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

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PREFACE

The experiment reported here was conceived and directed by Professor G. Lyman Duff in the Department of Pathology, McGill University, Montreal.

Technical assistance in routine chemical determinations was afforded by Misses C. McGuire, L. Dickie and S. Jaques. The preparation of histologic material was performed by the technical staff under the direction of Mr. H. Nye. The photographic material was done by Mr. H. Coletta.

Particular assistance was given by Dr. K. Evelyn in the consideration of the experimental data, and by Dr. D. C. Wilson in matters relating to the experimental management of alloxan diabetes.

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INTRODUCTION

Any study which purports to investigate the pathogenesis of arteriosclerosis must be considered of interest to both pure and applied science. There are few fields of biological research which touch upon so many fundamental problems as does this. It matters little whether one is concerned with the interfacial behavior of films, with the metabolism of lipids, with the behavior of extracellular protein, colloids and gels, or with the problem of cellular movement and irritability, for all these and many more may find some application in the study of this disease. And similarly, if one is more immediately concerned with the understanding and prevention or amelioration of human disease, there are few diseases that are of more pressing social and economic importance at the present time. Arteriosclerosis and its accepted sequelae have come to constitute the major cause of death in the more civilized parts of the world. It is a disease whose lethal potentialities have been exposed by the social and medical progress of the last 50 years. It is a disease of the world's ageing population.

However much is known about arteriosclerosis in its various aspects, one cannot but realize that it remains a mystery. There is little understanding of its fundamental properties, and nothing is known concerning its prevention or cure in man.

Many experimental studies of the disease have been undertaken in the past - studies which provide a vast and confusing mass of information and little understanding. The present experiment was based upon the clinical observation that patients suffering from diabetes mellitus also suffered from a greater degree and an earlier incidence of arteriosclerosis and its accepted sequelae than did non-diabetic persons. It is apparent that a sufficient study of diabetes and of

arteriosclerosis in experimental animals might be expected to elucidate the association observed between the two diseases in man.

While the experimental concept is a simple one, it has proven to be technically impossible to accomplish in the past. There has existed for many years a simple method of causing suitable arteriosclerosis in one type of laboratory animal only, i.e. the rabbit. Unfortunately, there was no suitable method of rendering rabbits diabetic. Recently, a chemical technique of causing diabetes mellitus in rabbits has been discovered, and an experiment designed to study the effects of diabetes on arteriosclerosis has become possible of realization.

Such an experimental study is reported here. The results obtained lead to the unexpected conclusion that diabetes induced by alloxan has an inhibitory effect on the development of experimental cholesterol arteriosclerosis in the rabbit. Such a conclusion is, of course, diametrically opposed to the observations made in man, and hence to the conclusions which formed the hypothesis on which the experimental study was based.

There can be little reasonable doubt that both the experimental and the human observations and conclusions are valid. The apparent conflict between them requires explanation or at least delineation. It may be that the conflict is more apparent than real, or it may be that a valid difference exists between the behavior of the human and of the experimental animal.

In any case, it will be necessary to consider the experimental postulates as well as the observations based upon human disease at some length if one hopes to relate the experimental result to arteriosclerosis in man. From the data reported here it is apparent that no such relationship can be drawn, and one is forced to add the experimental result to the existing body of information concerning arteriosclerosis as an unexplained phenomenon. On the other hand, consideration of the experimental result as an isolated finding proves to be an interesting and instructive exercise in pure science.

PART I

THE DEFINITION OF ARTERIOSCLEROSIS

The word "arteriosclerosis" was coined by Jean Frederic Martin Lobstein (1829-1833). It was understood to signify those conditions in which the arteries of the body became abnormally hard. To-day, the term has much the same connotation and little progress has been made in rendering its definition more precise or accurate. Indeed, the exact connotation and denotation of the word "arteriosclerosis" are most difficult to state in any concise manner.

The medical literature contains many attempts to define this word, but one is invariably impressed with each author's failure to achieve a useful definition. In 1933 Ludwig Aschoff[1] stated: "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 ageing with resulting deformation of the lumen and brittleness of the vascular walls". Bell[2] offers the following definition by exclusion: "All forms of arterial disease except those that are frankly inflammatory in character are commonly called arteriosclerosis". Moschcowitz[3] 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". Winternitz[4] mentions the "constellation of processes" that is represented by the term "arteriosclerosis". Hueper[5] refers to "the degenerative and sclerosing arterial diseases known under the collective term of arteriosclerosis". And Leary[6] speaks of the "non-infectious chronic diseases of the arterial system".

Examples of the failure to define arteriosclerosis adequately may be multiplied endlessly. It is apparent that our ignorance concerning its nature is

so great, and our knowledge of it is so diffuse that no satisfactory definition is possible. One can only wish that when Page[7] wrote: "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", he had also mentioned its definition.

Boyd[8], in his teaching, refuses to recognize the "omnibus" term arteriosclerosis, and substitutes for it the three arterial diseases, i.e. atherosclerosis, Monckeberg's medial sclerosis, and diffuse hyperplastic arteriolosclerosis, that are most commonly included under the term. The term "atherosclerosis" was coined by Felix Marchand (1846-1928) and it is that subdivision of arteriosclerosis which is of particular and vital interest to us here. We shall, therefore, undertake a morphological description or "definition in extenso" of this term as it is understood from both the human, and experimental aspects. At the same time, however, we must bear in mind that we cannot avoid some intercourse with the arteriopathies known as Monckeberg's medial sclerosis and diffuse hyperplastic arteriolosclerosis. Because we choose to concentrate on the atherosclerotic aspect of arteriosclerosis for reasons which will become obvious, we do not thereby necessarily exclude any other type of arteriosclerosis from similar consideration. Indeed, workers such as Aschoff[15] and Efskind[16] find no fundamental differences between atherosclerosis and arteriosclerosis.

THE MORPHOLOGY OF ATHEROSCLEROSIS IN MAN[27]

The earliest lesions of atherosclerosis that have been described in man are found in the aortae of children. These lesions consist of small aortic intimal elevations and thickenings of a fatty nature. They are somewhat yellow in color, round or oval in shape and contained within the intima. They may become quite elongate, enlarged, arranged in rows or confluent. The general orientation is parallel with the length of the aorta. They may be located throughout the entire

length of the aorta but are 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. In these locations their orientation tends to be transverse to the long axis of the aorta. Such fatty flecks and streaks are also common in the posterior wall of the thoracic aorta where they occur as longitudinal streaks that are not connected with the mouths of the intercostal arteries. They are less common in the abdominal aorta and in the major musculo-elastic arteries.

Microscopically, such lesions show a mucoid or oedematous swelling with separation of the intimal constituents and an increase in the amount of basophilic ground substance present. This particular change has been postulated by some workers as the earliest demonstrable pathological finding, but it is by no means certain whether it is a primary or a secondary phenomenon. There is also found an increased number of cells within the intima. These cells are both fibrocytic and histiocytic in nature and have a high content of finely divided lipid material, rich in cholesterol, within them. At the same time similar extracellular lipid is found in the interstitial ground substance and in relation to elastic and collagen fibres.

It is of some importance to demonstrate whether the lipid found in these lesions occurs in an extracellular location before it may be seen within cells, or vice versa. There is no satisfactory evidence on this point, but it is my personal impression that extracellular lipid deposition may be found in the absence of intracellular fats. In any case, as the lesion progresses, a few lymphocytes may be found in the area and there occurs a slight proliferation of fibrous connective tissue.

It is generally accepted that the lesion described above is the precursor of atherosclerosis of adult type. However, various opinions have been expressed: Zinserling[9] maintains that the lesions are irreversible and progress to frank atherosclerosis. Virchow, and more recently Sanders[10] believed that

these lesions bore no actual or causal relationship to arteriosclerosis. Klotz and Manning[11], Leary[12] and many others take the intermediate view that some of these fatty lesions heal with restitution, while others, particularly in adolescence and the later age periods, progress to constitute well developed atherosclerosis. Inasmuch as the smallest lesions of adult atherosclerosis are indistinguishable from the larger lesions of childhood atherosclerosis, and inasmuch as there is an even and uniform morphological gradation from less severe to more severe lesions, one has no choice but to accept that there is no essential morphologic basis for not considering the two together. Nevertheless, we are not thereby permitted to postulate the pathogenesis of any given atheromatous lesion.

The gross appearance of adult atherosclerosis presents both quantitative and qualitative differences from that seen in the child. The lesions are larger, more confluent and of wider distribution. The most severe lesions are found in the abdominal aorta and about the mouths of vessels arising from the posterior aspect of the organ. The entire intima may be affected by atheromatous lesions. Similar lesions may be found, not only in musculo-elastic arteries, but also muscular arteries and even veins. The lesion shows two features, the one degenerative and the other reparative. Degeneration is exemplified by the accumulation of more and more lipid, both intracellular and extracellular in location. There is necrosis of cellular elements and products[15] with the formation of a yellow, lipoid-rich core or pool of debris. Such an area of necrosis may extend from the intima through the greater part of the media. Around this pultaceous centre are many fibrocytic and histiocytic cells rich in lipid, and lymphocytes and small round cells are common. The reparative reaction consists of the formation of an enveloping coat of fibrous tissue which often contains newly formed elastic fibrils. This fibrotic reaction is most marked on the intimal aspect of the lesions and confers upon it a pearly-grey color. Calcification of bony nature[17] may occur in relation to the lipid and collagenous material. There may be a partial reduct-

ion in the fat and sterol content of the lesion with replacement by fibrous tissue. Contrarily, the lesion may progress with destruction of intima or media, leading to ulceration of the endothelium and aneurysm of the vascular wall. There is an associated loss of elasticity of the aorta which Wilens[13] finds to be due in part to the restrictive action of the intimal plaques. However, this loss is also a function of age[13], for while it has been shown that the amount of elastic tissue remains nearly constant throughout life[14], it suffers a decreased elasticity with increasing age, both in the intact aorta[13] and as an isolated tissue[14]. Excess vascularity[4] and even frank haemorrhage are also frequent findings in areas of atheromatous change.

The age changes in the elastic tissue of the aorta are not the only changes of this nature occurring in aortas that also show atheromata. While there is a degree of calcification associated with advanced atheromatous lesions that may be extreme, there is also a lesser degree of medial calcification that appears to depend upon age. Blumenthal et al[18] have shown that calcification of the media is a function of age; that it precedes intimal plaque formation; that it is more intense in the vicinity of intimal plaques and in the abdominal portion of the aorta; and that intimal plaques do not occur in its absence unless some other medial disease such as syphilitic aortitis is also present. It is also indicated that the protein and colloid constituents of the aorta "age" and alter with the passage of time, and that such changes may be independent of the formation of atheromata. Lastly, a certain degree of medial fibrosis and intimal thickening by collagenous and elastic fibril formation appears to occur with age independently of atheromatous alteration[19].

The source of the cellular elements in atheromata is not agreed upon. It would appear that the fibrocytic elements arise in situ, but it cannot be denied that some of them may be derived from histiocytes or from endothelial cells. Leary[20,21,22] maintains that the lipid filled histiocytes are derived from the

reticulo-endothelial system and, distended with lipids, enter the affected area from the aortic blood stream. Once in the intima they discharge or transfer part of their lipid content. Others believe that they are merely phagocytes attracted to an area where cell debris and extracellular, particulate lipids and sterols are present. Altschul[23] suggests that both the fibrocytic and the histiocytic elements which contain lipid are derived from the vascular endothelium. On the other hand Efskind[16] finds that while the vascular epithelium is histologically different at the sites of predilection of atherosclerosis, that such differences as exist are secondary to other intimal changes. He does not relate the epithelial cells to the other cellular elements found in atheromata.

The lesion of human atherosclerosis may be described as a patchy, progressive, fatty, necrotic, reactive, proliferative, sclerotic and deforming lesion which is the pathogenetic derivative of the fatty flecks and streaks found in the aortae of children. It is a lesion that is not uncommon in childhood, is common in early adult life, and is almost invariably present in persons who are more than 50 years of age[24,25,26]. The earliest histological changes in such lesions are debatable, as is the derivation of some of the cytological elements that are present. Nevertheless, the main morphological changes which characterize atherosclerosis are well known, and will serve as a satisfactory definition in extenso.

THE MORPHOLOGY OF EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS IN THE RABBIT

Before considering the etiology and theories of causation of arteriosclerosis, it is advisable to consider the morphology of experimental cholesterol arteriosclerosis in the rabbit. The results obtained from feeding cholesterol to rabbits have had so profound an influence on modern conceptions of human arteriosclerosis that it is idle to consider any etiological or theoretical discussion of the human disease without first considering the experimental lesion.

The following description is taken largely from the works of Anitschkow[28,29] Leary[30] and Duff[31,32].

The gross lesions appear first as minute, slightly raised, round or oval, yellow-white specks that shine through the intimal surface of the aorta. The first area to be affected is just distal to the aortic ring and about the vessels arising from the arch. The lesions increase in size, become more sharply demarcated and circumscribed, and may attain a size of 1 or 2 millimetres in diameter. At the same time, new lesions appear in the thoracic aorta and show some tendency to localize on the posterior wall about and between the ostia of the intercostal arteries. Such localization, however, is not strictly maintained. As the lesions progress there is a tendency to confluence so that large plaques and longitudinally orientated streaks develop. The intima becomes thick, rough and nodular, and lesions extend to below the level of the renal arteries or even towards the bifurcation of the aorta. The distal lesions, however, are always less severe than the proximal ones. In advanced conditions the vessel may be subject to irregular dilatation and even aneurysm formation.

If the experimental procedure is arrested but the animal is allowed to live for periods of up to about 3 years, there occurs a partial resorption of the atheromatous areas, fibrous encapsulation, calcification and loss of elasticity.

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, especially cholesterol, are deposited in the affected area and are found in lipid filled histiocytes. The focus of reaction now constitutes an elevated plaque consisting of swollen ground substance, extra- and intracellular lipids and stellate cells of fibrocytic type which are also heavy laden with fat. As the lesion progresses, the foam cells of the deepest layers next to the internal elastic lamina enlarge in size and eventually undergo necrosis, release their lipid content and constitute an atheromatous "abscess". The superficial layers often show a multiplication of fibrocytic cells, several rows thick, which provide

a superficial encapsulation. Such fibrocytic zones, however, contain little collagen unless the lesion is allowed to resorb. The elastic lamina may also show some degenerative changes and fine fibrillary elastic fibres may be formed. The underlying media may show invasion by extracellular lipid and fat-containing foamy phagocytes, together with degenerative changes. A further medial lesion consisting of a focal necrosis of the inner third of the media with a small amount of extracellular lipid may be seen occasionally. Such medial lesions may be quite independent of any intimal alteration. All lesions except those of the earliest form contain some cells of the lymphocytic type. The whole process may be so marked as to constitute an intimal atheromatosis of confluent type that averages one or even two times the thickness of the underlying media (in the absence of any thinning of this media). Excess vascularity is not a prominant feature.

If the lesions are allowed to regress, there occurs a partial resorption of the extracellular and intracellular lipid content of the area. In small lesions this resorption may be complete, but in larger ones an atheromatous core of pultaceous and crystalline lipid and cholesterol material remains surrounded by some mononuclear cells and encapsulated by fibrous tissue. Calcification and the formation of elastic fibrils are common occurrences. Such regressing lesions show a greater tendency to affect the media than do those previously described.

The changes described above are not confined to the aorta, but are found in musculo-elastic and muscular arteries generally. They occur regularly in the coronary arteries, heart valves and pulmonary arteries. The intrarenal arteries and the cerebral arteries, however, are not affected.

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.

Duff[31,32] has made the most rigorous study of this aspect of the subject He finds, in contra-distinction to Anitschkow[29] and to Leary[21,22], 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 vessels[59] are exempt except under special circumstances[60] and the retinal arteries are also 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 hypertension 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 degre

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)[29] and who maintain that an absolute identity between the natural and experimental lesions is too exacting a requirement (Leary)[22]. Others[32] 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.

THE ETIOLOGY AND NATURE OF CHOLESTEROL ARTERIOSCLEROSIS

The discovery that a diet which contained a high content of cholesterol would cause deposition of cholesterol in various organs of the rabbit has led to many studies involving the experimental use of this substance. It has been found[2 that if cholesterol is fed in doses of about 0.5 grams to rabbits for about 90 days there results a deposition of cholesterol and other lipids in the cells of the reticulo-endothelial system, the liver, the adrenal and other organs, in the intima and media of the aorta and certain arteries, and in collagen tissue and fibrocytes. These alterations are accompanied by a hypercholesteraemia and a lipaemia.

It has been found that, generally speaking, the degree of cholesterol deposition which occurs depends on the amount and period of cholesterol feeding,

the degree of hypercholesteraemia attained, the nature of the medium in which the cholesterol is administered and the physical constitution of the rabbit subjected to the feeding procedure[5,29,33,35,36].

The rabbit is subject to a type of spontaneous medial arteriosclerosis which is in no way comparable to that which accompanies cholesterol feeding[34]. This lesion, consisting of a medial degeneration with fibrosis and calcification may contain microscopic amounts of fat, but is almost fat free in comparison to the lesions of atherosclerosis. The occurrence of this lesion, more comparable to the experimental lesions induced by substances affecting calcium metabolism[37,38], has been used by various authors as an invalid objection to the use of the rabbit as an experimental animal for the study of atherosclerosis. On the other hand, rabbits are rarely subject to spontaneous atherosclerosis. The literature contains very few reports of such lesions[39,40],[135] and our own experience has shown that spontaneous atherosclerosis of the most minimal degree is a very infrequent occurrence in otherwise normal animals.

It is the experience of many workers[41,31], and our own study confirms them, that minute doses of cholesterol which are insufficient to disturb the normal levels of cholesterol in the blood will not cause vascular atherosis. Similarly, large doses fed for the relatively short period of 1 or 2 weeks are also ineffective although a slight hypercholesteraemia may be induced. It has also been demonstrated that relatively small doses of cholesterol-containing substances which cause a mild degree of hypercholesteraemia may cause vascular atherosis after prolonged periods, in the absence of deposition of lipid substances in the parenchymatous organs[5].

While it has not been positively proven, all available evidence indicates that the production of atherosclerosis in the rabbit depends upon an abnormally elevated blood cholesterol level and a period of time which, although it varies inversely with the degree of hypercholesteraemia attained, is probably never less

than 40 days. The minimum effective degree of hypercholesteraemia is not accurately established. Certain experiments of Dr. D. C. Wilson[42] in this department as well as the present experiment suggest that levels of about 150 to 250 mgm.% are effective if sufficiently prolonged in time.

The hypercholesteraemia attained by feeding cholesterol is relatively constant in nature. There is no consistent or important alteration of the ratio of total to free cholesterol regardless of the level to which the cholesterol content of the blood may rise. At the same time there is an increase in neutral fats in the blood which is irregular, but roughly proportional to that of cholesterol. Lecithin increases in proportion to the increase in neutral fats[33,35,43]. The other phosphatides, cephalin and sphingomyelin, in man at least[43], are independent of hyperlipaemic conditions which involve neutral fats, lecithin and cholesterol. So far as I am aware no studies of the serum esterases such as monobutyrin or tributyrin, or of serum lipase, phosphatase or amylase have been undertaken in the cholesterol-fed rabbit.

The physico-chemical states of cholesterol in the blood have been studied by various authors in both man, the rabbit, and other animals. The available data has been reviewed by such authors as Bills[44], Thannhauser[43], Hueper[5], and Hober[45]. The group working with Schonheimer[46] has shown that cholesterol is absorbed from the intestinal tract and that it is also formed by the organism from short-chain carbon compounds. The closely related phytosterols, except ergosterol, however, are not absorbed from the intestinal tract. Alimentary absorption of cholesterol depends upon the presence of fats[36], fatty acids and the emulsifying action of bile salts - espedially cholic or glycocholic acids[47]. The absorbed fats and cholesterol pass from the intestine to the thoracic duct, while a small portion passes directly to the liver. Cholesterol appears to be absorbed in both free and esterified forms, appearing in the lymph in the proportions of two parts esterified and one part free cholesterol.

