zinc Insulin						
17	2.190	488	10	38	28	74	-
28	2.010	444	86	160	74	46	1
42	1.910	446	18	22	4	18	-
59	1.940	406	6	16	10	62	-
71	2.090	452					-
76	Cholesterol in Oil Feeding						

Course: In progress.

GRAPH #1

Animal #7. No alloxan given.

The graph shows the normal variations of blood sugar and serum cholesterol values. Oral pure cholesterol was fed during the terminal 90 days, producing a considerable increase in total cholesterol but relatively less increase in the free cholesterol so that the ester cholesterol fraction is increased disproportionately. The blood sugar is unaffected. Atherosclerosis was present in the aorta.

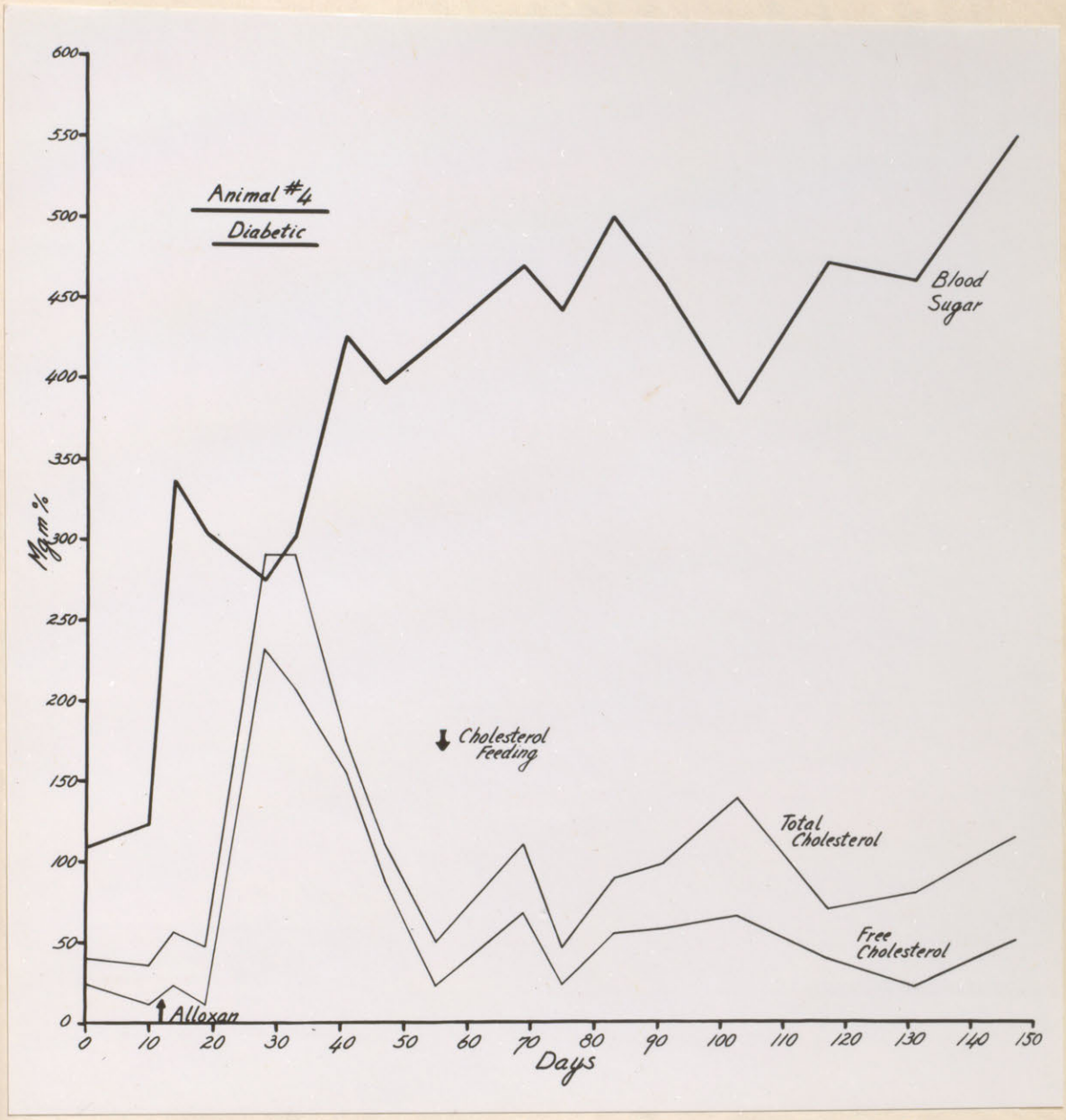

Animal #7
Control
Total Cholesterol
Blood Sugar
Free Cholesterol
Cholesterol Feeding
Mg m %
300
250
200
150
100
50
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
Days

1.

GRAPH #2

Animal #4. Diabetic.