Although cholesterol is rather freely converted from free to esterified forms by the action of cholesterol esterase which occurs in the liver, duodenum, and other organs, and perhaps in the serum and reticulo-endothelial cells, the body is known to effect few other changes[43,44]. The relatively constant ratio of ester and free cholesterol which is found in the blood probably depends on a cholesterol esterase equilibrium system which is controlled chiefly by the liver. Small amounts of dehydrocholesterol are found in the tissues, so that small amounts of cholesterol appear to be reduced at the 5, 6 double bond in the process of intermediary metabolism. The isomer of dehydrocholesterol, coprosterol, occurs in the intestinal tract only, and is not found in the tissues. Similarly, allocholesterol, the unsaturated stereo-isomeric sterol of coprosterol, is not present in the tissues. Coprosterol evidently originates in the intestine through the action of bacteria which may be able to change the steric configuration on atom 5. In this process cholestenone is also formed from cholesterol. It, too, has not been found in the body, so that the transformation of the cholesterol series to the coprosterol series appears to occur only outside of the body. The bile acids have the same steric configuration as coprosterol and are synthesized in the liver. Injection of allocholesterol or of coprosterol results in an increased excretion of bile acids. On the other hand, administration of "marked" cholesterol does not result in the presence of the tracer substance in the bile acids. The bile acids and their salts are surface active agents, and, by aiding the emulsification of cholesterol in fat, they enhance the rate of its alimentary absorption.

The fact that the nucleus of the sex hormones is similar to that of cholesterol has led to the supposition that they might be formed from cholesterol. It has been shown that pregnandiol can be formed in this way[48], but it appears that the great bulk of the physiologically active and inactive sterols which occur in the body are formed independently of cholesterol. Vitamin D, because of its structural characteristics has been similarly considered. It would appear, however

that it arises from the absorption and subsequent irradiation of ergosterol alone, and not from cholesterol.

Cholesterol is not only absorbed from the alimentary tract, but it is also excreted by the same path. The main routes of excretion are through the intestinal wall and in the bile fluid. Minor amounts are excreted by the desquamation of cells, in the sebum, milk and urine(5). The ability of the intestine to excrete cholesterol is not the same in all animals. The herbivorous animals excrete it less rapidly than the carnivorous ones. It may be that the experimental effects obtained by feeding cholesterol to such animals as rabbits and chickens depend in large part on their relative inability to excrete excess cholesterol through the intestine.

The role of cholesterol in metabolism is quite unknown. Because it occurs throughout the animal and in most of the plant kingdoms, it has been considered as an essential substance. Either directly or indirectly it plays some role in fat transportation by virtue of its ability to form esters with such fatty acids as stearic, oleic and palmitic acid. Thannhauser suggests that the correct mixture of lipids in a cell depends upon cholesterol, and in particular, upon its properties as a hydrophobic colloid.

The physico-chemical properties of cholesterol have been investigated in an irregular fashion(5). Both free and esterified cholesterol are anisotropic. It has the formula $C_{27}H_{46}O$. The molecular weight is 386.36. It crystallizes from alcohol with IH_2O. The crystals are white, unctuous, pearly leaflets. The specific gravity is 1.067 and it melts at 148-149°C. when anhydrous. The rotation is $(a)_D^{15}$ - 31° in 2% ether solution. As a hydrophobic colloid, it is held in colloidal suspension by hydrophilic colloids such as lecithin or protein. Cholestol plasma protein addition products occur to such an extent that 70% of the plasma cholesterol can be precipitated with the protein when the latter are salted out. Membranes which do not allow the passage of proteins also are impermeable to cholesterol in the biological fluid tested. Apparently cholesterol is associated with protein

by means of secondary valences, by opposite electrical charges holding protein and lipid aggregates together, and by simple entanglement in the protein micelle. In its dispersed state it possesses both oxidase and reducing activity. It forms interfacial films, is an electric insulator and hinders interfacial exchange or membrane permeability. Hueper[72] has shown that it reduces the speed of oxygenation of erythrocytes in hypercholesteraemic rabbits.

The stability of the colloid cholesterol solution is, as might be expected, influenced by factors which affect the hydration, dispersion and stability of the hydrophilic colloids such as albumin. This, for example, might explain the action of such ions of the Hofmeister series as iodine and thiocyanate upon the effects of hypercholesteraemia. The SS and SH groups in the protein decrease its precipitability while the NH_2 groups have an opposite effect. Loeper and Parrod[69] claim that sulphur maintains cholesterol in solution. The addition of a surface active agent such as bile salts increases its dispersion. Vitamin C has surface active properties in cholesterol-lecithin mixtures but not in water alone[70]. The addition of dextrose, galactosides, of cholesterol or other hydrophobic colloids to cholesterol sols increases the instability of the sol and causes the aggregation of cholesterol into larger particles. This latter reaction is said to be responsible for the opalescence of hypercholesteremic plasma.

While much further work needs to be done in order to gain an understanding of the physico-chemical properties of cholesterol and the relation of these properties to the lesions of experimental cholesterol atherosclerosis and human atherosclerosis, it is apparent that its behavior as a hydrophobic colloid and as a substance which forms interfacial films, may be important to the development of such lesions.

A further and very important finding in experimental cholesterol atherosclerosis depends upon the "susceptibility" of the test animal to the experimental procedure. Of all the various species of animals subjected to cholesterol feeding, only two, the rabbit[30,31] and the chicken, have been found subject to the

induction of hypercholesteraemia and atherosclerosis. The rabbit is a vegetarian animal while the chicken is an omnivore. Carnivores are immune to the effects of cholesterol feeding unless it is combined with some other experimental procedure such as thyroidectomy[52]. A hypercholesteraemia, however, may be elicited in dogs, rats, man and monkeys by the use of protein deficiency combined with cholesterol feeding[53] or by combined cholesterol-lecithin feeding in the form of egg yolk[54,55] or by lecithin and fat diets[68]. The reason for this species variability (or constancy) in relation to cholesterol feeding is not apparent. It might be due to a failure of absorption in those animals that are insusceptible, but available data do not indicate that this is the case. Rather it would appear that it is due to an inability of the susceptible animals to excrete cholesterol from the intestine. It might also be due to the fact that the insusceptible animals can catabolize the absorbed cholesterol. There is no evidence to support this view. The fact that those animals which are immune to the induction of a persistent hypercholesteraemia and atherosclerosis do not escape some effects of cholesterol feeding is important. It has been shown for example that rats[54,57], guinea-pigs[56] and monkeys[32] may acquire deposits of anisotropic lipid in such organs as the liver and spleen during the course of cholesterol feeding. Nevertheless, they are, with the possible exception of the guinea-pig, quite insusceptible to the development of experimental cholesterol atherosclerosis.

A more pertinent variation in response to cholesterol feeding, however, occurs in the rabbit itself. Some rabbits develop neither a marked hypercholesteraemia nor atherosclerosis when fed amounts of cholesterol that are usually considered adequate. The reason for such cholesterol resistance has not been determined, but may well depend on the ability of such an animal to excrete the excess of cholesterol with and without absorption. Pollak[58] studied 40 rabbits fed 0.5 gms. of cholesterol daily in dry form for 12 to 15 weeks. He found that the blood cholesterol level of 7 rose to 400 mgm.% after 5 weeks of feeding but that it then fell

to normal levels at 12 weeks. Nine rabbits showed a rise to 600 mgm.% at 7 weeks, a decline to 12 weeks, and a rise to the former maximum at 15 weeks. Ten rabbits showed a rise to 700 mgm.% at 6 weeks and then a less rapid rise to about 1000 mg.% at 15 weeks. Fourteen rabbits showed a slow steady elevation of the blood cholesterol to a low maximum of about 430 mgm.%. He found that hypercholesteraemia was not necessarily followed by atherosclerosis, but that the effective atherosclerotogenic threshold varied from animal to animal. Nevertheless, the generality that "the higher the blood cholesterol level, the greater the degree of atherosclerosis developed" was found to be true. The same author[61] has observed that age and weight factors are important in the development of experimental cholesterol atherosclerosis. He has demonstrated that very young rabbits developed less atherosclerosis than old rabbits although the degree of induced hypercholesteraemia was comparable in both groups of animals. Similarly Weinhouse and Hirsch[33,35] found that there was no absolute correlation between the cholesterol content of the serum of the organs or of the aorta of any given cholesterol fed rabbit. It is certainly true that rabbits vary in their individual response, not only to a given oral dose level of cholesterol, but also in the response of their fixed tissues to a given level of circulating cholesterol and lipid. The reason for such variations is important to know, but is quite unexplained at present. While our ignorance of the mechanisms involved are masked in phrases such as "individual variation" or "constitution effect", it is obvious that some other factor or factors in addition to simple chemical hypercholesteraemia are concerned in the deposition of cholesterol in the organs and vessels of rabbits fed cholesterol. Moreover, the deposition of cholesterol in the organs and in the vessels does not necessarily occur pari passu or in a constant ratio.

From the foregoing discussion only two major conclusions may be drawn. It is apparent that cholesterol feeding is a sine qua non of the development of atherosclerosis; neither it nor hypercholesteraemia, however, are the only factors concerned in the production of the lesion.

Numerous theories have been advanced to explain the occurrence of atherosclerosis in rabbits fed cholesterol and/or in man. A review of the numerous factors which have been implicated is presented in the following table constructed by Hueper[62]. It deals with all varieties of arteriosclerosis.

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.

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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, omphalo-mesenteric 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 - <u>Colloidal Plasmatic Disturbances Resulting in the Formation of Films and Precipitates on the Intima and Causing Thereby an Impairment of the Exchange</u>

III - 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)).

Some of the items tabulated above are applicable to arteriosclerosis other than atherosclerosis. Those factors most closely related to the present discussion and experiment are contained in section III. Suffice it to point out that the table contains the following items of immediate experimental interest:- acidosis, consumption of excessive amounts of liquids, hyperlipaemia in diabetes mellitus, hyperlipoidemia with excessive dietary intake of lipoid, and carbohydrate plasmatic disturbances.

Consideration of this table suggests immediately that the study of arteriosclerosis has acquired an aspect of confusion which all but precludes rational theorization. It is not surprising, therefore, to find that no adequate theory of the cause and nature of arteriosclerosis or of atherosclerosis exists at present. As pointed out by Hueper(5), any theory of the genesis of arteriosclerosis must provide a plausible explanation for the following features of the disease:

1. Its progressive increasing incidence and severity with advancing temporal age.
2. The not infrequent occurrence of lesions in relatively young persons and its occasional absence in old persons.
3. Its irregular and patchy distribution in the vascular tree.
4. Its variability as a spontaneous disease in various animal species.
5. The variable morphology of the lesions.
6. The etiological relationship to sex.
7. Its occasional positive association in some cases with definite endogenous and exogenous factors.
8. The chemical composition of the lesions and the basis of it.
9. The available data derived from animal experimentation including the effectiveness of various causal agents and of various modifying agents.
10. The positively associated antecedent and postcedent phenomena.

The senescence theory of the genesis of arteriosclerosis (L.Aschoff) is based upon the increasing incidence and severity of arteriosclerosis with advancing age and from the fact that non-pathological age changes can be demonstrated in the vascular tree. Additional unspecified modifying factors are accepted as operative in some cases, but the essential feature is pathological ageing of the blood vessels. The underlying changes are dehydration and demixture of the colloids of the vascular wall with their ultimate precipitation and secondary deposition of substances such as calcium or cholesterol and other waste metabolites. The theory does not explain the distribution or the different degree of development of lesions in various vessels of the same patient, the fact that lesions are not necessarily

correlated with temporal age, or the fact that sex factors are obviously operative. It is somewhat difficult to reconcile the important proliferative aspect of arteriosclerosis with the concept of senescence. Moreover, there is no valid reason why similar senescent changes with comparable, if not identical lesions should not occur in other collagenous tissues of the body beside the vascular system as a regular finding. That time is an important factor cannot be disputed, but time is not synonymous with the concept of senescence.

It has been suggested that mechanical trauma is the oasis of the genesis of arteriosclerosis. This theory states that mechanical forces derived from the pulsation of the arterial column of blood, from the impingement of the moving blood stream upon irregularities in the vascular configuration, from intrinsic movement of the vessels themselves and from extrinsic movement of and pressure upon the vessels, result in a separation and even a distruction of the cellular and extracellular elements of the vessel wall. Such damage allows an abnormal state of permeability to arise, so that excessive amounts of interstitial fluids pass into and accumulate in the vessel wall with a subsequent precipitation of cholesterol and other substances in the affected area. Because the tissues about the affected region are metabolically active, proliferative responses can and do occur.

The theory can, in part, explain certain factors related to the distribution of atheromatous changes - as for example, those occurring about the mouths of the intercostal arteries. It, however, is not acceptable as an explanation of the localization of many arteriosclerotic lesions. In addition, many of the objections raised against the senescence theory may be raised with equal force against the mechanical theory. This is not to say that mechanical factors play no part in the genesis of arteriosclerosis. Observations upon the arteriosclerotogenic effectiveness of generalized and pulmonary hypertension, of arteriovenous aneurysms, and of experimental and accidental trauma to blood vessels are too numerous to be disregarded. Nevertheless, as a general theory of the primary

genesis of arteriosclerosis, it is quite inadequate.

A toxic theory based upon human and experimental observations has been proposed. This theory claims that toxic substances of bacterial and other origin, when introduced into the organism, cause death or degeneration of various elements of the vascular wall. These alterations then favor the deposition of inert metabolites and the occurrence of proliferative phenomena. The chief difficulties with this theory are that there is no convincing evidence relating human arteriosclerosis and toxic agents and that experimental lesions produced by such methods are primarily medial in location. Moreover, it does not adequately explain the patchy distribution, localization or varying morphology of the lesions.

Winternitz et al have proposed that arteriosclerosis is essentially a disease of the vasa vasorum of the blood vessels. They observe that large atherosclerotic lesions of the aorta are abnormally vascular and suggest that, just as occlusion of the small blood vessels of an organ such as the kidney will lead to necrosis and scar tissue formation, so will occlusive disease of the vasa vasorum they observe have a similar effect. The deposition of cholesterol and other substances, as well as proliferative activity is a secondary phenomenon to local ischemia of the vessel wall. The difficulties encountered with this theory are that no such vasa vasorum are anatomically demonstrable in normal aortae, and the smaller vessels which are subject to arteriosclerosis have no vasa vasorum. Moreover, large veins should be subject to a somewhat similar disease process. It should also be pointed out that it is the intima which is affected primarily and principally by atherosclerosis and the intima apparently receives its oxygenation from the lumen which it lines. Their theory has recently been somewhat modified by Katz and Dauber by combining it with that of Leary.

This latter author has advanced, during his later period, the idea that atherosclerosis arises by invasion of the vascular endothelium and intima by lipid filled histiocytes derived from the reticulo-endothelial system in the liver, spleen and other organs. The lipid containing cells then migrate to and are arrest

ed by the internal elastic membrane. Lipid material is transferred to and partly metabolized by fibrocytic cells. The theory is based upon observations made in cholesterol fed rabbits and has been supplemented by observations on human material. It has recently received strong theoretical support from Gordon[63] who points out certain physical principles of the behavior of particulate matter in a flowing column of fluid which support the concept. However, even he has difficulty in comprehending a situation in which "should this obese cell ..... perhaps adherent to the endothelium, then pass a tentative pseudopod - why one cannot speculate - into the intima, ... " Several major criticisms may be levelled against this concept. Atherosclerosis in the rabbit can be produced without saturation of the reticulo-endothelial system by lipids. There is no definite ratio between the amount of lipid deposited in the reticulo-endothelial system and parenchyma of such organs and that found in the vascular system. Many animal species that are insusceptible to experimental cholesterol atherosclerosis acquire fatty depositions in organs such as the liver. In man, fat filled reticulo-endothelial cells are seldom seen regardless of the degree of atherosclerosis found. Moreover, in the lipidoses in which the reticulo-endothelial cells of the organism are filled with kerasin or sphingomyelin in the same morphological manner in which those of the rabbit are filled by cholesterol, these "Gaucher", or "Niemann-Pick" cells do not cause an overwhelming atherosclerosis of the type which would be expected[43,34]. I am not aware of the existence of an "iron atherosclerosis" in conditions in which a reticulo-endothelial haemosiderosis occurs.

Hueper[5] has recently placed the conceptions of the genesis of arteriosclerosis on a different basis. It is his contention that all available experimental and morphological data may be construed as causing a vascular anoxemia. This anoxemia is in the nature of a chronic and cumulative condition which results in degeneration of the affected elements and the subsequent deposition of various inert substances with or without the mediation of phagocytic endothelial cells. The anoxemia may be medial in location due to contraction of the vasa vasorum or

some other factor which impairs the circulation or oxygen transfer in the affected area, or it may be endothelial and intimal in location due to factors which inhibit oxygen transfer such as films formed by the dedispersion of macromolecular substances such as cholesterol. This theory is most interesting and difficult of assessment. It is somewhat difficult to understand why organs such as the aorta or coronary arteries which contain the most highly oxygenated blood in the body should be so subject to intimal arteriosclerosis when they are theoretically more subject to disease of the vasa vasorum and medial degeneration. It might be argued that the turbulence of the aortic blood stream favors the deposition of films, but against this conception one may stress that the pulmonary artery, which contains relatively anoxemic blood and also encloses a turbulent blood stream, is relatively immune to the development of atherosclerosis. Moreover in calcific disease of the aortic valve in which condition the turbulence of the blood stream issuing from the left heart is exaggerated, there is no particular tendency to aggravate arteriosclerosis of the aorta(65). Some authors(2) even consider that it has a protective effect on the development of aortic atherosclerosis, especially in the arch. One is not impressed with any relationship existing between chronic anaemic states in man and the associated degree of arteriosclerosis. Similar observations may be made concerning chronic anoxemic states of pulmonary or cardiac origin. Hypotension is said to be a factor causing circulatory anoxemia (Hueper), yet a substance such as thiocyanate which has a pharmacological action similar to the nitrites(71) is apparently capable of inhibiting experimental cholesterol atherosclerosis(66,67). It cannot be denied that anoxemia may play an important role in the genesis of arteriosclerosis, especially of the calcific types, but it is not an adequate explanation for many of the major features of this disease.

A review of the above theories indicates that all, except that of Leary, contain the idea that there is an alteration in the vascular substrate which allows the secondary deposition of such substances as cholesterol and the other chemical constituents of atheromatous lesions. Such changes in the substrate are considered

variously as senescent, toxic, traumatic, nutritional or anoxemic. Each is derived from and explains some feature or features of the disease. None is free from more or less serious criticism.

A theory derived from Virchow and amplified by Anitschkow approaches the problem in a somewhat different manner. This so-called imbibition is the basis of the deposition of cholesterol and other atheromatous substances which is considered as secondary by the other theories. It maintains that the deposited substances are contained in the interstitial juices of the fluid which infiltrates and flows among the cells of the vessel wall, passing from the blood in the lumen to the adventitia. Certain natural barriers such as the elastic laminae tend to halt the flow of such fluids or juices. In its purest form this theory considers that various substances such as cholesterol are in a state of unstable dispersion in the blood and tissue juices and are precipitated from solution as they flow through the vessel wall. This would naturally occur most readily under conditions such as hypercholesteraemia in which the stability of the colloid sols was decreased. The impure forms of the theory, as we have seen, postulate some hypothetical or actual precedent pathological change in the substrate which allows the desolution of the substances in question by chemical or physical mechanisms.