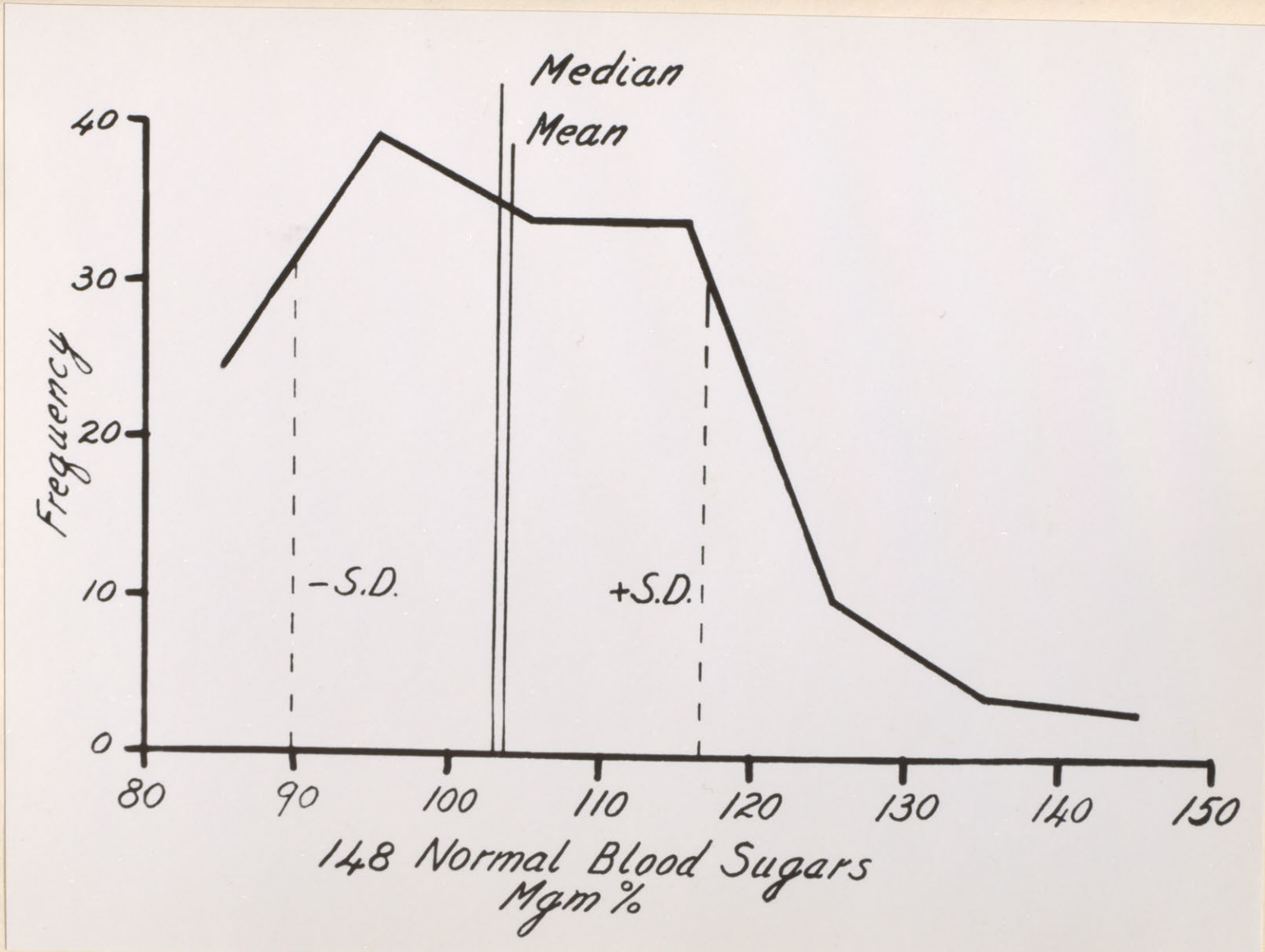
The marked, rapid, and continuing increase in blood sugar after alloxan administration is shown. There is also a moderately severe disturbance of cholesterol levels, the increase in the free level being almost as large as that in the total cholesterol level, so that the ester cholesterol is disproportionately low. Cholesterol feeding appears to have had a very slight effect on the serum cholesterol content, while the blood sugar level has become unusually high during the period of pure cholesterol feeding. Most minimal atherosclerosis was present in this animal's aorta.


Animal #4
Diabetic
Blood Sugar
Cholesterol Feeding
Total Cholesterol
Free Cholesterol
Alloxan
Mgm %
Days
600
550
500
450
400
350
300
250
200
150
100
50
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150

2.

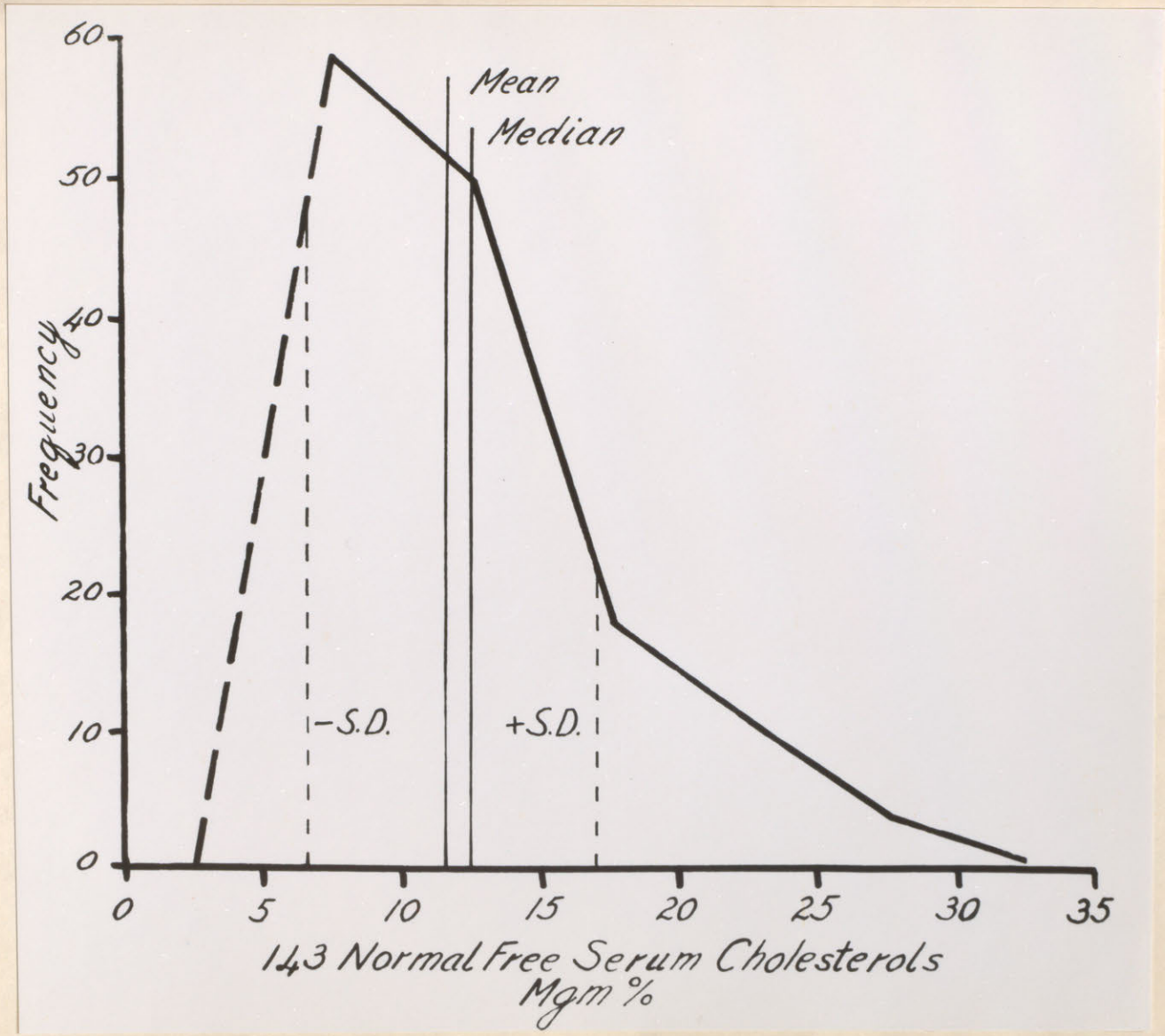
GRAPH #3

Frequency distribution of 148 normal blood sugar values at a class interval of 10 mgms.%. Based on 40 normal animals.


Median
Mean
40
30
20
10
0
Frequency
-S.D.
+S.D.
80
90
100
110
120
130
140
150
148 Normal Blood Sugars
Mgm %

GRAPH #4

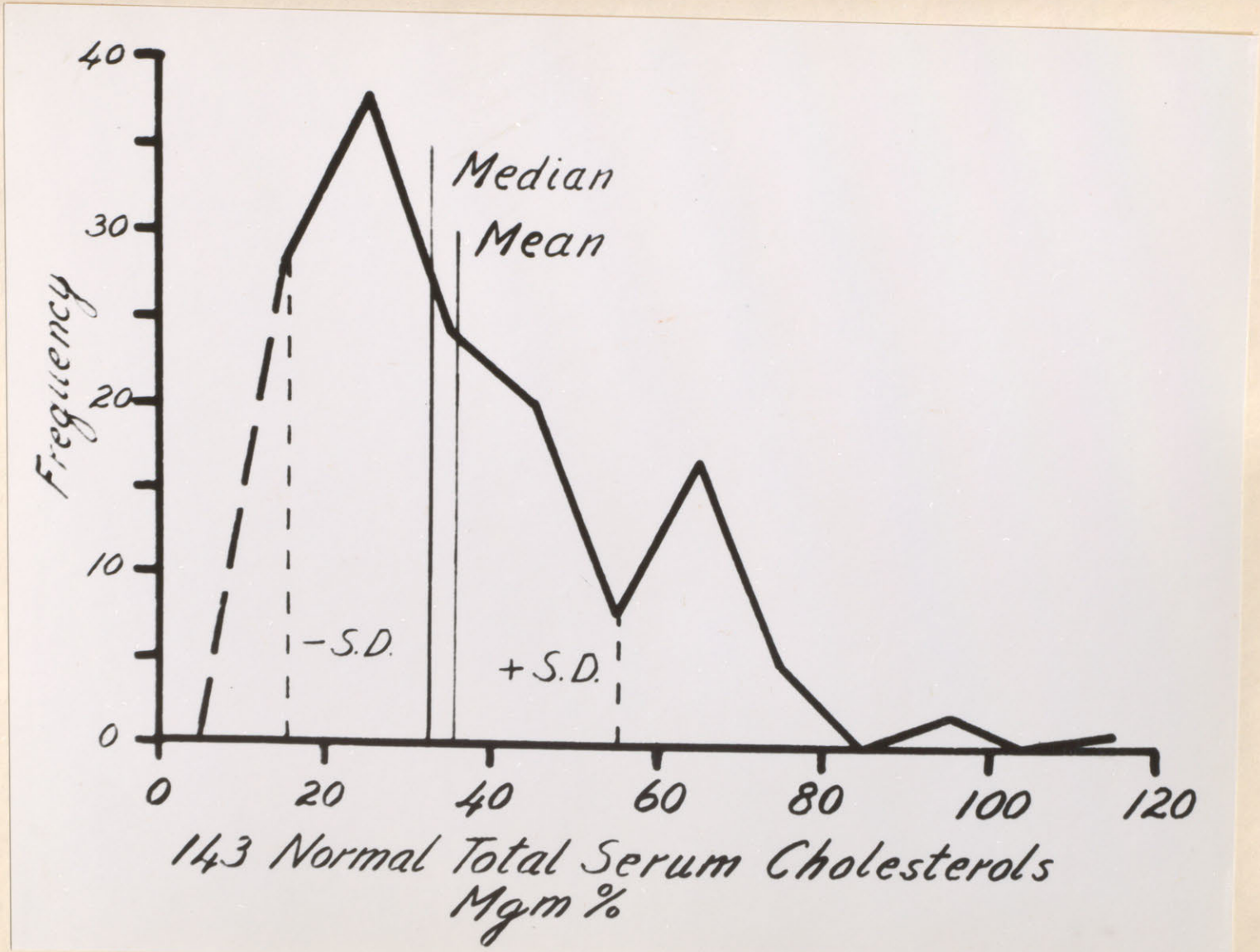
Frequency distribution of 143 normal free serum cholesterol levels at a class interval of 5 mgms.%. Based on 40 normal animals.