It is evident that the only factor common to all theories is found in the imbibition concept. As an isolated concept it is not a more adequate explanation of many of the features of arteriosclerosis than is any other, since the factors which affect the rate and degree of imbibition - aside from the rate of interstitial fluid flow and the cholesterol, etc. content of this fluid - are not specified. If one considers the ten criteria previously listed as the minimal requirements of any theory of the genesis of arteriosclerosis, then this theory is found to be lacking. Its faults, however, are different from those of the previous theories. The latter all contain features that are incompatible with fact. The imbibition theory cannot be criticized because of conflict with fact - except that one would expect to find a lesion comparable to atherosclerosis else-

where in the collagen tissues of the body. The criticisms which can be made are those which derive from our almost total ignorance of the factors which affect imbibition and dedispersion of colloid and other sols. For example, sex differences and species differences in relation to atheromatous lesions remain rationally inexplicable phenomena for the present. One particular virtue of the imbibition theory for our present purposes is that it is applicable without distinction to human and to experimental cholesterol atherosclerosis.

As a rational basis for further pursuit of the problem, particularly from the experimental point of view, it is only necessary to emphasize certain facts. Atherosclerosis is the expression of a reaction between the vasculature, the interstitial fluids and the reticulo-endothelial system. Of these three, the histiocytic, but not the endothelial aspect of the reticulo-endothelial factor, appears to be secondary and least important. An experimental approach may be based upon the induction of alterations in (a) the endothelium, (b) the interstitial fluids (or plasma), and (c) the vascular wall. It appears probable that any alteration of any one of these factors will also induce alterations in at least one of the remaining two factors. Nevertheless, it is also probable that predominant effects attributable to each of the three factors separately may be isolated by a properly constructed series of experiments. Moreover, it is indicated that the reaction which finds morphological expression as atherosclerosis may be exaggerated or held in abeyance by a suitable alteration in any one of the reactants.

STUDIES ON THE LIPIDS OF THE BLOOD AND BLOOD VESSELS

The relationships between hypercholesteraemia and the production of experimental cholesterol arteriosclerosis in the rabbit, as well as the fact that cholesterol and other lipids are an integral part of the lesions of atherosclerosis in man, have led to numerous investigations of the variations in the blood and tissue content of these substances in man and animals in health and disease.

Page et al[73] studied the blood lipid and cholesterol levels of 66

clinically healthy males of 20 to 90 years of age. Age variations were not found to influence the amount or composition of the plasma lipids. Peters and Man[74] analyzed the interrelations of serum lipids in normal persons and found that the maximum variation of cholesterol encountered in any one person was 80 mgm.%. They also found that the ratio of cholesterol to lecithin (lipid phosphorus) was more constant than either of its constituents. The ratio of free to total cholesterol varied from 0.24 to 0.32. The free fat content of the serum was not definitely correlated with either the cholesterol or the lecithin levels.

On the other hand in abnormal patients Kountz et al[75] in studying the blood cholesterol levels in elderly persons found that elderly females had higher blood cholesterol levels than did males. The males, however, had an earlier and higher incidence of atherosclerosis. The average cholesterol level for the whole group, 214 mgm.% by the Bloor method, may be considered as high normal or slightly elevated. Peripheral arteriosclerosis was commoner in the presence of low rather than high cholesterol levels. Similarly, Rafsky and Newman[76], who studied the blood cholesterol in patients of 62 to 104 years of age, found that about two-thirds of their patients had abnormally elevated cholesterol levels; Poindexter and Bruger[77], have studied the association between heart disease and blood cholesterol. They analyzed 33 normal persons, 18 suffering from rheumatic heart disease, 24 with arteriosclerotic and 19 with hypertensive arteriosclerotic heart disease. The serum total cholesterol level was elevated in the arteriosclerotic groups, but not in the rheumatics. The ratio of free to total cholesterol was not altered. From a study of 59 patients with angina pectoris with an average age of 55 years, and 54 normal persons with an average age of 34 years, Davis et al[78] concluded that the cholesterol, lecithin (lipid phosphorus) and fatty acid content of the serum was higher among the angina patients than in the control group. Their figures show a considerable degree of overlap between the two groups, but also show levels among the angina sufferers that are markedly above those of the control group. Leibig[79] studied 80 patients, finding that the serum cholesterol was

elevated in 61% of those with generalized atherosclerosis in 77% of aortic atherosclerosis cases, in 87% of nephrosclerosis cases and in 77% of those with hypertension. The alterations found varied from a 12 to 25%, elevation of serum cholesterol above a maximum normal of 200 mgm.%. Steiner and Domanski[80] followed 15 patients with arteriosclerotic disease of the coronary arteries and 15 control patients at bi-monthly intervals for about 2 years. They found maximum limits of 308 to 409 mgm.% and 214 to 334 mgm.% in the serum cholesterol of the two groups during the two year period. The group with coronary artery disease averaged 355 mgm.% of serum cholesterol while the control group averaged 255 mgm.%. There was a greater individual fluctuation in these values among the coronary group than was noted among the controls. The same authors[81] studied the degree of atherosclerosis found at autopsy in 54 patients with chronic glomerulonephritis. Thirty of these patients had had one or more determinations of their blood cholesterol content. The degree of atherosclerosis was markedly increased over that found in a control series, both in the aorta and in the coronary arteries. In general, the content of cholesterol in the blood had been moderately to markedly elevated. They also noted while studying 18 living glomerulonephritics, that all at some time or other presented a moderate to marked hypercholesteraemia, although their blood cholesterol was normal at other times. Lande and Sperry[82] approached the problem somewhat differently, analyzing the post mortem serum cholesterol in 123 acceptable cases of sudden death. Since many of their cases were traumatic, an unassessed factor of haemoconcentration or haemodilution must have been present. They were unable to show any correlation between the blood cholesterol and the vascular cholesterol contents. Breyfogle[83] made a different approach to the problem again. In a series of 1493 autopsies, including 162 cases of coronary artery disease, he found 363 cases of gall bladder disease. The associative factor between the two was +0.55. Although he does not account for this association it may be pointed out that gall stones are frequently of a cholesterol nature and are sometimes considered as a reflection of a disordered cholesterol metabolism

in the body as a whole.

It would appear that the blood cholesterol is not affected by age per se, and that while it is subject to wide variations in groups of individuals, it is relatively constant in any given individual. There is some evidence that it is elevated in persons suffering from atherosclerosis or its sequelae, but such evidence is not clear cut or decisive. Some of this confusion is probably related to the uncertainty of the results obtained with current methods of cholesterol analysis. Some may be implicit in the problem. Nevertheless, if any trend is indicated, then one must consider that there is a positive association between arteriosclerosis and an elevated blood cholesterol level.

The blood cholesterol has been studied in several other clinical conditions. The variations occurring in the lipidoses have been described by Thannhauser[43] and need not concern us further. It may be noted, however, that cases of essential xanthomatosis with elevated blood cholesterol levels and "xanthomatosis" or atherosclerosis of the blood vessels have been described. On the other hand there are cases such as that of Chapman and Kinney[84], a case of "Idiopathic Hyperlipaemia", in which the total blood lipids including the cholesterol were elevated 6 or 7 times above normal, and yet in the course of a careful autopsy, no mention is made of the aorta. One can only presume it was not atheromatous. This case is of general interest in that very little fat was found stored in the reticulo-endothelial system and it was noted that lipase was absent from the blood.

The variations occurring in the blood cholesterol under conditions of altered thyroid function have received considerable attention. Brøchner-Mortensen and Møller[85] examined 51 patients with thyrotoxicosis, finding that the blood cholesterol generally lay within normal limits, but that the average value of 184 mgm.% was somewhat lower than normal. In their material as a whole, no correlation was found between the metabolic rate and the blood cholesterol level, but by method of repeated examination it was shown that there was an inversely proportion-

al variation between the two in 32 of the cases. There was a rather marked subsequent rise in the blood cholesterol of 7 patients who later underwent thyroidectomy. Man et al found that, as a general rule, the serum cholesterol and phospholipid levels of hyperthyroid patients increased when they were treated with iodine or by thyroidectomy. Conversely, Greene[87] found the blood cholesterol levels elevated in myxoedema. Upon treatment with thyroid hormone the blood cholesterol levels were reduced in reciprocal relation to the induced increase in the basal metabolic rate. Using a somewhat different approach, Bruger and Rosenkrantz[88] studied the relation of the basal metabolic rate to arteriosclerosis. All patients were 55 years of age or older. There were 223 patients with clinical evidence of arteriosclerosis and 70 patients without. The basal metabolic rate of the patients with arteriosclerosis average -3 to -4 while that of the non-arteriosclerotic patients was +3 to +4. It is apparent that while the metabolic rate definitely influences the blood cholesterol level, it is by no means so certain that the basal metabolic rate is generally correlated with the development of atherosclerosis, or is closely correlated with the blood cholesterol level[113]. In cases of clinical thyroid dysfunction there may be a correlation with arteriosclerosis for atherosclerosis and coronary occlusion are not uncommon in myxoedema, and it is a common clinico-pathological impression that coronary occlusion is extremely rare in hyperthyroidism.

There is no satisfactory evidence that a simple diet high in cholesterol content influences either the blood cholesterol level or is atherosclerotogenic. It is true that a very transient postprandial hyperlipaemia and even hypercholesteraemia may occur, but it is not usually regarded as significant. Okey and Boyden[89] have shown that the blood cholesterol level falls during the menstrual period and that this fall is preceded or followed by higher levels than are usual for the individual concerned. This change is presumably hormonal rather than dietary. Bruger and Oppenheim[90] gave 500 cc. of cream to 7 normal and 13 obese

persons without influencing the free or total cholesterol levels. Fasting did not affect the blood cholesterol of a normal control group. The nutritional role of cholesterol in arteriosclerosis was studied by Shaffer[91]. He examined 100 patients who had been on a "milk and cream" ulcer diet for 5 years. Nine had coronary artery arteriosclerosis. In a corresponding control group of 500 the incidence of the corresponding disease was 10.5%. No endocrine disease was present. The effects of general nutrition has been studied at autopsy also, but these do not directly relate the blood cholesterol and arteriosclerosis. Dublin[92] found that in a study of 192,304 cases with 13,350 deaths, diseases of the heart and blood vessels were two to three times as common in obese persons as they were in underweight ones. The incidence of these diseases in persons of average weight lay about half-way between the two previous groups. Grothel et al[93] studying the etiological factors in 134 cases of arteriosclerosis, concluded that excessive nourishment was a causal or associative factor of some importance. In a recent study Wilens[94] analyzed the autopsies of 395 obese persons, of 372 of average nutrition, and of 483 of poorly nourished patients. All were 35 years of age or older. Advanced atherosclerosis was twice as common in the obese as in the poorly nourished group, while intermediate weights showed an intermediate incidence of this lesion. The findings were independent of age, sex, heart weight, hypertensio and diabetes. He substantiates Anitschkow from his own experience in the statemen that atherosclerosis is uncommon in the undernourished population of Costa Rica, and he quotes Oppenheim and Snapper as finding a reduced incidence of atherosclerosis among poorly nourished Chinese. French and Dock[95] studied 80 cases of fatal coronary occlusion in young soldiers aged 20 to 36 years. Overweight, which was present in 91% of these cases was the most striking associated factor.

It would appear that the degree of atherosclerosis is definitely associated with variations of the normal body weight; it is not clear, however, that the nutritional state or the diet has any direct influence upon the content of

cholesterol in the blood.

Extensive studies of the behavior of the blood cholesterol in diabetic patients have been made. The first observation of lipemia in association with diabetes was made during the period when blood-letting was a routine practice. About 100 years later it was found that the blood cholesterol was elevated also. In 1916 Bloor(96) studied 38 diabetics and found that while the individual blood lipids retained their interrelations, all of the lipoids were increased. Denis(97) found a cholesterolemia in diabetes only, and not in other conditions. User and Karr(98) found cholesterolemia in diabetes, and found that insulin prevented marked aberrations from normal. Various authors(99-111) 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(101). He found that 187 untreated, fully established diabetics had an average cholesterol of 242 mgm.%. A similar group of 163 very early diabetics had an average level of 212 mgm.%, and a group of 128 potential diabetics had an average cholesterol of 166 mgm.%. 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 of, 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 patient 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.

It would appear, therefore, that diabetics suffer from a hypercholesterolemia at one time or another, 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

Halliday[112] studied the lipid, carbohydrate and moisture content of the liver in diabetes mellitus at autopsy. He was unable to demonstrate any uniform alterations in the normal content of fatty acids, their iodine number, the content of phospholipids, total cholesterol, glycogen or free sugar. The effect of post mortem changes, however, obscure the interpretation of this data.

Before leaving the question of blood cholesterol, it is of interest to note two or three points. The most important is the question of the nature of the substance analyzed as cholesterol. Most of the determinations quoted have depended upon a colorometric reaction between cholesterol, acetic anhydride and sulphuric acid. The cholesterol is obtained by lipid extraction and may or may not be precipitated by means of a saponin such as digitonin as a further refinement. It should be noted that, not only is the colorometric reaction subject to many influences such as time and temperature which make accuracy difficult, but it is also not specific. A similar reaction for example is observed with various

phytosterols, steroid hormones, onocol and onoketone. The developed color is not pure, but is a complex of brown, blue and green whose color mixture changes during the time of color development. The inaccuracies of the method are less important than its lack of specificity, for while there is no real reason to question that a "chemical" hypercholesteraemia is a true hypercholesteraemia, there is, so far as I am aware, no proof that we are actually dealing with cholesterol and not some closely related substance in many of the conditions in which an altered cholesterol metabolism is said to be a feature. Moreover, there is no suitable data relating to the physico-chemical state of the cholesterol in these conditions. There is some fragmentary evidence that the physico-chemical state of cholesterol in the blood in man is subject to the influence of unspecified factors. Kraus and Kalal[114] found that while the total content of cholesterol in the blood does not change on standing in vitro, that the ester fraction may increase, decrease or remain constant. They postulated an "esterase activity". Schonholzer[115] exposed plasma to crystalline cholesterol, studying whether it was absorbed or precipitated from the plasma. He found that the ability of the plasma to take up cholesterol decreased with age, was always a negative value after the age of 70 years and was independent, in part at least, of the absolute cholesterol content of the plasma. Perhaps the most obvious observation relating to the physico-chemical state of cholesterol is that of in vivo and in vitro lipaemia. It would appear important to study this whole aspect of the problem further.

The morphological observation that atherosclerotic vessels contain lipid and anisotropic crystalline material has been amplified by chemical analysis. The early chemical studies have been reviewed by Wells[118]. Rosenthal[116] analyzed the total fat content of 500 aortae. He found that the fat content of the aorta, although increasing with age, was directly proportional also to the degree of severity of the atherosclerosis. In smooth aortae the fat content at age 25 to 30 averaged 0.065 gms. increasing in a roughly linear manner to 0.182 gms. at 61 to

70 years. In slight to moderate atherosclerosis the figures were 0.202 and 0.517 gms. respectively, while in moderate to severe atherosclerosis they were 0.662 (at 31 to 40 years of age) to 1.275 gms. Weinhouse and Hirsch[117] separated the intima and the media of the aorta and analyzed their content of moisture, lipid, free total and ester cholesterol, phosphatides, galactoside, calcium and ash. They used 25 aortae from persons aged 12 to 84 years. They found that the lipid and calcium content of the media increased with age, and that this increase was not correlated with the degree of intimal atherosclerosis. The normal intima had more lipid and less calcium than the normal media. With increasing severity of the intimal atherosclerotic lesions the proportion of free and ester cholesterol increased. After the onset of necrosis in the lesions the proportion of ester cholesterol decreased. With increasing severity of atherosclerosis there were also increased proportions of ether insoluable phospholipids and calcium, but decreased proportions of ether soluable phospholipids, galactosides and fatty acids. Since the proportions of the lipid constituents in the normal intima and in the early atherosclerotic intimal lesions corresponded closely to those of the blood, they were felt to constitute a non-selective infiltration. Lehnherr[119] analyzed similar constituents in 25 aortae from non-diabetic persons, 25 from diabetic patients and 6 children's aortae. It was his conclusion 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 aortae from diabetic and non-diabetic persons. The diabetic specimens differed in that they contained exaggerated lipid and calcium contents. Zeek[120] analyzed 11 aortae finding that the morphologic changes of atherosclerosis were accompanied by corresponding chemical changes of the type already described. In a study of the acetone soluable lipid of the atheromatous aorta, McArthur[121] found that the composition of the phospholipid-free fat from atheromatous intimal tissue was as follows: stearic acid 2.9%; palmitic acid 14.6%; oleic acid 65.2%; linoleic acid 9.4%; arachidonic acid

2.1% and petroleum insoluable acids 5.8%. On comparing his data with that obtained from studies of the chemistry of the blood, he concluded that the main difference between the two lies in the fact that the proportion of glycerides is much lower in the atheromatous material than in the blood, and that they either are not imbibed by the intima or are decomposed in the lesion. Bruger and Chassin[122] analyzed the cholesterol content of the renal arteries and thoracic aorta in hypertensive and non-hypertensive patients in a series of 37 consecutive autopsies. They state that there is a positive correlation between the degree of hypertension and the cholesterol content of the thoracic aorta and renal arteries. The ratio of the cholesterol content of the arteries and aorta was relatively constant in hypertensives of various ages but it decreased with increasing age in non-hypertensives. Faber[123] analyzed 65 aortae. In 29 normals there was a logarithmic correlation of cholesterol content with age. In 9 cases in which there had been a hypercholesteraemia exceeding 300 mgm.%, 7 showed a greater amount of cholesterol in the aorta than was expected. Of 13 hypertensive persons with normal blood cholesterol levels, 10 showed an excessive content of cholesterol in the aorta. It may be noted in passing that several authors[33,35,124,125] have made more or less detailed analyses of the aorta of the rabbit in experimental cholesterol atherosclerosis with findings experimentally comparable to those in man.

Consideration of these data indicates that the proportion of lipid materials found in normal and atheromatous aortic intima closely approximates that found in the blood. The cerebrosides, as demonstrated by both McArthur and Weinhouse and Hirsch, are reduced in atheromatous material, but one should remember that Thannhauser[43], working with large quantities of sera and stromata, has been quite unable to demonstrate any cerebroside whatsoever in the blood. Whether Weinhouse and Hirsch were analyzing for galactose (cerebroside) or some other substance with reducing power would be difficult to say, but it is at least fair to state that they demonstrated a progressive reduction in the quantity of a reducing substance in a lipid extract of increasingly severe atheromatous disease of th

intima of the aorta.

The data also indicates that the fat and cholesterol content of the aorta increased in proportion to any increase in atherosclerotic disease; that conditions of hyperlipaemia, including diabetes, enhance the content of these substances in the aorta without alteration of the various lipid proportions; and that hypertension in the presence of a normal cholesteraemia is associated with an increase in the cholesterol content of the aorta.

ATTEMPTED ALTERATIONS OF THE BLOOD LIPIDS AND OF EXPERIMENTAL ARTERIOSCLEROSIS

Because of the fact that cholesterol feeding in the rabbit elicits changes in the blood lipids and vascular tree of this animal, and because of the variations in the blood lipids in various diseases of man, many attempts to alter such induced or spontaneous changes have been made. Some of these attempts have had a rational basis, some have not. In general, most of them have yielded apparently conflicting results, and derive their importance from that fact.

The blood lipids of dogs under conditions of acute and chronic fasting have been studied by Entenman et al[126]. No lipaemia or increase in total cholesterol, fatty acids or phospholipids was observed in acute or chronic fasting. Acute fasting of up to 30 days duration and a weight loss of 30% did not produce any striking changes. However, chronic fasting of over 5 months duration and a weight loss of about 50% was accompanied by a 30% reduction of these blood lipids. Artom and Freeman[127] gave rabbits a fatty meal consisting of 10 grams of olive oi and found a slight increase in blood lecithins and a slight reduction in cephalins. The results were rather inconclusive. Member and Bruger[36] studied the effect of olive oil upon the absorption and deposition of cholesterol in the rabbit. Eighte experimental and a similar number of control rabbits were used. They found that those animals receiving olive oil as well as cholesterol had a slightly higher cholesteraemia and a very appreciably increased content of cholesterol in the live and aorta. The effect of bile acids upon cholesterol feeding in the rabbit was

studied by the same authors[47] using 75 female rabbits of which 39 were controls. They found that cholic acid or glycocholic acid when fed with cholesterol markedly increased the amount of cholesterol in the whole blood and aorta compared to the effects of feeding cholesterol alone. Dehydrocholic, hyodesoxycholic and desoxycholic acids did not have this effect. Cholic acid was peculiar in that it increased the ester cholesterol fraction disproportionally. Corwin[68] shared the common experience of finding cholesterol feeding ineffective in the dog, however, lecithin feeding did produce a cholesteraemia, and combined lecithin and fat feeding resulted in levels of 700 mgm.% in about 6 weeks. This effect of lecithin feeding in dogs and man was studied by Steiner and Domanski[55] using an egg yolk powder consisting of 14% lecithin and 8% cholesterol. In 8 patients with rheumatoid arthritis and 2 with chronic nephritis, they were able to produce repeated moderate elevations of the blood cholesterol by 6 to 10 weeks of egg yolk powder diet. Dogs were fed this diet for about 1 year with a moderate hypercholesteraemia but no atheromata resulting.