60
50
40
30
20
10
0
Frequency
Mean
Median
-S.D.
+S.D.
0
5
10
15
20
25
30
35
143 Normal Free Serum Cholesterols
Mgm %

4.

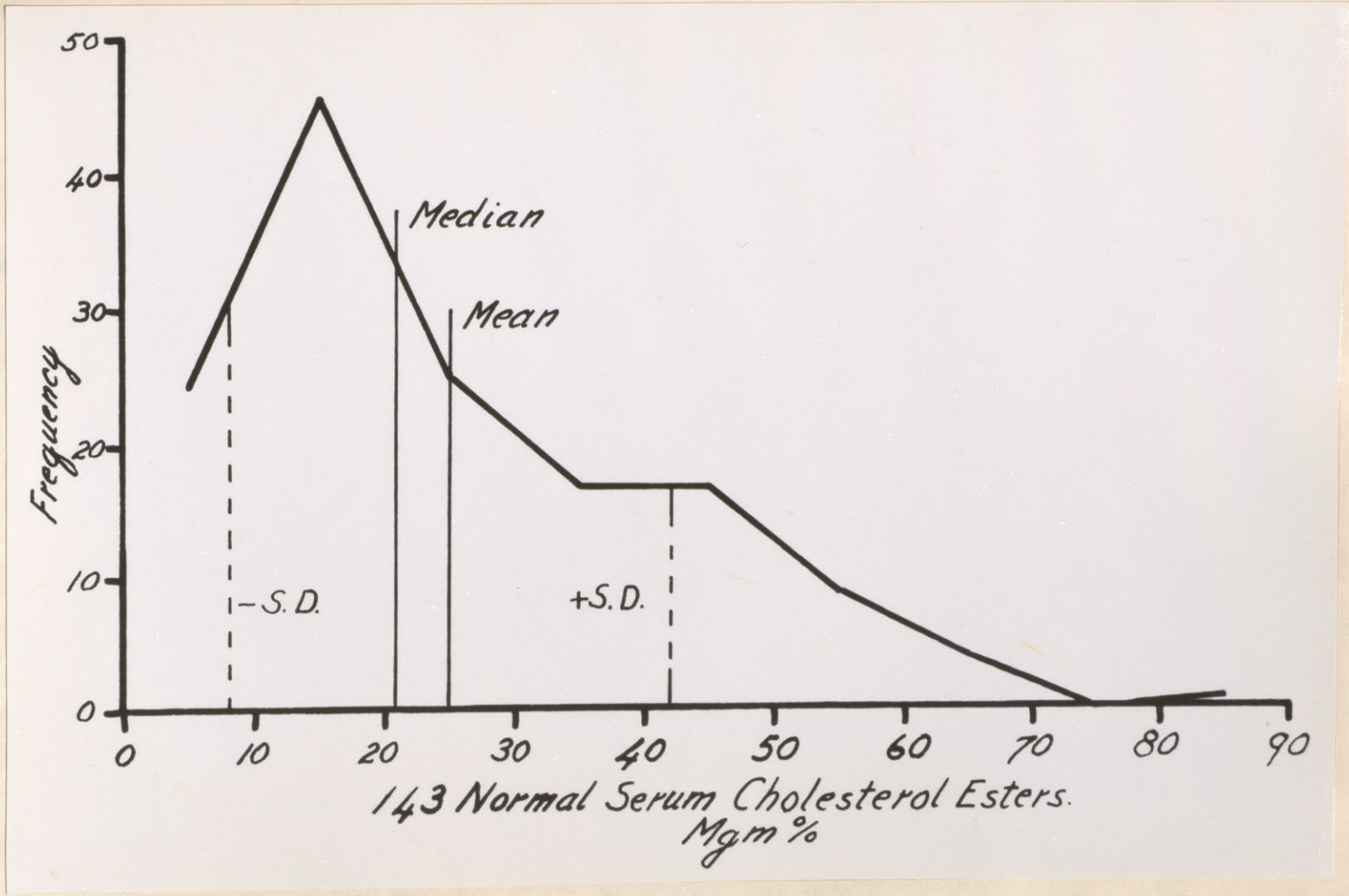
GRAPH #5

Frequency distribution of 143 normal serum total cholesterol levels at a class interval of 10 mgms.%. Based on 40 normal animals.


40
30
20
10
0
Frequency
Median
Mean
-S.D.
+S.D.
0
20
40
60
80
100
120
143 Normal Total Serum Cholesterols
Mgm %

GRAPH #6

Frequency distribution of 143 normal serum ester cholesterol levels at a class interval of 10 mgms.%. Based on 40 normal animals.


50
40
30
20
10
0
Frequency
Median
Mean
-S.D.
+S.D.
0
10
20
30
40
50
60
70
80
90
143 Normal Serum Cholesterol Esters.
Mgm %

GRAPH #7

Frequency distribution of 143 normal serum ester cholesterol per cent of total cholesterol values at a class interval of 10%. Based on 40 normal animals.

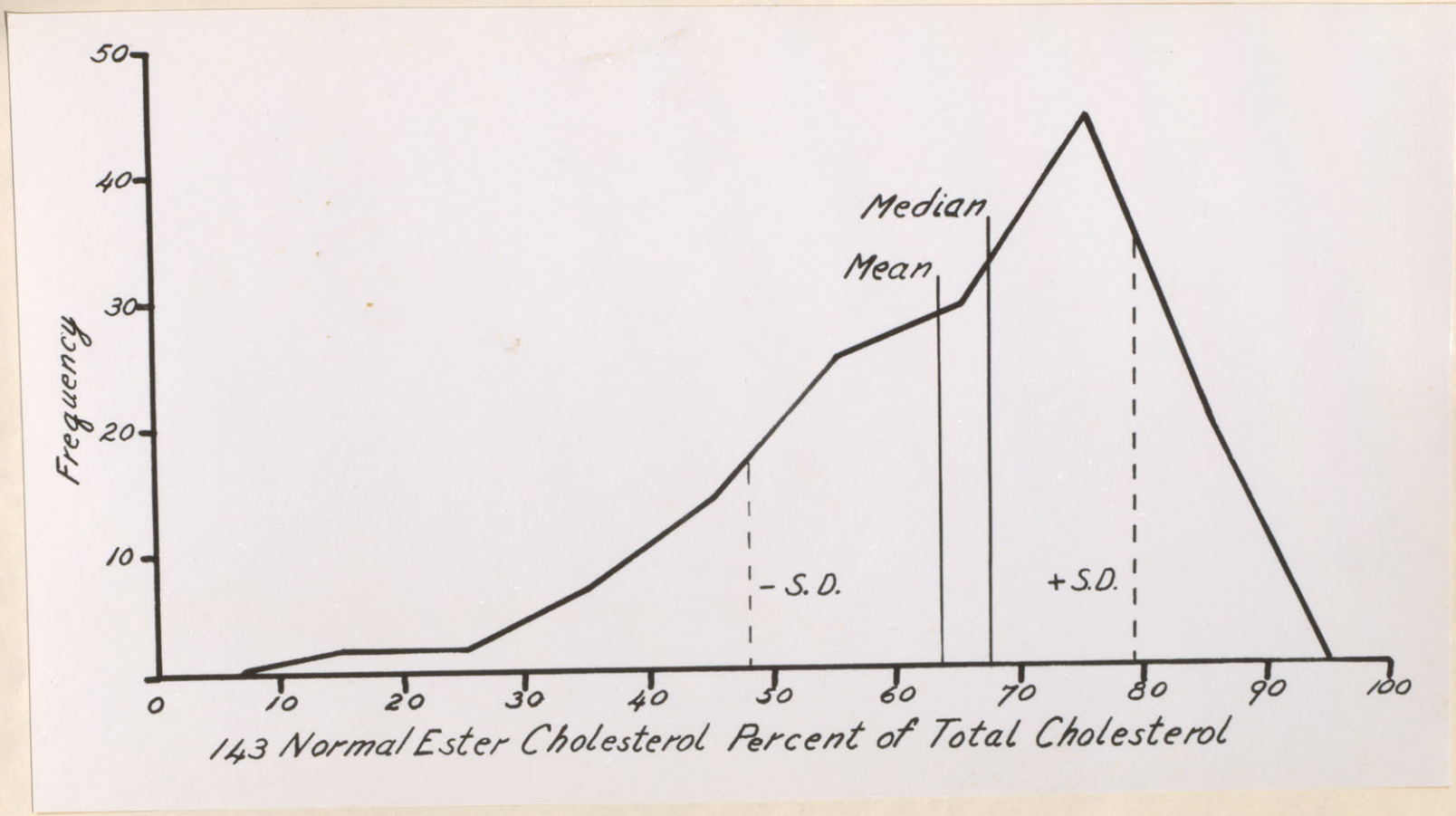
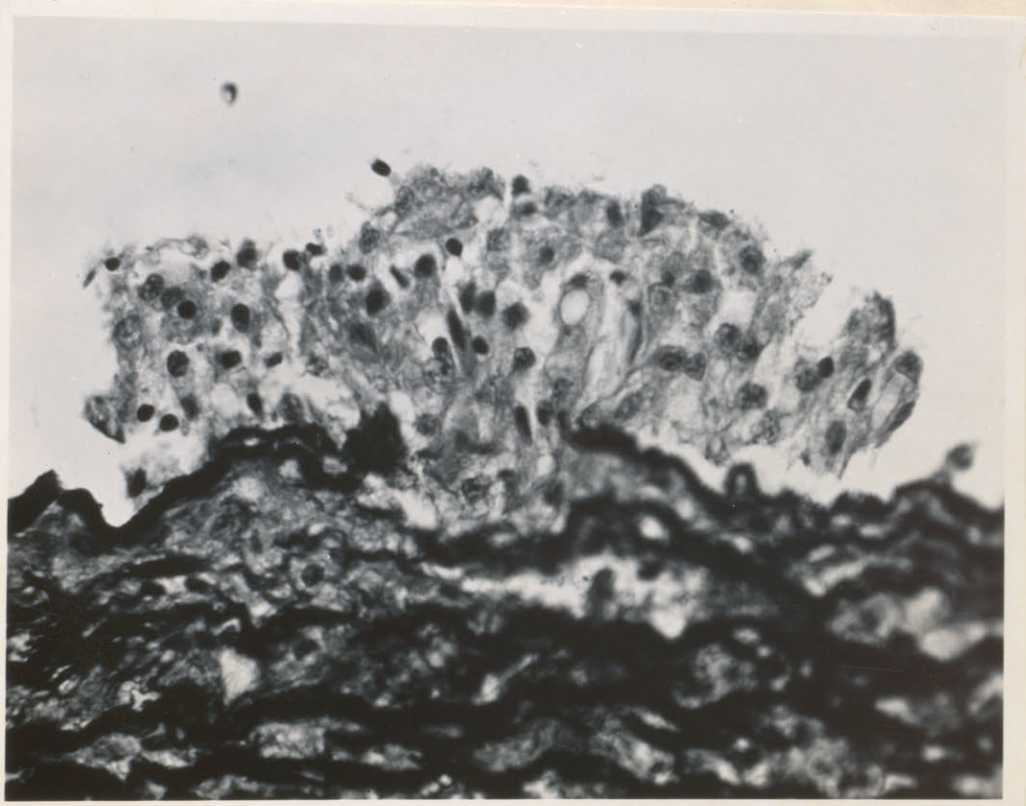