On the other hand the effect of prolonged lecithin feeding in hypercholesteraemic conditions has been studied by various authors. Adlersberg and Sabotka[128] gave soya lecithin (20% lecithin; choline, inositol, fatty acids, etc.) to 5 hypercholesteraemic patients with such conditions as diabetes mellitus, hepatic cirrhosis and psoriasis. They achieved a striking decrease in serum cholesterol levels with relapse of the condition when the lecithin therapy was discontinued after an interval of a few months. They also found (J. Nutrition 25, 1943) that feeding soya lecithin to persons with a normal cholesterol level produced an ephemeral effect only. In a somewhat similar experiment Steiner and Domanski[129] fed soya lecithin to 8 patients with high normal to moderately elevated (546 mgm.%) cholesterol levels. Significant decreases in blood cholesterol resulted, but could only be maintained for some 5 weeks. Gross and Kesten[130] found the substance effective against the hypercholesteraemia of two patients with xanthomatosis and one with necrobiosis lipoidica diabeticorum. Kesten and Silbowitz[131] tested its

effect in cholesterol-fed rabbits and tested choline as well. Their results are interesting but scarcely significant. They reduced the expected degree of hypercholesteraemia and also reduced the incidence of atherosclerosis. Choline was less effective than soya lecithin in this respect.

The effect of choline or betaine as inhibiting agents in the development of fatty livers caused by feeding rats cholesterol has been studied by Best and Ridout[132] who find that choline causes a decrease in the glyceride and cholesterol ester fractions of the liver, the effect on the glyceride fraction preceding that on the cholesterol ester portion. Baumann and Rusch[133] studied the effect of choline hydrochloride on the cholesterol content of the blood, liver and aorta of cholesterol fed rabbits. They were unable to demonstrate any effect of choline on the development of atheromas after up to 4 months of combined choline and cholesterol feeding. However, in one group of rabbits given 240 mgm. of cholesterol and 300 mgm. of choline daily, while there resulted satisfactory atheromatous lesions of the aorta, the livers of these animals did not contain any more cholesterol than did those of a control series of animals that did not receive cholesterol. Steiner[134] studied a similar problem, finding that choline did not prevent the hypercholesteraemia induced by feeding cholesterol to rabbits. However, it was noted that it appeared to delay the onset of atheromatous changes up to an experimental period of about 80 days. After 80 days no difference was noted in the atheromata of the control and experimental groups. Meeker and Kesten[135] studied the effect of a high protein diet on experimental atherosclerosis in rabbits. They found that soya flour had a protective action against cholesterol feeding: that cholesterol, when supplemented by defatted casein, gave a greater degree of atherosclerosis than was expected, and that casein supplement alone caused a hyper cholesteraemia and atheromatosis indistinguishable from that due to cholesterol feeding.

The effect of alterations in thyroid function upon the general and the lipid metabolism has stimulated several experimental studies. A fatty diet of cotton seed oil causes enlargement of the thyroid in rabbits[136] but oils which

contain iodine such as cod-liver oil do not have this effect. It usually requires 60 to 90 days of a diet containing about 10 cc. of oil to produce thyroid overgrowth. The effect is presumably due, in part at least, to iodine depletion. Turner has investigated the relation of the thyroid and of iodine to experimental cholesterol atherosclerosis in the rabbit. In his first experiment[137] he demonstrated that both whole thyroid and large amounts of potassium iodide were effectiv inhibitors of marked hypercholesteraemia and atherosclerosis induced by feeding cholesterol to rabbits for 90 to 120 days. Potassium bromide and potassium chloride were ineffective in both respects. He[138] also demonstrated that cholesterol feeding produced hypercholesteraemia and atherosclerosis in thyroidectomized rabbits, and that potassium iodide exerted no protective action against cholesterol feeding in the absence of the thyroid. Rosenthal[139] on the other hand used very small amounts of potassium iodide or organic iodine and found that he obtained contrary effects, and moreover, in a group of rabbits which had been fed cholesterol for 7 weeks before iodine was introduced into their diet for the remainder of the 120 day period, he found an exaggerated degree of hypercholesteraemia and atherosclerosis. The apparent conflict in these two reports was confirmed in a single experiment by Breusch and Thiersch[140] one year later. They fed cholesterol to rabbits for a period of 6 months and at the same time gave substances such as calcium iodide, di-iodotyrosin and thyroxin. Their data indicate that iodine does not affect the cholesterol level of normal animals, small doses (1 to 3 mgms.) exaggerate induced hypercholesteraemia and large doses (25 mgms.) depress it. The degree of induced atherosclerosis was similarly affected. Turner and Bidwell[141] investigated further and found the ability of potassium iodide to inhibit induced hypercholesteraemia was temporary only, and that the cholesterol "escaped" after about 4 months with a resulting hypercholesteraemia and atherosclerosis. They observed that the administration of potassium iodide to rabbits with an existing hypercholesteraemia caused a further marked rise in the blood cholesterol. On the other hand, if dried thyroid were given to such animals there resulted a sharp but

transient fall in the blood cholesterol which then rose to new high levels. It was also noted that if thyroid or thyroxin was given to thyroidectomized, cholesterol and potassium iodide-fed animals, there was a delay in the induction of hypercholesteraemia and atherosclerosis. Both Meeker and Kesten[142] and Turner and Bidwell[143] investigated the effect of potassium iodide on the regression of induced atheromata and hypercholesteraemia. Their data indicated that the rate and nature of the involution of the atheromata was probably unaffected. However, there was a definite delay in the regression of the hypercholesteraemia, a depression of the ester: free cholesterol ratio, and a delay in the return to normal of the overweight, cholesterol-rich adrenal glands. Turner et al[144] indicate that the blood cholesterol of normal rabbits may be increased 19% by thyroidectomy; that the level in hypercholesteraemic rabbits is raised 137% by thyroidectomy, and that thyroidectomy abolishes cholesterol resistance. They also found that thyroxin reduced hypercholesteraemia before and after thyroidectomy. In contrast to their observation that the thyroid was necessary to the effective counter-action of cholesterol feeding by potassium iodide, they observed that potassium iodide would exaggerate existing induced hypercholesteraemia in the presence or absence of this gland. They also noted that a single injection of insulin would reduce an existing hyperlipaemia, and that thyroidectomy, either did not abolish this effect or appeared to enhance it. In connection with cholesterol resistance, it is interesting to note that the natural resistance of the dog to orally induced hypercholesteraemia and atherosclerosis can be overcome by thiouracil medication[52]. Bruger and Fitz[145] gave the thyrotropic factor of the anterior pituitary lobe to rabbits which were being fed cholesterol. Their result seems to indicate that the hormone enhanced the degree of both hypercholesteraemia and atherosclerosis. Unfortunately, they used too few animals to make their result acceptable. Turner and De Lamater[146] tested the action of thyrotropic hormone in cholesterol fed rabbits of 3 types: control, thyroidectomized and partially thyroidectomized. A reduction of 20 to 25% of the blood cholesterol occurred in all rabbits.

One is at a loss to interpret these results in a satisfactory and compact manner. It would appear that iodine compounds are capable of very definite actions upon cholesterol metabolism. The only contrary report is that of Page and Bernhard[147] who fed the di-iodide of ricinsterolic acid and cholesterol to rabbits for 110 to 180 days. They used 6 experimental and 6 control animals. The blood lipids were, if anything, higher in the iodide-cholesterol treated group than in the group receiving cholesterol alone. However, the degrees of atherosclerosis were slight and moderate respectively in the two groups. Further analysis of their experiment indicates that for the first 100 days they were giving the equivalent of 6.2 to 15.5 mgms. of iodine and that thereafter 46.5 mgms. of iodine were given. It might be expected therefore, that such a dose schedule would promote hypercholesteraemia. The difference in the observed degrees of induced atherosclerosis cannot be explained, but they are not statistically significant when one considers the variability of the response of rabbits to cholesterol feeding. The mechanism of iodine and thyroid activity upon experimental cholesterol atherosclerosis is obscure, but it is apparent that any protective activity is dependent upon a lowering of the blood cholesterol level.

Before turning from the question of thyroid function and lipid metabolism, three fragments of information may be noted. If thyroxine is given to lactating cows, the yields of milk and milk fats are increased. The nature of the milk remains unchanged, but the plasma lipids are decreased 10 to 20%[148]. In experimental hyperthyroidism and hypothyroidism in rats and rabbits, there is evidence to indicate that thyroid hormone shifts cholesterol to and from the blood and body tissues, rather than having a specific effect on cholesterol metabolism[149]. If lipaemia is induced in dogs by the process of plasmapheresis, such a lipaemic reaction is found to be the same in control and in thyroidectomized animals[150].

While the associations of diabetes mellitus, disturbances in lipid metabolism and atherosclerosis have been subject to extensive study in man, much less related work has been done in experimental animal diabetes. Kendall et al[151]

have found that a transient period of hypercholesteraemia and hyperlipaemia may occur in the early phases of diabetes induced by injecting rabbits with alloxan. The blood lipids parallel the blood cholesterol levels in general, but they may increase more or less than the cholesterol does. Their work is confirmed by this laboratory which has shown also that the hypercholesteraemic condition may be recurrent, and that the ester:free cholesterol ratio is markedly reduced during the hypercholesteraemic episode. Gibbs and Chaikoff[152] studied totally depancreatectomized dogs and insulin deprivation. Diabetic hyperlipaemia was a regular finding only in those animals that received raw pancreas before and after insulin deprivation. It was uncommon in those animals which received no raw pancreas. In some cases of hyperlipaemia only the triglyceride fraction was increased and the cholesterol and phospholipid levels of the blood were unchanged. It was also determined that a hyperlipaemia occurring when the dogs were receiving both insulin and raw pancreas showed an increase in the cholesterol portion, especially its ester fraction. Dragstedt et al[153] found an incidence of arteriosclerosis of the aorta of 13% of 80 depancreatized dogs under insulin control, on high fat diets and in lipocaic deficiency. They consider such an incidence of arteriosclerosis most unusual. On the other hand Lukens and Dohan[162] maintained pituitary diabetes in a dog for 5 years. While there was glomerulosclerosis at autopsy, the aorta was not mentioned, and presumably was normal. They quote a report by Fisher of arteriosclerosis in a partially depancreatized dog. Huber et al[154] in an unsatisfactorily controlled experiment, found that cholesterol-induced hyperlipaemia and atherosclerosis was inhibited by feeding rabbits lipocaic. However, Vermeulen et al[155] using much larger numbers of rabbits were unable to confirm Huber's findings. They determined that the blood cholesterol levels of the cholesterol fed animals was not influenced by lipocaic treatment; that comparable degrees of atherosclerosis developed in the presence or absence of lipocaic supplement; that the non-cholesterol lipid fractions of the blood of the lipocaic treated animals were reduced, both relatively and absolutely, and that the livers of these animals contained less lipid

and cholesterol than did those of rabbits receiving cholesterol alone.

Before leaving the literature concerning diabetes it may be noted that insulin, in man at least, given in massive and repeated doses, has no effect on the normal levels of blood lipids(156). A curious and interesting effect of insulin has been demonstrated in dogs by Ralli and Sherry(157). These authors used normal and depancreatized diabetic dogs kept on a vitamin-C free diet. The average plasma levels of vitamic C were 0.71 mgm.% and 0.53 mgm.% in the two groups of animals and the average urinary excretions were 20.6 mgm. and 3.6 mgm. per kilogram of body weight. The diabetic animals thus had a lower plasma and urinary content of vitamin-C. On injecting insulin into these animals it was found that the plasma ascorbic acid was reduced for 5 to 6 hours following the injection and that the excretion of vitamin-C was also reduced. The effect was about the same in diabetic and non-diabetic animals. One cannot clearly speculate concerning the importance of this observation in terms of atherosclerosis, but the known role of vitamin-C in the production of intercellular substance(70) makes the observation one of interest. One may also note in passing, and because of the possible role of the reticulo-endothelial system in the genesis of atherosclerosis (Leary), that the phagocytic power of the blood phagocytes is markedly reduced in man and cats under conditions of uncontrolled diabetes or diabetic acidosis(158).

The studies related to experimental diabetes and lipid metabolism need to be expanded, but there is evidence at hand to indicate that both the islet and acinar elements of the pancreas are capable of modifying fat metabolism. The only satisfactory experimental demonstration of an association with arteriosclerosis is that of Dragstedt and co-workers who found an excessive incidence of the disease in depancreatized, diabetic, lipocaic deficient dogs maintained on a high fat diet.

In addition to the hormones of the pancreas and the thyroid gland, the effects of another series of endocrine substances has been investigated. These are derived from the gonads. Ludden et al(159) investigated the effect of testosterone propionate and estradiol dipropionate on male and female rabbits subjected to chole-

sterol feeding for 100 days. The male rabbits developed the expected degrees of hypercholesterolaemia and atherosclerosis. The female animals, however, acquired only a minimal hypercholesteraemia and the cholesterol content of their aortae was practically normal. The effect occurred with either testosterone or estradiol administration. Bruger et al[160] repeated the experiment using castrate female rabbits. They concluded

(1) that castration per se did not alter the content of cholesterol in the aorta or blood,

(2) cholesterol feeding had its usual sequelae in castrates, and

(3) testosterone and estradiol were ineffective as innibitors of experimental cholesterol atherosclerosis or hypercholesteraemia in castrate female rabbits.

A contrary type of experiment was done by Lindsay and co-workers[161] who implanted pellets of diethylstilbestrol into young chickens. The chickens developed an extreme hyperlipaemia, a moderate to marked hypercholesteraemia and an extensive atheromatosis.

Again, it is apparent that further experimentation is necessary to elucidate the significance of these observations. Nevertheless, the gonadal hormones appear to have a marked influence upon lipid metabolism and a secondary association with experimental atherosclerosis. Unlike the hormones of the pancreas and thyroid, the presence of the endocrine organ appears to be necessary for this effect to manifest itself. Why a synthetic ovarian "hormone" such as stilbestrol should act contrarily to true gonadal hormones is not obvious. An analogy with human arteriosclerosis has been drawn because of the observation that men are two or three times as liable to coronary occlusion as women.

The effects of a large number of miscellaneous substances - organic and inorganic chemicals, vitamins, physiological material, etc. - have been studied by various authors interested in arteriosclerosis and related fields.

Based upon the idea that iodine prevented experimental atherosclerosis because it was a member of the Hofmeister series of ions, Malesoff[66] studied the

effect of a related ion. He gave a series of rabbits cholesterol and potassium thiocyanate. Four rabbits served as control animals for the cholesterol feeding, four were given cholesterol and 20 mgm. of thiocyanate daily, four were given cholesterol and 60 mgm. of thiocyanate daily. All were thyroidectomized at the beginning of the experiment. The control rabbits developed severe atherosclerosis, the second group developed moderate sclerosis and the group that received the larger dose of thiocyanate developed no atherosclerosis. Hueper(67) repeated the experiment using 4 control and 5 experimental rabbits with intact thyroid glands. His findings suggest that thiocyanate does not inhibit the induction of hypercholesteraemia, but does inhibit the development of atherosclerosis. Eberhard(163) fed ethyl alcohol and cholesterol to rabbits. His results suggest that alcohol inhances induced hypercholesteraemia while diminishing the deposition of cholesterol in the aorta and liver. The differences demonstrated, however, were slight and could be appreciated by chemical analyses only; grossly visible differences in the development of atherosclerosis were not apparent. On the other hand, Wilens(174) has concluded that alcohol has no influence on the development of human atherosclerosis. His data is based on a study of autopsies performed on 519 chronic alcoholics.

In order to test whether diabetic acetonemia might be related to arteriosclerosis, Short et al(164) fed rats large amounts of aceto-acetic acid for 6 to 12 months. The rats had acetone in their expired air whenever tested, but no vascular lesions resulted. Christianson(165) injected such substances as human fat, fatty acids, soaps and cholesterol into the media of the aorta of experimental animals. A chronic inflammatory and granulomatous reaction arose with the frequent production of secondary lesions of fibrotic type in the intima. Hurst(166) described a marked hyperplasia of vascular endothelium in rabbits infected with infectious myxoma. Thiersch(167) combined myxoma infection with cholesterol feeding. The disease is one that causes proliferation of the reticulo-endothelial system. Thiersch found that myxoma lesions do not localize in atheromatous areas; that the

disease is not influenced by the existence of atheromatosis and that the development of the atheromata does not appear to be accelerated by infectious myxoma. Flexner et al[168] studied the effect of dietary supplements of thiamine hydrochloride and/or ascorbic acid on the development of experimental cholesterol atherosclerosis. No effect on the expected hypercholesteraemic or atherosclerotic changes was noted. Bruger[169] studied the effect of vitamin-E (d, l-alphatocopherol) on experimental cholesterol arteriosclerosis and found that it increased the deposition of cholesterol in the aorta by about two times. The amount of cholesterol in the liver was not so increased. Unfortunately, the number of animals is rather small so that the result is somewhat unconvincing. Hueper[170] studied the effect of various detergents upon experimental cholesterol atherosclerosis finding that certain agents tend to increase the effects of cholesterol feeding while others decrease them. Again, there are far too few animals to enable one to draw any useful conclusion. A miscellaneous series of possible adjuvants to cholesterol feeding were studied by Jobling and Meeker[171] who fed rabbits with cholesterol for about one month, combining this with streptococcus toxin, ammonium chloride, artificial fever, anaphylactic shock, and intravenous uric acid injection. No atheromata were found in any case. Raab[172] used an extract of adrenal cortical lipids and adrenalin ("lipoid-adrenalin compound") or an extract of one litre of human "arteriosclerotic and hypertensive" serum as a preparatory treatment method before feeding 0.5 grams of cholesterol daily to rabbits for 14 days. A moderate degree of atherosclerosis resulted whereas no atherosclerosis might be expected from cholesterol feeding alone. Member et al[173] were unable to show statistically significant variations of the serum cholesterol levels of patients immediately or a short time after treatment with acetyl-B-methylcholine. Pollak[59] attempted unsuccessfully to produce cerebral atherosclerosis in rabbits by feeding cholesterol combined with treatment with epinephrine, histamine and arterial and venous ligation. The effect of the so-called active principle of the artichoke has been investigated in man by Schonholzer[115] who has demonstrated that it increases the

ability of the serum to take up crystalline cholesterol in vitro.