50
40
30
20
10
Frequency
Median
Mean
- S.D.
+ S.D.
0
10
20
30
40
50
60
70
80
90
100
143 Normal Ester Cholesterol Percent of Total Cholesterol

FIGURE I

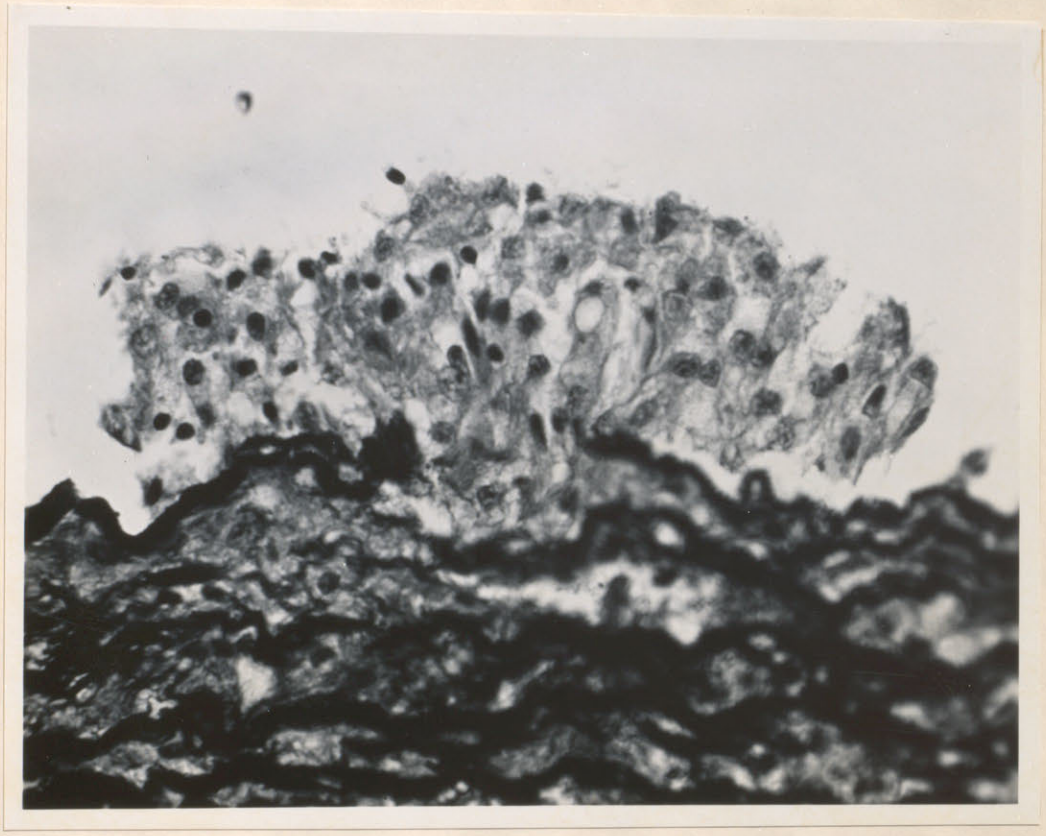
Photomicrograph of aortic atherosclerotic lesion in animal #26. Note the defect in the intimal elastic lamina and the presence of foam cells in the superficial part of the media. Verhoeff elastic tissue - haematoxylin van Gieson stain. X 590