The action of various mechanical and pressure factors in experimental cholesterol atherosclerosis has received some attention. Harrison has conducted two very interesting experiments. The first of these involved the sequential production of arteriosclerosis by the use of cholesterol and of vitamin-D[37]. If atheromata were developed by cholesterol feeding and the animal were then caused to develop medial aortic calcification by the use of vitamin-D, the calcification occurred between but not under atheromatous areas. If, on the other hand, medial calcification were induced and then cholesterol atherosclerosis were caused, the latter occurred between but not over calcified areas. It is very interesting to note that one of his experimental rabbits was resistant to the induction of arteriosclerosis of either type. In a second experiment Harrison[175] performed a unilateral lumbar sympathectomy in rabbits which were then subjected to experimental cholesterol atherosclerosis. The femoral arteries of the two limbs of the same animal were then examined for atheromata. The vessel on the side subjected to sympathectomy suffered more atherosclerosis, both by morphological examination and by chemical analysis, than did that on the intact side. Presumably both vessels were exposed to the same blood content of cholesterol. Wilens[176] placed silver cuffs about muscular arteries causing thereby a local periarterial fibrosis and loss of medial musculature. There was also an intimal fibrosis at the lips of the cuff. When cholesterol atheromatosis was caused, more lipid accumulated at the lips of the cuff than anywhere else in the vessel concerned. The same author has studied the effect of postural hypertension on the development of atherosclerosis in the rabbit[177]. By placing rabbits upright he was able to induce an increase in the blood pressure, as measured at the ear artery, of about 35%. This increase continued throughout the period of postural change. On inducing cholesterol atherosclerosis it was observed that there was an increase in the severity of the induced lesions and a tendency to shift them towards the distal aorta. The experiment comprised only 6 experimental and 7 control animals and must be regarded with cautio

Dill et al[178,179] in ill-controlled experiments caused hypertension in rabbits by placing a wire loop about the aorta above the renal vessels. Atheromatous plaques were found proximal to the constricting loop. The animals were receiving a diet containing 0.2 gms.% of cholesterol and moreover, the experimental result lacks statistical significance. No valid conclusions can be drawn from their work with reference to atherosclerosis. In passing we may also note that Dominguez[180] has demonstrated that fluctuations of the systolic blood pressure beyond normal are not necessary for the production of experimental cholesterol atherosclerosis in the aorta of the rabbit. Similarly, atherosclerosis of extreme degree is not accompanied by an elevation of blood pressure outside of the normal range.

It is difficult to summarize the foregoing data in an intelligent manner. Hormones derived from the thyroid, pancreas, gonad and possibly from the adrenal can influence lipid metabolism, and whatever effect they induce appears to be reflected in a proportional way in the development of atherosclerosis. Mechanical factors influence the development of atherosclerosis in a manner that appears to be completely independent of the hypercholesteraemia to which the vessel wall is exposed. This is not to say that hypercholesteraemia is not an essential factor, but rather it is to emphasize that it is not the sole factor concerned. The two brief experiments related to thiocyanate medication acquire great interest, for they constitute the only available suggestion that it is possible to induce a marked hypercholesteraemia in a rabbit, and, at the same time inhibit the development of experimental cholesterol atherosclerosis. One can only wish that further relevant data were to hand.

ARTERIOSCLEROSIS AND DIABETES MELLITUS IN MAN

Extensive observations of the degree and incidence of arteriosclerosis have been made in cases of diabetes mellitus in man. These observations have been derived from autopsy, roentgenologic and clinical material, and, in total, they represent many thousands of cases. While the compilations of such cases may prove

to be statistically impressive, it is vital to remember the premises on which these cases were studied. The majority of the material to be discussed was considered to be arteriosclerotic in nature because (a) there was evidence of an acute or chronic impairment of the circulation of the heart or legs, or (b) because there was roentgenologic evidence of arterial calcification, or (c) because there was clinical evidence of a hypertensive and arteriosclerotic cardio-vascular syndrome. The least part of the material is derived from direct anatomo-pathological observation of the arterial lesions in question. There can be no doubt that the roentgenologic data is valid evidence of calcific disease of the arterial media, and as such it constitutes a true type of arteriosclerosis. Any evidence of atherosclerotic disease, however, is not immediately established by proving the presence of calcific medial sclerosis. The fact that an impaired circulation of the heart or legs which causes myocardial infarction or gangrene may generally premise arterial disease, does not prove that such disease is necessarily atherosclerotic in type. It is, however, quite true that such lesions are generally accepted as sequelae of atherosclerotic vascular disease. The clinical signs and symptoms of hypertensive and arteriosclerotic cardio-vascular disease are well established, but it nevertheless remains true that a certain primary diagnosis of atherosclerotic vascular disease is not possible; rather the diagnosis of atherosclerosis is implicit in some other primary diagnostic impression.

While there is a definite association between vascular disease and diabetes mellitus, there is no good evidence that either is the causal factor of the other. It is generally true that clinical arteriosclerosis and diabetes mellitus exist independently; it is only in the minority of cases that the two become associative. It will become evident from the data to be discussed that when the two are associative, the association is quite striking, but this observation must not cause us to neglect those many cases in which the two diseases appear to be independent. It is generally held that diabetes promotes or exaggerates the development of arteriosclerosis, but certain respected authors suggest that vascular

disease is the cause of diabetes[181,182], or that both diseases represent the expression of some more basic constitutional fault[183].

A full appreciation of the incidence and degree of arteriosclerosis among diabetics is hampered by a lack of adequate control material. Some authors provide control material for their studies, others do not. Perhaps the best control material may be obtained from the routine autopsy studies of Willins et al[26] and of Ophuls[25]. In any consideration of such material, however, it is necessary to remember that the assessment of degrees of arteriosclerosis is probably as much subjective as objective. Unfortunately, practical objective means of measuring degrees and variations of arteriosclerosis do not exist.

Warren[184] states that arteriosclerosis in diabetes is fundamentally and predominantly atherosclerotic in type. This opinion has had a profound influence on thought and theory regarding the genesis of arteriosclerosis in diabetes for many years, and has become generally accepted as a true statement. Nevertheless, important contrary evidence is available. Bruger[185] finds that there is no essential difference in the type of arteriosclerosis found in diabetic and non-diabetic persons. Lisa et al[186] examined a large series of limbs amputated because of arteriosclerotic vascular disease in diabetic and non-diabetic patients. They were unable to distinguish between vessels obtained from diabetic and from non-diabetic persons. Vartiainen[187] found very little difference in the degree or type of generalized arteriosclerosis in 75 diabetic and 75 non-diabetic autopsies. Many authors have studied the excessive occurrence of calcific medial arteriosclerosis in diabetic persons by X-ray, indicating that medial arteriosclerosis is a finding of major importance in diabetes mellitus. Indeed, when one considers the available evidence it becomes apparent that arteriosclerosis in diabetes is not necessarily predominantly and fundamentally atherosclerotic in type. Certainly the atherosclerotic component may be exaggerated at the expense of the other components of arteriosclerosis in those patients who have a marked hypercholesteraemia, but it would appear that the statement of Warren is not a valid general

conclusion.

Apparently an association between diabetes mellitus and angina pectoris was first noted in 1864. In an analysis of coronary sclerosis in 77 fatal cases of diabetes and in a control group of 450 non-diabetics, Blotner[188] found coronary sclerosis in 45% of the diabetics (all over 34 years of age) and in only 21% of the control series. Death was attributable to the heart in 43% of the diabetics. Hart and Lisa[189] found that the sex ratio of coronary sclerosis in 2,798 autopsies was 1.25:1, male:female. In their series of diabetics the ratio reversed, becoming 1:1.05. A similar tendency in the sex ratio was noted in arteriosclerosis of the aorta and kidney, and in gangrene of the lower extremity. Root et al[190] studied the autopsies of 349 diabetics and 3400 non-diabetic persons, finding coronary occlusion in 32% and 6% respectively. Narrowing of the coronary arteries without occlusion was present in 19% and 14% respectively, while insignificant sclerosis occurred in 49% of the diabetic and 80% of the non-diabetic cases. They found a sex ratio of coronary occlusion of 5:1, male:female in the non-diabetic group, and of 1:1 in the diabetic series. Rosenblum[191] studied 214 diabetic patients of whom 24 had evidence of coronary disease at an average age of 57.3 years. Females were as liable to this lesion as were males. He quotes Meakins and Eakin as finding coronary occlusion in 62 of 6548 consecutive autopsies; and Sutton and Brandes as finding coronary sclerosis in 11.1% of 3,040 autopsies. Dry and Tessmer[192] examined 130 hearts from diabetic persons of 40 years of age or over. Coronary occlusion was present in 15 while severe coronary artery sclerosis was found in an additional 53. Lisa et al[193], from autopsy examinations of 193 diabetics and 2092 non-diabetics all over 40 years of age, found coronary sclerosis in 70 and 60% respectively. The degree of this sclerosis was moderate in 35 and 48%, and severe in 65 and 52% respectively. Myocardial infarction was found in 30% of diabetics and in 22% of non-diabetics. In a study at this Institute, W. E. Finkelstein[194] has found that the deaths of 13.4% of 232 diabetics were due to coronary artery disease, while the corresponding lesion occurred in 5.16% of 1686 non-diabet-

ics of 35 or more years of age. Enklewitz[195] found coronary thrombosis to be twice as frequent in diabetic as in non-diabetic persons. There were 92 diabetics in his series. Nathanson[196] studied 100 diabetic and 249 non-diabetic autopsies. In persons of 50 years of age or more, severe coronary sclerosis was found in 52.7% of the diabetics and in 8.2% of the control group. The sex-ratios were male:female, 1.8:1 and 3:1 respectively. Wilder[197] found advanced coronary artery sclerosis in 34% of 49 diabetic autopsies. Root[198] found coronary arteriosclerosis in 60% of 55 diabetic autopsies.

The variation in the figures quoted above provides ample evidence of the subjective nature of opinions expressed with reference to the degree (and hence incidence) of arteriosclerosis. Nevertheless, it remains true that diabetic persons suffer a considerably greater degree of coronary artery arteriosclerosis than do non-diabetics. It is apparent also that the usual sex differential of the condition is abolished by the presence of diabetes. Lastly it may be noted that coronary occlusion has an even higher relative incidence among diabetes than severe coronary artery arteriosclerosis. It must also be remembered that arteriosclerosis of the coronary arteries is predominantly atherosclerotic in type.

Arteriosclerosis of the vessels of the legs has been extensively studied. Lisa et al[186] examined legs amputated because of circulatory insufficiency in 55 diabetic and 51 non-diabetic persons. Gangrene was present in 39 and 31 of the diabetic and non-diabetic limbs respectively. If one assumes an incidence of diabetes of between 0.5 and 1.0% of the general population, it is apparent that there is a very striking incidence of peripheral circulatory deficiency among diabetics. Naide[199] found 11% of diabetics under 50 years of age and 34% over 50 years of age who had evidence of peripheral vascular disease. The patients were under satisfactory insulin control. Dry and Tessmer[192] found severe peripheral arteriosclerosis in 28% of 182 autopsies diabetics. Gangrene or trophic ulcers were found in 31 of these cases. Rosenblum[191] found evidence of gangrene in 19 of 234 diabetic patients. Scott[200] reported 107 cases of amputations because of

arteriosclerotic gangrene, finding diabetes present in 99 cases. Meyers and Altshuler[201] found an incidence of 43% of peripheral vascular disease among 74 diabetics; less than one-half of these were symptomatic. Solley[202] reported 39,370 hospital admissions of which 702 were diabetic: 30 of these had gangrene of the lower limbs. Hart and Lisa[189] found the sex ratio of gangrene, male:female was 5.63:1 among non-diabetics while it was 1:1.04 among diabetics. Vartiainen[187] found 13 examples of gangrene in 75 diabetic autopsies and only 2 among a similar group of control necropsies. Eisile[203] 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[204] 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[205] analyzed 58 cases of diabetic gangrene occurring among a group of 1008 diabetics, and reviewed some of the relevant literature. In addition Kramer found 28 threatened and 89 potential cases of gangrene among his 1008 diabetic cases. Morrison and Bogan[206] 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 was rare in normal persons of less than 40 years of age, while for the age period of 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 calcifica-

tion, 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 increased with the patient's age and with the duration of his diabetes, and that the severity of the lesion increased in a corresponding manner. They believe, therefore, that diabetes is an etiological factor in the production of peripheral vascular calcification. Wilder[197] found that gangrene was responsible for 14 of 81 diabetic deaths in the age period of 39 to 75 years. Shepardson[207] 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[100] 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[208] found calcified peripheral arteries in 55 of 71 diabetic patients. Collens and Wilensky[209] 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[210] 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. Lehnhoff and co-authors[211] examined 60 cases of diabetic gangrene, 30 from the pre-insulin era and 30 from the insulin era. They found an earlier onset of gangrene in the pre-insulin era, and a more severe degree of arteriosclerosis in the post-insulin period. Finkelstein[194] found an incidence of gangrene of 12.5% among 232 diabetics at the Pathological Institute, McGill University, Montreal.

It is apparent from the many fragments of data presented above that diabetic patients are particularly liable to gangrene and calcific arteriosclerosis of the legs. It is not so evident that atherosclerosis of the vessels of the extremities is a lesion of importance in relation to these conditions.

Information concerning the aortae of diabetic persons is both morphologic and chemical. Lehnherr[119] 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[193] 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[184] reports an incidence of arteriosclerosis of 85.2% in 264 diabetics of all ages at autopsy, while among 108 diabetics of less than 51 years of age he found 65.7% to have arteriosclerosis. Rabinowitch[101] using a combined method[212] 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[197] reports arteriosclerosis of grade 2 or more (on a scale of 4) in 41 of 51 autopsies on diabetic individuals. Page and Warren[213] report arteriosclerosis in 7 of 11 young diabetics, ages 12 to 33 years, at autopsy.

The overall picture of vascular disease in human diabetes leads one to

conclude that the vascular system of the diabetic is strikingly prone to acute occlusion or chronic progressive stenosis, and that it is also subject to a high incidence and degree of medial calcification. It would also appear that atherosclerosis of certain vessels is a little more common and rather more severe than it is in non-diabetic persons. There is evidence that certain vessels - such for example, as those of the circle of Willis[193] - are not in any way more subject to atherosclerosis in the diabetic than in the non-diabetic. Indeed, except insofar as vascular occlusion implies atherosclerotic disease, there is but little evidence that the diabetic person suffers from more than a slightly increased degree and incidence of this lesion. It is important to note that vascular disease in the human diabetic is not only more frequent and severe than it is in the non-diabetic, but that it has a much earlier age onset and has a reduced sex-ratio. It is customary to interpret the data listed above as evidence that diabetes accelerates and exaggerates the genesis of arteriosclerosis. There is, however, no logical compulsion to accept this interpretation. Indeed, one may oppose it - without much force it is true - by the observation that the majority of diabetics do not suffer from an abnormal degree or incidence of vascular disease. It has been suggested that vascular disease causes diabetes mellitus, but again the majority of patients with vascular disease do not become subject to diabetes mellitus. A further explanation that has been advanced is that both diseases are conjointly secondary to some third factor, tending to occur together, but also capable of existing independently under suitable circumstances.

The acceptance of any explanation of the positive association between vascular disease and diabetes mellitus in man must await further investigation.

SUMMARY - PART I

A few broad generalizations may be drawn from the foregoing review. The disease known as 'arteriosclerosis' was found to be an entity of uncertain pathogenesis which, in one of its aspects (atherosclerosis) was found to be morphologically similar to, but by no means identical with, an experimental disease caused by feeding cholesterol to rabbits. Hypercholesteraemia was found to be a sine qua non of the experimental disease, but it was not considered to be the only important factor concerned in the development of experimental cholesterol atherosclerosis. Many factors were demonstrably capable of altering the chemical quantity and the physical state of cholesterol in the body fluids. Similarly, many factors were found capable of altering the genesis of experimental cholesterol atherosclerosis by altering or inhibiting a condition of chemical hypercholesteraemia. Very little evidence could be found which indicated that it was possible to alter the genesis of experimental cholesterol atherosclerosis without altering or inhibiting a condition of chemical hypercholesteraemia.

It was found that vascular disease of arteriosclerotic type was a common concomitant of diabetes mellitus in man. And, it was also noted that hypercholesteraemia was commonly associated with diabetes mellitus. Evidence was obtained that, at least in some cases of diabetes, there was a co-existance of an excessive cholesteraemia and an excessive degree of arteriosclerosis. It was also determined that the commonly expressed opinion that diabetics suffer from an excessive degree and incidence of atherosclerosis was well founded but probably exaggerated.

From these facts no conclusion was drawn with reference to the genesis of arteriosclerosis or atherosclerosis in man or animals. It is felt that the imbibition theory of atherosclerosis is the most satisfactory explanation of the genesis of atherosclerosis that is available at present. It is, however, the theory about which there is the least relevant knowledge.

ADDENDUM

The following papers have come to my attention since the main body of the text of this thesis was written. They are included here in abstract form because of their inherent interest in the field of vascular pathology.

Bevans, Abell and Kendall[214] report the production of intimal atherosclerosis in rabbits by the intravenous injection of colloidal cholesterol. Colloidal solutions of 2.5% cholesterol stabilized with 1% sodium stearate may be injected in the amount of 0.5 grams of cholesterol leading to an increase of 250 to 300 mgm.% in the blood cholesterol within a few minutes. This level then falls by about 50% in 6 hours and then rises to about 150 to 200 mgm.% above the base line at the end of 24 hours. Normal levels are regained within one week. By daily injection small but grossly visible lesions were produced in 13 days and extensive lesions were produced in 36 days.

Davis and Baker[215] used 16 dogs to study the blood iodine in alloxan diabetes. One group received a dietary supplement containing 0.5 mgm. of iodine daily, the other did not. It was found that if the dose of alloxan given to the dogs were sufficient to cause an elevation of blood sugar, there also occurred an increase in total blood iodine. If the dogs became permanently diabetic the blood iodine remained high. Greater increases were observed in the presence of iodine supplement than in its absence.

Rinehart and Greenberg[216] report the production of a fibrotic type of arteriosclerosis in 4 rhesus monkeys subjected to pyridoxine deficiency for from 5 to 14 months. Lesions were encountered in the arteries of the renal hilus and and in the coronary arteries.

Steiner, Bevans and Kendall [217] have recently amplified a previous report by Steiner and Kendall concerning the use of thiouracil and cholesterol to

produce atherosclerosis in dogs. Using 2 young dogs fed 10 grams of cholesterol and up to 1.2 grams of thiouracil per day for one year they produced hypercholesteraemia and extensive arteriosclerosis, including arteriosclerosis of the large cerebral arteries.

Marsters, Leonards and Meyers[218] have analyzed the chemical composition of 64 human thoracic aortae. The water content showed no significant change with age but was decreased in severe arteriosclerosis. Creatine (an index of smooth muscle) and elastin tended to decrease with age while collagen content increased. The differences were not statistically significant. Marked increased with age were found in the cholesterol, calcium and phosphorus contents. There was a good correlation between the chemical and morphological findings. Occasionally the cholesterol fraction was excessively increased, but usually a parallelism existed between it and those of calcium and phosphorus.

Marx and Lipsett[219] were unable to show that extracts of normal rat liver were able to destroy cholesterol in vitro. However, by using extracts of livers from rats fed cholesterol for several weeks they were able to demonstrate an enzymatic destruction of cholesterol.

Kellner, Correll and Ladd[220] studied the effect of a surface active agent, polyoxyalkylene sorbitan mono-oleate, Tween 80, on the blood cholesterol and the development of atherosclerosis in cholesterol fed rabbits. They found that rabbits fed Tween 80 and cholesterol developed blood cholesterol levels 2 to 3 times as high as those obtained by feeding cholesterol alone and also exhibited an earlier and somewhat more severe degree of atherosclerosis. The effect of the wetting agent on the blood cholesterol level of normal rabbits was negligible.

PART II

REPORT OF EXPERIMENT

In view of the information that has been summarized in the foregoing portion of this thesis, it was concluded that there was an interrelation between arteriosclerosis and diabetes mellitus in man. The hypothetical question of an experimental demonstration of such a relationship was, therefore, proposed, and alloxan diabetes in the rabbit was made the experimental instrument of choice. A basic experiment was undertaken by Dr. D. C. Wilson of the Pathological Institute, McGill University, who studied the effect of alloxan diabetes upon the vascular system of a large number of otherwise normal rabbits. His work, as yet unpublished, indicated that the diabetic state per se induced no significant vascular pathology after a duration of as long as one year. It was, therefore, decided to propose the following two questions:

1. Does alloxan diabetes influence the induction of experimental cholesterol atherosclerosis in the rabbit?
2. Does alloxan diabetes influence the regression of existing experimental cholesterol atherosclerosis in the rabbit?

Two suitable experiments were, therefore, designed which would show a positive, negative or neutral effect in this regard, and which would also show whether cholesterol feeding and its sequelae had any effect on alloxan diabetes. Inasmuch as the experiments are distinct entities they will be dealt with separately.

THE EFFECT OF ALLOXAN DIABETES ON THE INDUCTION OF EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS IN THE RABBIT

Over 120 rabbits were used during a period of three years for this experiment. Of these, only 58 completed satisfactory experimental courses, and only these will be considered in detail.

Domestic white rabbits of both sexes were used. The rabbits were purchased on the open market from different dealers, and both long and short eared

varieties were used. The rabbits were all young adults weighing from 2.0 to 3.0 kilograms at the time the experiment was begun. Their exact ages were unknown. Only one rabbit (#8) was fully grown. The rabbits were divided into convenient experimental groups or series of about 8 to 10 animals for experimental purposes and every animal in any one series received exactly the same treatment (with the exception of being rendered diabetic) that was given to any other animal of that series. Moreover, all chemical studies of a given series were done in groups as far as this was possible.

Upon entering the animal house, the rabbits were placed in individual cages, given the standard diet of Purina Rabbit Chow and water ad lib., and allowed to acclimatize to their new environment for a period of not less than 7 days. The animals were then examined for evidence of disease, were sexed, weighed and at least one, but more commonly two control determinations of their serum cholesterol and blood sugar values made.

After satisfactory evidence was obtained that the animals were normal, a random selection of one-half to two-thirds of the group was made, these animals were injected with alloxan, and the remainder were kept as control animals. It may be noted that in some of the later series of animals studied, the control rabbits were added to the series after a satisfactory group of diabetic rabbits had been obtained. Once these animals had been added to the series, however, their treatment was exactly the same as those of the others in all respects.

Alloxan monohydrate (Eastman Kodak Co.) was prepared as a 5% solution in distilled water. It was not sterilized, and was prepared immediately before use. This solution was then injected into the lateral ear vein of the selected rabbit. The dose was calculated at 200 mgm. of alloxan per kilogram of the non-fasting weight of the rabbit. The rate of injection was about 1 cc. or 50 mgm. of alloxan in 5 seconds. One series of animals (series 14A and 14B) received only 150 mgm. of alloxan per kilogram of body weight. It was noted that different batches of alloxan varied somewhat in the properties of the solution which they formed. Some

solutions developed a marked but transient pink color, others did not. Some smelled rather strongly of acetic acid, and others did not; and some made peculiar cracking noises upon the addition of water, while others did not. No differences in the diabetogenic effects of these solutions were detected.

The injection of alloxan was made in the non-fasting state between 9 and 10 o'clock in the morning. This was followed by subcutaneous injection of 2 units of protamine zinc insulin and an intravenous injection of 20 cc. of 20% dextrose in water at 2 o'clock of the afternoon, and this therapy was repeated at 9 o'clock of the same night. Thereafter the rabbits were treated once daily with about 4 units of protamine zinc insulin and 20 cc. of 20% destrose for periods of 7 to 14 days. The control animals received neither insulin nor sugar. Occasional sugar and acetone determinations were made on the urine during this period, and the therapy was varied according to the individual animal's requirements. No attempt was made to control either hyperglycaemia or glycosuria, the chief efforts being directed towards amelioration of acetonuria, diabetic coma and the occasional example of hypoglycaemic convulsions.

Following withdrawal of insulin and dextrose therapy, the surviving animals were allowed to live for a period of 40 to 50 days without any further treatment. Food and water were given ad lib. This "waiting period" was chosen because previous experience had shown (Wilson, D.C.) and (Kendal et al)(151) that rabbits may suffer a marked diabetic hypercholesteraemia shortly after the injection of alloxan. Such a hypercholesteraemic phase however regresses within about 40 days, and it may be noted that all of the animals considered here were in a normal quantitative cholesteraemic state before further experimentation was undertaken.

During this period of time, frequent routine determinations of the blood sugar and serum cholesterol levels were made on all animals. The animals, controls, diabetics and non-diabetic-alloxan-resistants, were then subjected to cholesterol feeding. Series 1 received cholesterol 0.5 grams per dose, in pure form (Merck or B.D.H.) in gelatin capsules (Parke Davis No. 00). The remainder of

the series received cholesterol dissolved in vegetable oil (sun-flower seed oil or corn oil). The oil solutions were administered by means of a soft rubber ureteral catheter, 14F., employed as a stomach tube. They were prepared as 3.3 or 5.0% solutions of cholesterol, dissolved by heat and fed at room temperature or at about 40°C. It was found most convenient to dissolve and maintain the 5% solution at 60°C., allowing it to cool somewhat before feeding. Regardless of what form or solution of cholesterol was used it was constant for a given series of animals. The cholesterol feeding procedure was varied in frequency, dose and duration among various series of animals, but was exactly constant within a given series. It was found that certain animals would not tolerate a daily dose of 0.75 grams of cholesterol in 15 cc. of warm vegetable oil. Such animals were afflicted with diarrhoea that could be induced at will by the feeding procedure. It was found best to discard them.

Upon completion of a suitable feeding period of from 52 to 91 days, the surviving animals were killed by air embolism and autopsied. The duration of a given experiment was frequently governed by the death, in coma, of a diabetic animal, necessitating the sacrifice of the remainder of the animals in the series. The tissues were fixed in formol-saline, Helly's fluid or Bouin's fixative, and selected sections were submitted for routine or special histological study. The liver, spleen, adrenal and kidney were examined in frozen as well as in paraffin section. The heart and aorta were removed en bloc, stained with Scharlach R and the fatty intimal deposits that were demonstrated by this technique were recorded by means of schematic drawings and graded on an arbitrary scale of 0 to 4. The heart and the entire length of the aorta were then studied in paraffin sections stained by the Verhoeff-van Gieson and Mallory's phosphotungstic acid methods. Routine histologic studies were made on several other tissues including the thyroid, gonads and pancreas.

Chemical determinations were made at intervals of at least 2 to 5 weeks and samples were obtained after a fast period of about 18 hours. However, all de-

terminations made in series 14A and B were derived from postprandial samples. These determinations comprised free and total serum cholesterol and the content of sugar in the blood. The blood sugar technique used is that given in "Notes on Operation of the Evelyn Photoelectric Colorimeter", Rubicon Co., Philadelphia, Pa. It involves the use of a sample of blood of 0.1 cc. volume. The method was found to be eminently satisfactory, having an error of less than 5% on replicate determinations. The cholesterol method used was that of Schoenheimer and Sperry (Sperry, W.M., Dept. Biochemistry, New York State Psychiatric Institute and Hospital, N.Y. Revised 1945), involving the precipitation by digitonin of the cholesterol extracted from 0.5 cc. of serum, and the development of the Liebermann-Burchard color reaction. In our hands replicate estimations were sometimes as much as 35% of the lower value in error between extremes. On the other hand occasional multiple analyses varied as little as ± 5% of the mean. Difficulties with this technique have been mentioned previously. Undoubtedly, as a special technique, greater accuracy can be obtained, but as a routine method for handling thousands of cholesterol determinations too much stress must not be laid upon the accuracy of an individual determination. All colorometric determinations were made by the photoelectric method. Blood samples were obtained by incisions of the central artery and vein of the ear after causing vasodilatation by the application of xylol.

Occasional spot tests for acetone and sugar in the urine were done by means of commercial reagents, Galatest and acetone test, marketed by the Denver Chemical Mfg. Co., Montreal. The lipaemic condition of the blood was estimated by inspection of the whole blood before and after clotting in a centrifuge tube. The lipaemia was graded on an arbitrary scale of 0 to 4.

The six animals of series 14B were injected with colloidal thorium dioxide (Thorotrast, Heyden Chemical Co. N.Y.) 1 to 4 days before death, in order to study the behavior of a heavy colloidal substance in the rabbit. The amount used varied from about 2 to 8 cc. per kilogram of body weight. Certain of the animals were subjected to roentgenography during the time of intravenous or intra-

cardiac injection of the thorium solution in an effort to visualize the presence of aortic atheromata. The tissues were subjected to histological study after the animals were killed.

A further exploratory experiment was begun upon the serum of some of the diabetic and non-diabetic animals from various series. The technique, modified from Schonholtzer, consisted of exposing 3 cc. of fresh serum to 60 mgm. of crystalline cholesterol at a temperature of 37°C. for two days. A control tube of serum was similarly treated, and after filtering, the cholesterol content of the sera was determined in the usual manner. It was hoped to demonstrate a difference in the behavior of the diabetic and non-diabetic sera in the presence of crystalline cholesterol by this method.

RESULTS AND CONCLUSIONS

The protocols of all of the individual animals as well as the experimental and feeding schedules are contained in the appendix. Schematic representations of the 0-4 scale of atherosclerosis used in grading aortae and of the aortae of those animals which completed the full experimental schedule are also appended. A table of average and other relevent values found among the 58 animals under consideration is given below. Percentage distribution diagrams and four graphs of the experimental behavior of 4 animals are also contained in the appendix.

TABLE I

SUMMARY OF EXPERIMENTAL DATA. THE EFFECT OF ALLOXAN DIABETES ON THE INDUCTION OF EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS.

Series	Rabbit	Experimental Type *	Sex	Days of Cholesterol Feeding	Total Dose of Cholesterol (gm.) and Av. dose per day	Weight at Commencement of Cholesterol Feeding (kgm.)	Weight at Cessation of Cholesterol Feeding (kgm.)
1	1	D	F			2.450	1.980
	4	D	M			3.150	2.640
	5	C	F	91	38.0 (0.42)	3.540	4.060
	7	C	F			3.080	3.360
	8	C	M			2.840	2.760
3	35	D	M			2.980	2.610
	40	D	M			2.120	1.890
	41	D	M	91	16.25	2.740	2.920
	38	C	M		(0.18)	2.560	3.200
	39	R	M			3.080	3.510
4	52	D	M			2.530	2.240
	53	D	M	90	31.0	2.090	2.500
	45	C	M		(0.34)	3.260	4.660
	46	C	M			3.090	3.750
5	55	D	M			3.480	3.450
	57	C	M			4.380	5.250
	59	C	M	89	48.5	3.400	3.680
	62	C	M		(0.54)	3.290	3.130
	54	R	M			4.450	4.990
	60	R	M			3.940	4.310
6	69	D	M			2.580	1.840
	70	D	F			2.730	2.600
	66	C	F			3.900	4.140
	74	C	F	90	46.25	3.620	4.400
	64	R	M		(0.52)	3.040	3.230
	72	R	M			3.430	3.780
	75	R	F			3.550	3.980
7	81	D	M			2.470	2.890
	78	C	M			3.500	4.060
	84	C	M	76	51.75	3.720	4.090
	77	R	M		(0.68)	3.660	4.230
	79	R	M			3.120	-
	85	S.D	M			3.700	4.000

* D= Diabetic; C= Control; R= Alloxan Resistant; S.D= Mild Diabetic.

TABLE I (cont'd)

Series	Rabbit	Experi-mental Type *	Sex	Days of Chole-sterol Feeding	Total Dose of Cholesterol (gm.) and Av. dose per day	Weight at Commencement of Cholesterol Feeding (kgm.)	Weight at Cessation of Cholesterol Feeding (kgm.)
8	88	D	M			3.070	3.380
	92	D	M			3.100	2.190
	96	C	F	82	60	4.050	4.200
	98	C	F		(0.73)	3.730	4.510
	90	R	F			3.850	5.090
9	99	D	F			2.690	3.310
	108	C	M	89	65.25	2.800	4.170
	109	C	M		(0.73)	3.170	4.360
	110	C	M			3.500	4.510
10	116	D	M			2.220	1.590
	117	D	M			2.370	1.880
	120	C	F	53	39.75	4.580	5.150
	122	C	F		(0.75)	3.500	4.590
	123	C	F			3.020	3.720
	119	R	M			3.320	3.630
14A	195	D	M			2.230	2.050
	199	C	M	52	39.0	2.280	2.630
	201	C	M		(0.75)	2.410	2.070
	184	R	M			3.240	3.580
14B	192	D	M			2.960	3.360
	200	C	M			2.060	2.830
	202	C	M	82	51.75	2.490	4.100
	203	C	M		(0.63)	2.340	4.170
	186	R	M			2.940	3.110
	194	R	M			3.570	4.500

* D= Diabetic; C= Control; R= Alloxan Resistant; S.D= Mild Diabetic.

TABLE I (cont'd)

SUMMARY OF EXPERIMENTAL DATA. THE EFFECT OF ALLOXAN DIABETES ON THE INDUCTION OF EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS.

Series	Rabbit *	Average Values During Cholesterol Feeding Period					Lipaemia (0-4)
		Blood Sugar Mgm.%	Cholesterol				
			Free Mgm.%	Total Mgm.%	Ester Mgm.%	Ester %	
1	1 D	517	18	53	35	65	0
	4 D	472	45	90	45	50	0.8
	5 C	96	25	84	59	71	0
	7 C	96	33	130	97	74	0
	8 C	100	27	103	76	74	0
3	35 D	508	68	114	46	41	0
	40 D	583	89	111	22	20	0
	41 D	397	13	32	19	60	0
	38 C	109	11	30	19	63	0
	39 R	108	14	32	18	59	0
4	52 D	501	51	71	20	28	0
	53 D	473	20	42	22	52	0
	45 C	116	11	23	12	51	0
	46 C	115	24	55	31	56	0
5	55 D	452	99	277	178	64	2.0
	57 C	114	15	63	48	77	0
	59 C	118	66	167	101	60	1.4
	62 C	111	97	316	219	69	1.8
	64 R	110	59	140	81	58	1.0
	60 R	116	60	123	63	51	1.0
6	69 D	355	192	396	204	51	3.7
	70 D	354	57	159	102	64	1.7
	66 C	114	155	334	179	53	1.7
	74 C	123	139	349	210	60	2.0
	64 R	116	104	359	255	70	1.8
	72 R	108	89	232	143	62	1.2
	75 R	115	100	367	267	73	1.8
7	81 D	375	171	401	230	57	4.0
	78 C	113	121	425	304	71	1.75
	84 C	118	70	272	202	73	1.25
	77 R	121	98	340	142	71	1.5
	79 R	136	159	472	313	66	2.25
	85 S.D	147	120	309	189	61	1.0

* D= Diabetic; C= Control; R= Alloxan Resistant; S.D= Mild Diabetic.

TABLE I (cont'd)

SUMMARY OF EXPERIMENTAL DATA. THE EFFECT OF ALLOXAN DIABETES ON THE INDUCTION OF EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS.

Series	Rabbit *	Average Values During Cholesterol Feeding Period					Lipaemia (0-4)
		Blood Sugar Mgm.%	Cholesterol				
			Free Mgm.%	Total Mgm.%	Ester Mgm.%	Ester %	
8	88 D	444	505	1075	507	53	3.8
	92 D	398	315	703	388	55	3.2
	96 C	126	164	562	398	71	1.6
	98 C	117	192	602	410	68	1.6
	90 R	115	235	656	421	64	2.0
9	99 D	392	135	346	211	61	1.2
	108 C	124	55	221	166	75	0
	109 C	128	123	450	327	72	1.0
	110 C	122	143	593	450	75	1.2
10	116 D	481	219	423	204	47	3.75
	117 D	427	101	197	96	49	2.75
	120 C	119	64	210	146	67	0.5
	122 C	131	105	316	211	67	1.0
	123 C	129	111	389	278	72	1.5
	119 R	111	32	77	45	59	0.25
14A	195 D	329	220	726	506	69	2.0
	199 C	136	41	120	79	66	1.0
	201 C	153	414	1728	1314	76	1.3
	184 R	240 **	14	48	34	71	0
14B	192 D	335	459	1970	1511	76	1.6
	200 C	145	344	632	288	46	1.25
	202 C	137	179	334	155	46	0.75
	203 C	136	191	491	300	61	1.25
	186 R	177	612	1849	1237	66	1.5
	194 R	127	75	357	282	80	0.8

* D= Diabetic; C= Control; R= Alloxan Resistant; S.D= Mild Diabetic.

** Blood Sugar values over 300 mgm.% for 6 weeks after injection of alloxan. Terminal blood sugars normal.

TABLE I (cont'd)

SUMMARY OF EXPERIMENTAL DATA. THE EFFECT OF ALLOXAN DIABETES ON THE INDUCTION OF EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS.

Series	Rabbit *	Content of Sudanophilic Substance			Average Dose of Cholesterol per day per kgm. (mgm.)
		Aorta 0-4	Liver 0-4	Spleen 0-4	
1	1 D	0	0	0	191
	4 D	0	0	1	145
	5 C	1	1	1	110
	7 C	1	1	0	131
	8 C	1	0	0	150
3	35 D	0	-	-	64
	40 D	0	-	-	90
	41 D	0	-	-	64
	38 C	0	-	-	62
	39 R	0	-	-	55
4	52 D	0	-	-	142
	53 D	0	-	-	146
	45 C	0	-	-	85
	46 C	0	-	-	100
5	55 D	1	0	0	159
	57 C	2	0	1	113
	59 C	3	2	0	154
	62 C	4	4	4	169
	54 R	3	3	3	115
	60 R	3	-	1	132
6	69 D	1	1	1	237
	70 D	1	1	-	193
	66 C	4	2	-	130
	74 C	4	1	1	130
	64 R	4	4	3	168
	72 R	4	3	2	144
	75 R	4	1	0	137
7	81 D	0	0	-	252
	78 C	4	4	3	179
	84 C	2	4	2	174
	77 R	3	4	2	174
	79 R	4	4	3	-
	85 S.D	1	2	2	174

* D= Diabetic; C= Control; R= Alloxan Resistant; S.D= Mild Diabetic.

TABLE I (cont'd)

SUMMARY OF EXPERIMENTAL DATA. THE EFFECT OF ALLOXAN DIABETES ON THE INDUCTION OF EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS.

Series	Rabbit *	Content of Sudanophilic Substance			Average Dose of Cholesterol per day per kgm. (mgm.)
		Aorta 0-4	Liver 0-4	Spleen 0-4	
8	88 D	1	1	2	228
	92 D	1	1	-	281
	96 C	4	4	2	178
	98 C	2	2	3	178
	90 R	3	4	3	162
9	99 D	1	1	1	243
	108 C	1	2	1	209
	109 C	3	2	2	192
	110 C	4	2	4	182
10	116 D	0	1	2	394
	117 D	0	0	0	357
	120 C	1	1	1	153
	122 C	1	1	1	187
	123 C	2	2	1	215
	119 R	1	2	0	215
14A	195 D	0	2	0	357
	199 C	1	4	2	300
	201 C	3	4	4	381
	184 R	0	1	1	221
14B	192 D	2	2	3	203
	200 C	1	1	2	252
	202 C	3	1	1	191
	203 C	4	4	4	191
	186 R	4	4	3	210
	194 R	1	2	2	158

* D= Diabetic; C= Control; R= Alloxan Resistant.

Before considering the data further, it is necessary to note the criteria and standards used, as well as the heterogeniety of the experimental procedure.

The diabetic state was judged by the features of polyphagia, polydipsia, polyuria, weight loss, increased urinary excretion of nitrogen, glycosuria, acetonuria, lenticular cataract formation (or the early fissuring of the lens detectable by ophthalmoscopic examination) and by hyperglycaemia. Of these features only weight changes and blood sugar variations were studied as a strict routine, the others being noted merely in a casual manner. As a general rule, the diabetic animals ate, drank and excreted two or three times as much as the control animals. Few diabetics gained weight, and they were never comparable to the control animals in this respect. Nitrogen (urea) excretion was studied in only one emaciated diabetic. The amount of urea excreted was about 50 times greater than in a control animal. Lenticular cataract and fissuring were noted occasionally, but were not observed and recorded accurately. Urinary sugar was present whenever it was tested for. A few quantitative estimations were made which indicated that some rabbits were excreting as much as 24 grams of sugar in the urine in 24 hours. Qualitative tests for acetone in the urine were usually positive during the first 2 or 3 weeks of diabetes, and were irregularly positive but usually negative during the remainder of the diabetic state until coma supervened, at which time they became positive. No systematic study of acetonuria was made.