I

FIGURE I

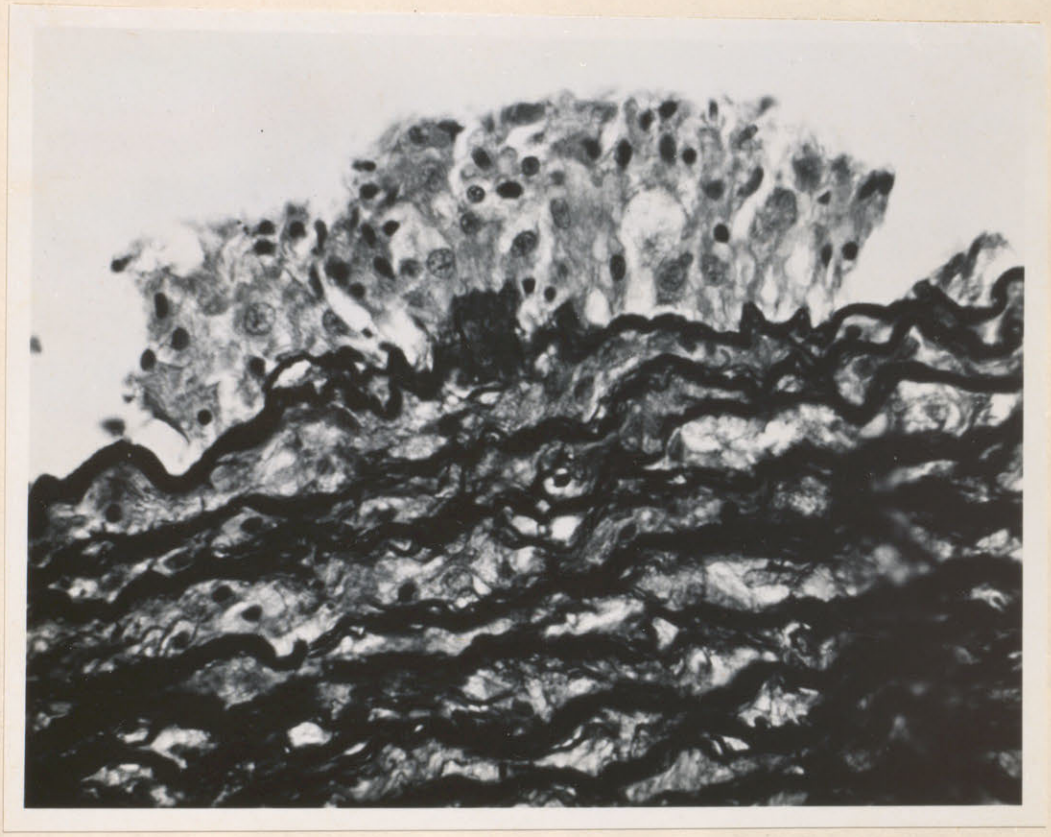
Photomicrograph of aortic atherosclerotic lesion in animal #26. Note the defect in the intimal elastic lamina and the presence of foam cells in the superficial part of the media. Verhoeff elastic tissue - haematoxylin van Gieson stain. X 590



I

FIGURE II

The same lesion that was seen in Figure I is seen in a closely adjacent section. Note the deformity of the intimal elastic lamina at the point where a defect was seen in Figure I. Verhoeff elastic tissue - haematoxylin van Gieson stain. X 535



II

FIGURE III

Photomicrograph of the pancreas of animal #35. Marked hydropic changes are seen in the tubular system. One mildly hydropic and atrophic islet is seen. Gmori granule stain. X 133