The chief criterion of the diabetic state was the quantity of sugar in the blood. An average level of 300 mgm.% or more after a short fast period was taken as the minimal requirement which would allow an animal to be classed as diabetic. Eighteen animals were in this class. The existence of the diabetic state was confirmed at autopsy by demonstrating the presence of the pathology of alloxan diabetes in the pancreas and by finding glycogen vacuolation of the convoluted tubules of the kidney.

In addition to the animals which became diabetic when injected with

alloxan, a further 12 animals proved to be refractory to its diabetogenic action. The criteria of resistance were merely that both the fasting and non-fasting blood sugars of these animals should be within normal limits before cholesterol feeding was begun, and that there should not occur either hyperglycaemia or glycosuria thereafter. Such animals however may have been more or less diabetic for a period of up to 6 weeks. Commonly, they showed hyperglycaemia and glycosuria for about 3 weeks. Clinically, once classed as resistant, they showed none of the features of alloxan diabetes. These resistant animals, of course, received insulin and dextrose therapy as did the diabetic ones.

An animal was accepted as a normal control if the fasting blood sugar did not exceed 150 mgm.% and the non-fasting blood sugar did not exceed 180 mgm.%. Twenty-eight animals were in this class. The mean of 148 determinations made on 40 normal rabbits during the early phases of this experiment was 103.5 mgm.% $\pm$ 13.6 mgm% of blood sugar. A systematic drift in this figure appeared during the later phases of the experiment, and the mean rose to about 116 mgm.%.

Similar observations of the serum cholesterol values were made on normal animals yielding a mean serum free cholesterol of 11.5 mgm.% $\pm$ 5.4; a total cholesterol of 35.7 mgm.% $\pm$ 19.6; an ester cholesterol of 24.8 mgm.% $\pm$ 16.7; and an ester % of 63.6%. No drift was apparent in these figures during the experiment. It was arbitrarily accepted that an animal had a normal cholesterol level if the average total cholesterol did not exceed 70 mgm.%. The diabetic and alloxan resistant animals occasionally showed elevations of the free and total cholesterol levels shortly after receiving alloxan (see protocol #26). However, all animals had normal serum cholesterol values before receiving dietary cholesterol.

The criteria of atherosclerosis have already been mentioned. The reader is referred to figure 1 of the appendix for an example of the scale of grading used. Each drawing represents a degree of sclerosis approaching the upper limit of the class in which it is placed. The grading was confirmed by microscopic examination. It will be noted that the scale of grading is not linear, but that grade

1 implies that less than about 7% of the aortic surface is involved; grade 2 implies less than about 20% involvement; grade 3 indicates less than 65 or 70% involvement and grade 4 constitutes the remainder. It is obvious that such grading is crude and it is not surprising that some aortae proved difficult of accurate assessment. On the other hand, the differences between the mid-point of any one grade and the next is very striking. It is to be noted that the distribution, size and the thickness of the lesions all entered into consideration in the process of classifying aortae. The diagrams of the aortae contained in the appendix are generally satisfactory. However, due to photographic variation, the occasional print is lighter or darker than it should be, and proves to be somewhat misleading. Essentially similar criteria were used to grade the amount of sudanophilic substance in the tissues of the liver and spleen.

Examination of Table I discloses a remarkable uniformity in the fact that diabetic animals suffer less experimental cholesterol arteriosclerosis than do control or resistant animals of the same series. The only exceptions occur in series 3 and 4 where no sclerosis was induced in any of the animals and in series 14B where diabetic #192 suffered a low 2 grade of sclerosis while the other animals varied from 1 to 4 in their degree of cholesterol atherosclerosis. The table also indicates that the diabetic animals possessed less sudanophilic material in their livers and spleens, than do the other animals, and the same condition was found to be true of the fat content of the kidney and adrenal. The chemical values given in this table are average figures based on bi-weekly observations during the period of cholesterol feeding. They include one "normal" determination made immediately before cholesterol feeding was begun. The high terminal cholesterol values obtained in some animals are thus masked. It may also be seen that the serum cholesterol values of the diabetic animals during the cholesterol feeding period were about as high as or higher than were those of the control or resistant animals in the same group. The lipaemic index of the diabetics was markedly higher than that of the other animals.

Aside from these general, but rather consistent observations, little other information can be gained by superficial examination of Table I. This fact arises from the marked heterogeneity of the experimental treatment and of constitution that exist among the various series of animals. It will be noted, for example, that the days of cholesterol feeding, the total dose of cholesterol fed, the average dose per day, the average dose per day per kilogram of average body weight during the feeding period, and the average total serum cholesterol level varied enormously.

It was, therefore, decided to attempt to pair suitable animals in as many relevant respects as possible in order to obtain well controlled data. A preliminary grouping was done in which the following features were standardized:

1. The dose of cholesterol per day
2. The number of days fed cholesterol
3. The average total cholesterol level.

The results obtained are given in the following abbreviated protocols in which some of the unpaired variables are included. The limits of the criteria of pairing are indicated in these protocols. Other variables can be added easily from Table I.

Diabetic #99 - Dose per day, 0.73 grams.

Duration 89 days. Average total cholesterol 346 mgm. Atherosclerosis 1. Lipaemia 1.2. Sex F. Liver fat 1. Splenic fat 1. Weight change +0.62 kgm.

Control #109 - Dose per day 0.73 grams.

Duration 89 days. Average total cholesterol 450 mgm. Atherosclerosis 3. Lipaemia 1. Sex M. Liver fat 2. Splenic fat 2. Weight change +1.19 kgm.

Diabetic #117 - Dose per day 0.75 grams.

Duration 53 days. Average total cholesterol 197 mgm. Atherosclerosis 0. Lipaemia 2.75. Sex M. Liver fat 0. Splenic fat 0. Weight change -0.49 kgm.

Control #120 - Dose per day 0.75 grams.

Duration 53 days. Average total cholesterol 210 mgm. Atherosclerosis 1. Lipaemia 0.5. Sex F. Liver fat 1. Splenic fat 1. Weight change +0.57 kgm.

Diabetic #70 - Dose per day 0.52 grams.

Duration 90 days. Average total cholesterol 159 mgm. Atherosclerosis 1. Lipaemia 1.7. Sex F. Liver fat 1. Splenic fat ?. Weight change -0.15 kgm.

Resistant #54 - Dose per day 0.54 grams.

Duration 89 days. Average total cholesterol 140 mgm. Atherosclerosis 3. Lipaemia 1.0. Sex M. Liver fat 3. Splenic fat 3. Weight change +0.54 kgm.

Control #59 - Dose per day 0.54 grams.

Duration 89 days. Average total cholesterol 167 mgm. Atherosclerosis 3. Lipaemia 1.4. Sex M. Liver fat 2. Splenic fat 0. Weight change +0.28 kgm.

Diabetic #116 - Dose per day 0.75 grams.

Duration 53 days. Average total cholesterol 219 mgm. Atherosclerosis 0. Lipaemia 3.75. Sex M. Liver fat 1. Splenic fat 2. Weight change -0.63 kgm.

Control #123 - Dose per day 0.75 grams.

Duration 53 days. Average total cholesterol 278 mgm. Atherosclerosis 2. Lipaemia 1.5. Sex F. Liver fat 2. Splenic fat 1. Weight change +0.70 kgm.

Diabetic #81 - Dose per day 0.68 grams.

Duration 76 days. Average total cholesterol 401 mgm. Atherosclerosis 0. Lipaemia 4.0. Sex M. Liver fat 0. Splenic fat ?. Weight change +0.42 kgm.

Resistant #194 - Dose per day 0.63 grams.

Duration 82 days. Average total cholesterol 357 mgm. Atherosclerosis 1. Lipaemia 0.8. Sex M. Liver fat 2. Splenic fat 2. Weight change +0.93 kgm.

Control #78 - Dose per day 0.68 grams.

Duration 76 days. Average total cholesterol 425 mgm. Atherosclerosis 4. Lipaemia 1.75. Sex M. Liver fat 4. Splenic fat 3. Weight change +0.56 kgm.

Diabetic #69 - Dose per day 0.52 grams.

Duration 90 days. Average total cholesterol 396 mgm. Atherosclerosis 1. Lipaemia 3.7. Sex M. Liver fat 1. Splenic fat 1. Weight change -0.74 kgm.

Resistant #64 - Dose per day 0.52 grams.

Duration 90 days. Average total cholesterol 359 mgm. Atherosclerosis 4. Lipaemia 1.8. Sex M. Liver fat 4. Splenic fat 3. Weight change +0.19 kgm.

Resistant #75 - Dose per day 0.52 grams.

Duration 90 days. Average total cholesterol 367 mgm. Atherosclerosis 4. Lipaemia 1.8. Sex F. Liver fat 1. Splenic fat 0. Weight change +0.43 kgm.

Control #66 - Dose per day 0.52 grams.

Duration 90 days. Average total cholesterol 334 mgm. Atherosclerosis 4. Lipaemia 1.7. Sex F. Liver fat 2. Splenic fat ?. Weight change +0.24 kgm.

Control #74 - Dose per day 0.52 grams.

Duration 90 days. Average total cholesterol 349 mgm. Atherosclerosis 4. Lipaemia 2. Sex F. Liver fat 1. Splenic fat 1. Weight change +0.78 kgm.

Diabetic #92 - Dose per day 0.73 grams.

Duration 82 days. Average total cholesterol 703 mgm. Atherosclerosis 1. Lipaemia 3.2. Sex M. Liver fat 1. Splenic fat ?. Weight change -0.91 kgm.

Resistant #90 - Dose per day 0.73 grams.

Duration 82 days. Average total cholesterol 656 mgm. Atherosclerosis 3. Lipaemia 2. Sex F. Liver fat 4. Splenic fat 3. Weight change +0.24 kgm.

Control #200 - Dose per day 0.63 grams.

Duration 82 days. Average total cholesterol 632 mgm. Atherosclerosis 1. Lipaemia 1.25. Sex M. Liver fat 1. Splenic fat 2. Weight change +0.77 kgm.

See also #4 and #8; #41, #39 and #38; #192 and #186 in the following group

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Consideration of the above paired data indicates that, within the limits of pairing, diabetes inhibits the induction of atherosclerosis. Sex is not an essential factor. Weight gain or loss is not an essential factor. There is no difference in the behavior of alloxan resistant and of control animals in terms of the atherosclerosis and lipaemia induced. The diabetics have less fat in the liver and spleen. The resistants may possibly have more fat in these organs than the controls. And, the diabetics have a more marked lipaemia than do the non-diabetics.

A similar form of pairing may be done with the further refinement of introducing the factor of dose of cholesterol per day per kilogram of body weight into the above type of grouping. The body weight employed was that obtained by adding the weight at the commencement of feeding to that at death and dividing by 2.

The protocols of the animals paired for (1) the average daily dose per kgm. of body weight; (2) the number of days fed; (3) the average total cholesterol level

during cholesterol feeding are given below. It will be noted that the limits of tolerance for pairing are narrower than in the previous case.

Diabetic #117 - Dose per day per kgm. 357 mgm.

Duration 53 days. Average total cholesterol 197 mgm. Atherosclerosis 0. Lipaemia 2.75. Sex M. Liver fat 0. Splenic fat 0. Weight change -0.49 kgm.

Control #199 - Dose per day per kgm. 300 mgm.

Duration 52 days. Average total cholesterol 120 mgm. Atherosclerosis 1. Lipaemia 1. Sex M. Liver fat 4. Splenic fat 2. Weight change +0.35 kgm.

Diabetic #203 - Dose per day per kgm. 203 mgm.

Duration 82 days. Average total cholesterol 1970 mgm. Atherosclerosis 2. Lipaemia 1.6. Sex M. Liver fat 2. Splenic fat 3. Weight change +0.40 kgm.

Resistant #186 - Dose per day per kgm. 201 mgm.

Duration 82 days. Average total cholesterol 1849 mgm. Atherosclerosis 4. Lipaemia 1.5. Sex M. Liver fat 4. Splenic fat 3. Weight change +0.07 kgm.

Resistant #77 - Dose per day per kgm. 174 mgm.

Duration 76 days. Average total cholesterol 340 mgm. Atherosclerosis 3. Lipaemia 1.5. Sex M. Liver fat 4. Splenic fat 2. Weight change +0.57 kgm.

Control #202 - Dose per day per kgm. 191 mgm.

Duration 82 days. Average total cholesterol 334 mgm. Atherosclerosis 3. Lipaemia 0.75. Sex M. Liver fat 1. Splenic fat 1. Weight change +0.61 kgm.

Diabetic #4 - Dose per day per kgm. 145 mgm.

Duration 91 days. Average total cholesterol 90 mgm. Atherosclerosis 0. Lipaemia 0.8. Sex M. Liver fat 0. Splenic fat 1. Weight change -0.51 kgm.

Control #8 - Dose per day per kgm. 150 mgm.

Duration 91 days. Average total cholesterol 103 mgm. Atherosclerosis 1. Lipaemia 0. Sex M. Liver fat 0. Splenic fat 0. Weight change -0.08 kgm.
This animal was adult.

Resistant #60 - Dose per day per kgm. 132 mgm.

Duration 89 days. Average total cholesterol 123 mgm. Atherosclerosis 3. Lipaemia 1. Sex M. Liver fat ?. Splenic fat 1. Weight change +0.37 kgm.

Control #7 - Dose per day per kgm. 130 mgm.

Duration 90 days. Average total cholesterol 130 mgm. Atherosclerosis 1. Lipaemia 0. Sex F. Liver fat 1. Splenic fat 0. Weight change +0.28 kgm.

Diabetic #41 - Dose per day per kgm. 64 mgm.

Duration 91 days. Average total cholesterol 32 mgm. Atherosclerosis 0. Lipaemia 0. Sex M. Weight change +0.18 kgm.

Resistant #39 - Dose per day per kgm. 55 mgm.

Duration 91 days. Average total cholesterol 32 mgm. Atherosclerosis 0. Lipaemia 0. Sex M. Weight change +0.43 kgm.

Control #38 - Dose per day per kgm. 62 mgm.

Duration 91 days. Average total cholesterol 30 mgm. Atherosclerosis 0. Lipaemia 0. Sex M. Weight change +0.64 kgm.

Diabetic #52 - Dose per day per kgm. 142 mgm.

Duration 90 days. Average cholesterol (total) 71 mgm. Atherosclerosis 0. Lipaemia 0. Sex M. Weight change 0.29 kgm.

Control #57 - Dose per day per kgm. 113 mgm.

Duration 89 days. Average total cholesterol 63 mgm. Atherosclerosis 2. Lipaemia 0. Sex M. Weight change +0.87 kgm.

Resistant #75 - Dose per day per kgm. 137 mgm.

Duration 90 days. Average total cholesterol 367 mgm. Atherosclerosis 4. Lipaemia 1.8. Sex F. Liver fat 1. Splenic fat 0. Weight change +0.43 kgm.

Control #74 - Dose per day per kgm. 130 mgm.

Duration 90 days. Average total cholesterol 349 mgm. Atherosclerosis 4. Lipaemia 2. Sex F. Liver fat 1. Splenic Fat 1. Weight change +0.82 kgm.

This system of pairing includes several of the animals that were included before, but also introduces several other animals, and at the same time excludes still others. Essentially the same conclusions as previously may be drawn except that the animals are almost all males so that sex becomes a pair factor rather than a variable. It may also be noted that the data is too meagre to allow any conclusion related to the fat content of the liver and spleen of the alloxan resistant

animals as opposed to the control animals. However, the data provides control material of a highly paired type from which several variables have been largely eliminated - variables which the literature has shown to be of importance. It should be pointed out that pairing on the basis of cholesterol fractions or ratios has not been undertaken. However, neither the literature nor the data of Table I indicate the necessity of such a procedure.

If the data is paired within a given type of experimental animal some relevant data can be obtained. For example, in the diabetic series #35 and #40 form pairs showing no atherosclerosis. Similar pairs are formed by #4 and #52, and #53 and #1; #99 and #69 form a pair each with a one plus degree of atherosclerosis. Among the resistants #75 and #64 are a pair each with four plus arteriosclerosis. No. 54 and #60 form a pair with three plus sclerosis, and #77 and #194 form a pair with three plus and one plus sclerosis respectively. Among the controls #66 and #74 form a pair with four plus atherosclerosis while #46 and #57 have 0 and two plus sclerosis respectively. No. 38 and #45 are a pair each with no sclerosis and #96 and #98 are a pair with four plus and two plus sclerosis respectively. There is thus a tendency for atherosclerosis to occur in a uniform pattern within a uniform population. A similar analysis of pairs with reference to lipaemia leads to the same conclusion. Sex does not appear as an important factor in either the diabetic group or the alloxan resistant group. In the control group sex is paired and does not constitute a variable.

No. 85, a male, was excluded from the above data because, while its fasting blood sugar value was normal, the postprandial blood sugar was about 250 mgm.%. It was, therefore, classed as a semi-diabetic. This animal can be paired with Resistant #77, Control #84 and Control #202. The atherosclerosis induced in these animals was: #85, 1; #77, 3; #84, 2; and #202, 3. The lipaemic index was: #85, 1; #77, 1.5; #84, 1.25; and #202, 0.75. The pattern appears to be intermediate to that described above. The reaction of this animal to cholesterol induced athero-

sclerosis is that of a diabetic, but the lipaemic reaction is more like that of a non-diabetic animal.

It was mentioned in the section on materials and methods that an attempt was made to demonstrate a difference in the in vitro reaction of serum from diabetic and non-diabetic rabbits to crystalline cholesterol. Unfortunately, the data obtained is too fragmentary to report at this time. The reaction is variable and may be of minor character. It is apparent that such a study will require duplicate or preferably triplicate analyses under more carefully controlled conditions than were employed in the present study.

A morphologic study of various of the tissues of the animals was made. It may be noted that gross autopsy findings exclusive of the aorta were not remarkable except for a few features. Diabetic animals showed - if weight loss were a feature - severe wasting of all tissues except the skeleton. Even those animals which gained some weight were poor in adipose tissue and muscle. There was a mild retardation of skeletal growth, the gonads and adrenals were small. The tissues were dry and, in the case of death in coma, dehydration was a marked feature. In the case of death in coma the bladder contained so little urine that it was usually necessary to test the bladder wall for evidence of acetonuria. In the diabetics also the post mortem blood was usually sufficiently lipaemic to render the organs a pale creamy color. Among the non-diabetics the only striking features were a prominent adiposity, a marked increase in size and pallor of the adrenals and a slight increase in heart size.

Microscopic examination of the aortae of diabetic and non-diabetic animals was made in detail, the entire length of the aorta being examined. Inasmuch as the lesions induced in the tissues of the control animals were not found to differ from those studied by other workers, there is no need to describe them in detail. It should be pointed out, however, that

1. the diabetic state was not found to cause any morphological changes in the aorta

or other blood vessels,

2. the vessels of alloxan resistant animals were similar to those of control and diabetic animals,
3. the atheromata induced in diabetic animals differed in no qualitative respect from those induced in non-diabetic animals,
4. any morphologic differences noted (i.e. disintegration of an atheroma) were implicit in the quantitative differences existing between the atheromata induced in the two groups of animals, and
5. these findings were true, not only of the aorta, but of other vessels such as the coronary arteries and the splenic arterioles.

It is also interesting to note that lipoidal deposits were found frequently in the minute splenic and hepatic arteries or arterioles. These deposits were both in and between the fibres of the media of the vascular wall, were in the form of fine globules and occurred in the absence of histiocytic macrophages. On a few occasions a diffuse, non-particulate infiltration of lipid was noted in large collagen masses in the absence of foam cells. It was also striking to note the occasional blood vessel in the parenchyma of the kidney filled with strongly sudanophilic blood but showing an entirely normal vascular wall. Fat filled phagocytes were found rarely in the circulating blood of various organs, occurring most commonly in the capillaries of the lung.