III

FIGURE IV

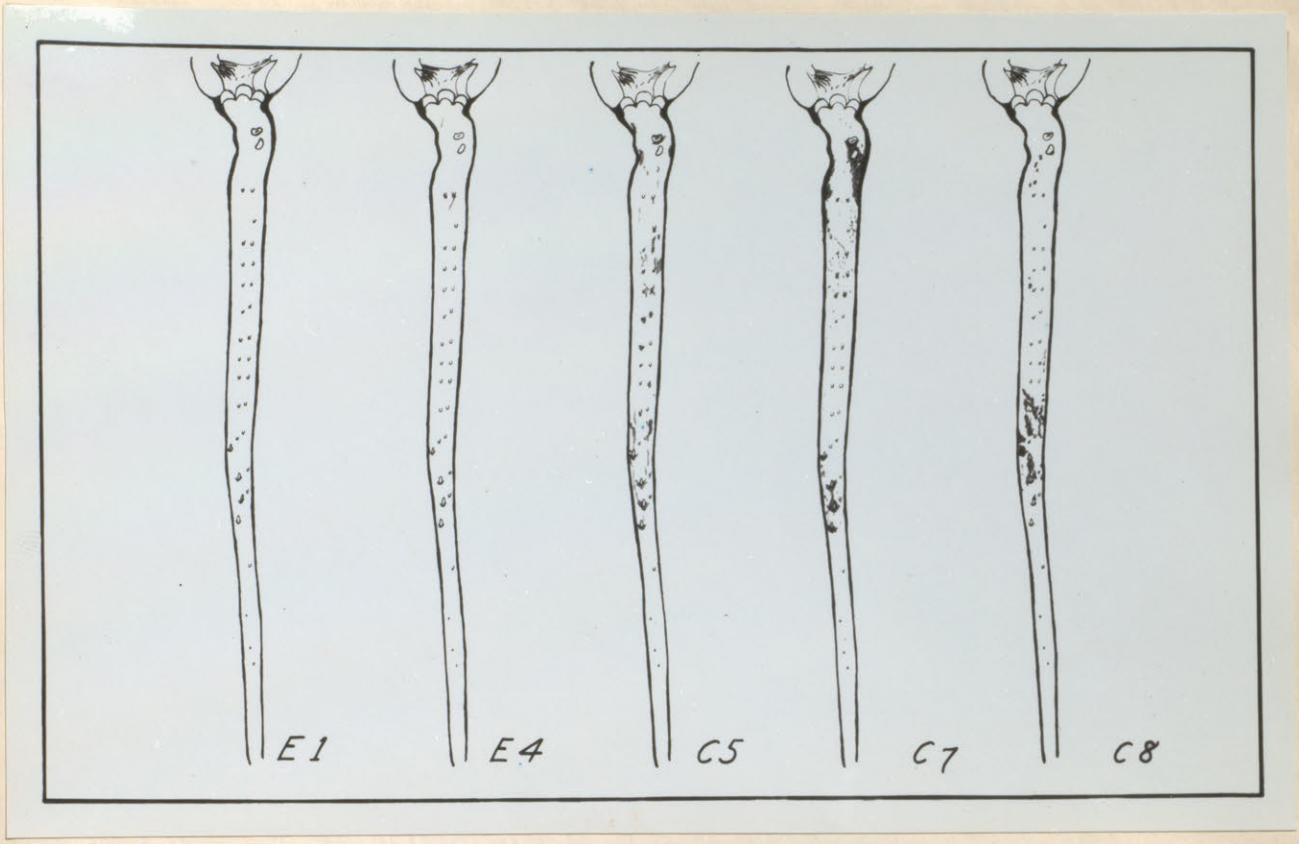
Photomicrograph of a medium size duct from the pancreas of animal #35. Note the branch tubule which shows marked hydropic changes. The hydropic degeneration stops quite abruptly at the point of origin of the tubule from the duct. Gmori granule stain. X 300

FIGURE V

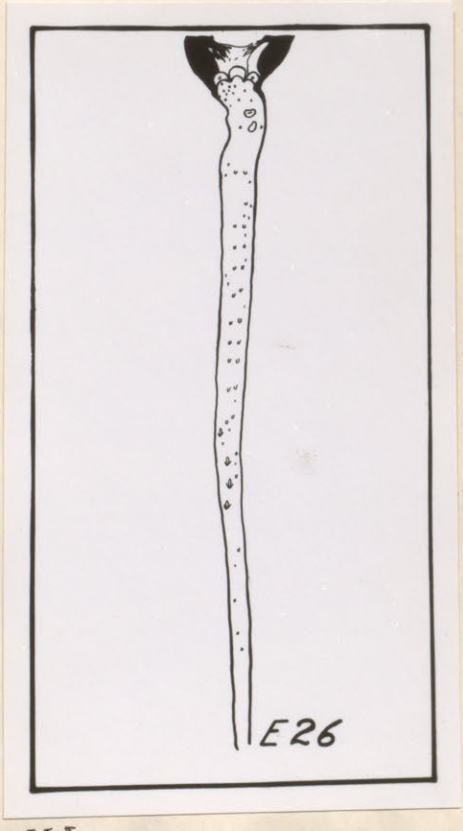
Standardized tracing of the degree and distribution of cholesterol atherosclerosis in the aortae of 5 animals fed pure cholesterol. E indicates a diabetic animal, C indicates a non-diabetic one.

FIGURE VI

Standardized tracing of the degree and distribution of atherosclerosis in an animal that suffered a severe hypercholesterinemia after an injection of alloxan. No cholesterol was fed to this animal. See Figures I and II.



V



VI