Lipid deposits in the parenchyma of the adrenal, liver and spleen did not differ qualitatively in the various types of experimental animals. Tissues stained with Nile blue sulphate - a product recently marketed by Ciba Co., Montreal was found to be most satisfactory - showed no qualitative differences in the type or distribution of the lipid substances present.

Examination of various other organs such as the thyroid and pituitary glands disclosed neither quantitative nor qualitative differences between the various types of animals. On the other hand, the pancreas, kidney and gonads of

the diabetic animals all showed morphological changes. The alterations of the pancreas have been described already from this laboratory and will not be dealt with further, although we may mention that β-cell granules may persist for up to 3 days following alloxan injection. The kidneys showed the glycogen vacuolation of Armani in those cases in which hyperglycemia was present for more than about 10 days or 2 weeks. The Kimmelstiel-Wilson lesion was not noted. The gonads were more or less atrophic. The change was best seen in the testes in which a reduction of miosis and mitosis as well as of the sperm count was the rule. In a few cases the atrophy was severe. Finally, it was found that when alloxan necrosis of the adrenal or liver occurred, exceptionally fine examples of mitonecrosis were seen in the affected areas.

A histologic study of the tissues of those animals (series 14B) injected with thorotrast 1 to 4 days before death proved most interesting. The data is fragmentary, but offers an indication for future experimentation. Thorotrast was identified in the reticulo-endothelial system generally, and was found in foam cells that also contained the lipid globules induced by cholesterol feeding. It was identified in the parenchymal cells of the liver and adrenal gland cortex. It was seen in the blood stream, especially in the glomerular capillaries and occasionally in a phagocytic cell flowing in the blood. The kidney of rabbit #186 showed the only example of "spontaneous" glomerulo-nephritis that has been observed in more than 300 animals in this department. The lesion was essentially one of epithelial cell proliferation and crescent formation and was surprisingly similar to the subacute or late acute glomerulonephritis of man. The crescent cells of this lesion contained some thorotrast. In some of the other animals the cells of the glomeruli had also taken up a small amount of thorotrast, but it could not be definitely determined whether these were endothelial or epithelial in nature, although the latter appeared more probable. Some thorotrast was seen in cells of the renal tubular epithelium.

The most interesting observation was that the substance entered the endothelial cells of the aortic intima (and of other arteries as well). It caused a progressive filling and swelling of the endothelial cell until it became ovoid to globular in shape. An actual transformation of this cell to a "foam cell" like those of an atheroma was not actually demonstrated, but was suspected. Underneath such cells the foam cells of existent atheroma frequently showed thorotrast in their cytoplasm. This alteration occurred only in the most superficial layer of foam cells. A similar change was also noted in foam cells lying deep to a perfectly intact and normal endothelium. There was a suggestion that the substance deposited predominantly in areas that were already atheromatous. However, it was also found in the endothelium of portions of vascular wall that were otherwise normal.

The possible significance of these observations will be discussed later.

THE EFFECT OF ALLOXAN DIABETES ON THE REGRESSION OF EXPERIMENT CHOLESTEROL ATHEROSCLEROSIS IN THE RABBIT

The materials and methods employed in this experiment were essentially the same as those detailed previously. The exceptions were as follows -

1. Cholesterol feeding preceded the use of alloxan;
2. Cholesterol feeding was terminated the day before alloxan was administered to the selected rabbits, and neither they nor the control animals received further cholesterol feeding;
3. The dose of alloxan was standardized at 150 mgm. per kgm. of body weight;
4. All blood samples were taken in the non-fasting state;
5. No anti-diabetic therapy was employed;
6. Animals were killed at progressively increasing intervals of time after the cessation of cholesterol feeding, up to a maximum regression period of four months;
7. The conditions of cholesterol feeding were more uniform, a dose of 0.75 grams in 15 cc. of oil being used throughout.

As before, the relevant data on the surviving animals is shown in Table II below. A frequency distribution chart and diagrams of the animals' aortae are contained in the appendix.

TABLE II

EFFECT OF ALLOXAN DIABETES ON THE REGRESSION OF EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS IN THE RABBIT

Rabbit	Experimental Type *	Sex	Days of Cholesterol Feeding	Total Dose of Cholesterol (grams)	Weeks of Regression	Weight at Commencement of Cholesterol feeding (kgm.)	Weight at Commencement of regression period (kgm)	Weight at End of regression period (kgm)
134	D	F	90			2.760	4.500	4.500
144	D	F	90	61.5	1	2.880	3.660	3.510
166	D	M	88			2.780	4.040	4.040
162	C		88			2.660	3.830	3.840
126	D	F	90	61.5	3	2.960	4.270	3.110
136	D	F	90			3.010	4.660	2.910
129	C	F	90	61.5	5	2.710		4.510
130	C	F	90			3.020		5.090
160	D	F	88	61.5	6	2.600	3.960	4.260
124	D	F	90	61.5	7	3.090	4.600	3.890
141	C	M	90			2.560		3.840
121	D	F	89	62.5	9		4.340	2.060
158	C	F	88	61.5		2.600	3.890	3.110
165	S.D	F	88			3.100	4.560	3.570
133	D	M	90			2.750	3.860	2.260
163	R	M	88	61.5	11	2.500	4.050	4.220
164	R	M	88			2.400	4.050	3.940
143	R	M	90			2.970	3.750	3.710
128	D	F	90			2.740	3.080	2.140
142	D	F	90			2.890	4.130	3.010
131	C	F	90			3.000	4.450	6.100
137	C	M	90	61.5	16	2.880	3.200	4.370
145	C	M	90			2.830	3.710	4.270
146	C	M	90			2.750		4.740
148	C	F	90			2.630	4.610	5.300
154	C	F	90			2.420	3.600	4.060
172	D	F	59			2.760	4.130	4.200
178	D	F	59			2.450	3.580	3.600
173	C	F	59			2.640	3.780	3.330
176	C	M	59	40.5	6	2.700	3.320	3.330
179	C	F	59			3.200	4.200	4.260
180	C	F	59			2.270	3.580	4.270
170	R	M	59			3.040	3.860	4.100

* D= Diabetic; C= Control; R= Alloxan Resistant; S.D= Mild Diabetic.

TABLE II (cont'd)

EFFECT OF ALLOXAN DIABETES ON THE REGRESSION

OF EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS IN THE RABBIT

Rabbit	Experimental Type *	Average Blood Sugar During Regression	Average Values During Cholesterol Feeding Period				Lipaemia (0-4)	Aortic Atherosclerosis (0-4)	Content of Sudanophilic Substance (0-4)	
			Cholesterol							
			Free mg.%	Total mg.%	Ester mg.%	Ester %			Liver	Spleen
134	D		90	277	187	67	0.8	1	4	3
144	D	570	134	382	248	65	1.0	4	4	2
166	D	-	-	-	-	-	0.6	3	4	-
162	C	122	174	398	224	56	1.4	3	2	3
126	D	357	163	471	308	65	1.8	3	2	2
136	D	384	144	426	282	66	1.0	2	2	2
129	C	134	161	516	355	69	1.25	4	1	1
130	C	148	107	291	184	63	1.0	3	1	1
160	D	319	194	472	278	59	1.6	4	3	1
124	D	425	96	307	201	65	0.9	3	1	1
141	C	-	-	-	-	-	0.5	1	1	0
121	D	353	106	261	155	59	0.9	2	-	-
158	C	112	140	440	300	68	1.8	4	1	1
165	S.D	286	135	430	295	59	1.2	3	1	1
133	D	410	218	525	307	68	1.5	4	1	1
163	R	138	124	318	194	61	1.0	2	1	1
164	R	175	81	208	127	61	1.0	4	1	1
143	R	163	163	487	324	61	1.8	2	1	1
128	D	396	183	479	296	62	1.4	2	1	1
142	D	488	232	480	248	52	1.8	2	1	0
131	C	133	-	-	-	-	1.0	2	1	0
137	C	126	83	298	215	72	1.0	4	1	1
145	C	120	126	371	245	66	0.5	4	1	1
146	C	111	88	325	237	73	0.7	2	1	1
148	C	124	143	382	239	63	1.5	4	1	1
154	C	127	72	240	168	70	0.5	3	0	0
172	D	309	140	320	180	56	0.6	3	0	0
178	D	373	88	262	174	66	0.6	3	0	0
173	C	130	138	424	286	66	0.6	4	0	1
176	C	120	41	187	146	78	0.6	3	1	0
179	C	128	145	452	307	68	1.3	3	0	0
180	C	125	71	214	143	67	1.3	2	1	0
170	R	124	83	270	187	68	1.0	4	1	1

* D= Diabetic; C= Control; R= Alloxan Resistant; S.D= Mild Diabetic.

An examination of the data suggests that the induction of alloxan diabetes does not alter the rate of regression of the lesions of experimental cholesterol atherosclerosis within the time limits examined, as judged by gross examination of the stained aorta.

As before it is advisable to test this conclusion under more closely controlled conditions. The animals whose protocols are presented below have been paired in respect to -

1. Dose of cholesterol per day per kgm. of average body weight,
2. Days of cholesterol feeding,
3. Days of regression or diabetes, and
4. Average total serum cholesterol level during the feeding period.

The additional factor of days of regression renders the number of obtainable pairs rather small.

Diabetic #144 - Dose per day per kgm. 207 mgm.

Duration 90 days. Average total cholesterol 382 mgm. Regression 1 week. Atherosclerosis 4.

Control #162 - Dose per day per kgm. 216 mgm.

Duration 88 days. Average total cholesterol 398 mgm. Regression 1 week. Atherosclerosis 3.

Diabetic #172 - Dose per day per kgm. 198 mgm.

Duration 59 days. Average total cholesterol 320 mgm. Regression 6 weeks. Atherosclerosis 3.

Resistant #170 - Dose per day per kgm. 197 mgm.

Duration 59 days. Average total cholesterol 270 mgm. Regression 6 weeks. Atherosclerosis 4.

Diabetic #178 - Dose per day per kgm. 222 mgm.

Duration 59 days. Average total cholesterol 262 mgm. Regression 6 weeks. Atherosclerosis 3.

Control #180 - Dose per day per kgm. 233 mgm.

Duration 59 days. Average total cholesterol 214 mgm. Regression 6 weeks. Atherosclerosis 2.

Diabetic #133 - Dose per day per kgm. 206 mgm.

Duration 90 days. Average total cholesterol 525 mgm. Regression 11 weeks. Atherosclerosis 4.

Resistant #143 - Dose per day per kgm. 202 mgm.

Duration 90 days. Average total cholesterol 487 mgm. Regression 11 weeks. Atherosclerosis 2.

Diabetic #142 - Dose per day per kgm. 194 mgm.

Duration 90 days. Average total cholesterol 480 mgm. Regression 16 weeks. Atherosclerosis 2.

Control #148 - Dose per day per kgm. 188 mgm.

Duration 90 days. Average total cholesterol 382 mgm. Regression 16 weeks. Atherosclerosis 4.

The above paired data does not demonstrate that alloxan diabetes alters the rate of regression of experimental cholesterol atherosclerosis up to the duration of regression examined.

The data does not allow the pairing of alloxan resistant and control animals.

If the control animals of this experiment are paired with those of the previous experiment on the same basis as detailed above, the following data are obtained.

Control #108 - Experiment 1

Atherosclerosis 1. No regression.

Control #180 - Experiment 2

Atherosclerosis 2. Regression 6 weeks.

Resistant #164 - Experiment 2

Atherosclerosis 4. Regression 11 weeks.

Control #154 - Experiment 2

Atherosclerosis 3. Regression 16 weeks.

Control #109 - Experiment 1

Atherosclerosis 3. Regression none.

Control #162 - Experiment 2

Atherosclerosis 3. Regression 1 week.

Control #173 - Experiment 2

Atherosclerosis 4. Regression 6 weeks.

Resistant #143 - Experiment 2

Atherosclerosis 2. Regression 11 weeks.

Control #62 - Experiment 1

Atherosclerosis 4. Regression none.

Resistant #170 - Experiment 2

Atherosclerosis 4. Regression 6 weeks.

Control #163 - Experiment 2

Atherosclerosis 2. Regression 11 weeks.

Resistant #64 - Experiment 1

Atherosclerosis 4. Regression none.

Control #148 - Experiment 2

Atherosclerosis 4. Regression 16 weeks.

The data does not indicate that any grossly detectible regression of atheromata has occurred in the second experimental procedure in either the control or in the non-diabetic animals. There is no evidence that the behavior of the alloxan resistant animals is different from that of either diabetic or control animals in this respect.

Histologic examination of the aortic lesions merely showed the changes of early regression that are described by other authors. As in the previous experiment no qualitative morphological differences were found. Moreover, no quantitative differences were noted. Examination of various other organs gave findings

similar to those of the previous experiment except with reference to the lipid content of the liver, spleen and adrenal. These organs in both diabetic and non-diabetic animals showed a progressive depletion of their excess lipid content until, from the 9th to the 16th weeks of regression, only traces of excess lipid remained. The rate of disappearance of the excess lipid appeared to be the same in both diabetic and non-diabetic animals.

It must be noted that the blood cholesterol levels of all animals had returned to normal levels before 40 days after the cessation of cholesterol feeding. Among the diabetic group, however, the average level about 2 weeks after the induction of diabetes and the termination of cholesterol feeding was appreciably higher among the diabetics than in the control series. This was due to the occasional occurrence of an early diabetic hypercholesteraemia.

Finally, it may be noted that none of the 8 animals in the group whose regression period was of 16 weeks duration showed lipid in the splenic arterioles while most of the animals from groups with lesser regression periods, had this lesion. The opposite result occurred with respect to atherosclerosis of the coronary arteries however, for these fibrotic and fatty lesions became progressively more common with the increasing duration of regression, until 6 of the 8 animals in the group of 16 weeks regression showed coronary artery atherosclerosis in the single block of heart that was examined. Diabetes was not shown to affect these results.

CONCLUSIONS AND DISCUSSION

It is concluded from the above data that within the terms of reference of the experimental procedure,

1. Alloxan diabetes exerted a marked but incomplete inhibitory effect on the induction of experimental cholesterol atherosclerosis in the rabbit.
2. Alloxan diabetes did not alter the rate or type of regression of experimental cholesterol atherosclerosis in the rabbit.

The first conclusion appeared to be independent of the form in which cholesterol was fed; of the dose used; of the weight of the animal; of the number of days of cholesterol feeding; of the average total serum cholesterol level attained; of the weight change during the experimental period; of the sex of the animal; of the morphology of the vasculature, thyroid gland and various other organs; and of the morphology of the resulting atherosclerotic lesions.

It was also concluded that alloxan diabetes inhibited the deposition of sudanophilic substances in the reticulo-endothelial system generally and in the parenchyma of the liver and adrenal gland. The diabetic animals were found to suffer from a greater degree of, and a more rapidly appearing condition of lipaemia than were non-diabetic ones. No differences were demonstrated between control and alloxan resistant animals in respect to the above conclusions so that they were found to be independent of the use of alloxan per se, and of the employment of insulin and dextrose therapy during the initial phases of the procedure followed in the first experiment.

The second conclusion was independent of the dose of cholesterol, of the duration of cholesterol feeding, of the weight of the experimental animal, of the average total serum cholesterol level attained during the feeding period, of the time of regression, of the morphology of the thyroid gland and other organs and of the degree of the atherosclerotic lesions. It was also found that the regression of other lipid deposits caused by the cholesterol feeding procedure was probably not affected by the presence or absence of the diabetic state. It was found

that grossly detectable regression of the atherosclerosis caused by cholesterol feeding was not demonstrable after a regression period of up to four months, but there was obvious microscopic evidence of regression of such lesions in both the aorta and in the splenic arterioles.

The conclusions and observations of other workers who have fed cholesterol to normal rabbits under similar conditions were, in the main, substantiated.

The experimental procedure involving the use of thorotrast led to the conclusion that the vascular endothelial cells were capable of either active or passive absorption of particulate matter from the blood stream and that the foam cells of cholesterol induced atheromata were capable of the same reaction. There was evidence suggestive of the conversion of endothelial cells to phagocytes of the "foam-cell" type, and of some localizing factor related to the presence of atheromata which tended to cause localization of thorotrast in these areas. Thorotrast depositions were found in both a diabetic and in non-diabetic animals without morphologic distinction, but it must be remembered that the diabetic animal #192 was the only diabetic animal in which atherosclerotic lesions of two plus degree were found.

The variable or unpaired features of this experiment were chiefly those related to -

1. The differential deposition of lipids in the diabetic and non-diabetic tissues,
2. The differential general clinical features dependent upon the diabetic state with the exception of weight changes.

It was, therefore, concluded that some factor or factors related to the diabetic state were capable of inhibiting the deposition of lipid substances in the tissues without inhibiting the development of the usual hypercholesteraemia associated with cholesterol feeding. Indeed, there is some evidence that the diabetic animals suffered a somewhat greater degree of hypercholesteraemia than did the non-diabetic ones, and they certainly suffered a greater degree of hyperlipaemia.

The data of the experiment does not allow a rational explanation of these facts. One is struck by the obvious conclusion that hypercholesteraemia alone is not a sufficient causal factor in the production of atherosclerosis in the rabbit. If we return to the concept of atherosclerosis as the product of a reaction between the blood, the interstitial fluids, the endothelium and the vascular wall, we shall be able to indicate at least possible explanations of the experimental result. It is possible that the diabetic state may have introduced some physico-chemical change into the blood (and the interstitial fluids) which might prevent the intra- or extracellular absorption of cholesterol. So little is known of the state of the blood in alloxan diabetes that it is futile to pursue this explanatory path further at this time. The hyperglycaemic state may be presumed to favor the precipitation of cholesterol. A lipaemic or acetonaemic state would favor the solution of cholesterol. One may consider that a dedispersed state of cholesterol could form a film over the endothelium which would prevent the entery of the substance into the vessel wall, but such a phenomenon could also favor endothelial phagocytosis and atheroma formation. A greatly increased solubility of cholesterol might be presumed to hinder atheroma formation. It is of some interest to note that the total blood iodine is apparently elevated in alloxan diabetes and it might possibly be expected that this factor has an inhibitory effect similar to that obtained by feeding potassium iodide to intact cholesterol fed animals, but it is to be noted that potassium iodide is an effective inhibitor of atherosclerosis, only if fed in large amount and that this effect is accompanied by a reduction of the quantity of cholesterol in the blood. Small amounts of potassium iodide appear to increase both the quantity of cholesterol in the blood and the degree of induced atherosclerosis. Nevertheless, the data relating to the action of the thyroid gland and of iodine on experimental cholesterol atherosclerosis are sufficiently confusing to make further study advisable. No morphological evidence of altered thyroid function was demonstrated in the present experiment.

As regards the role of the endothelium in the atherosclerotic reaction, very little can be said. There was no morphological evidence that the diabetic state had altered the vascular endothelium, even in its reaction to thorotrast. It may be indicated, however, that the diabetic state apparently reduces the phagocytic ability of the blood phagocytes. If one adheres to the lipophage theory of Leary or to the conception that vascular endothelial cell phagocytic activity are fundamental to the genesis of atherosclerosis, then the diabetic state might inhibit the development of atherosclerosis. The preliminary data obtained by the use of thorotrast, however, are somewhat opposed to this conception, for if the Leary theory is held then it is difficult to understand how foam cells containing thorium dioxide (specific gravity 9.7) could lie at the periphery of the axial blood stream and so invade the intima, while the endothelial concept lacks support from the present experimental data because thorium dioxide was found in the endothelial phagocytes of the only diabetic animal examined. It is possible, however, to interpret the data to indicate that the phagocytic ability of the endothelium, as demonstrated by thorotrast is fundamental to the genesis of atherosclerosis, and that its occurrence in the diabetic animal was due to the experimental conditions or to the fact that the diabetic state does not influence colloidal thorotrast in the same manner as it does blood borne cholesterol.

Consideration of the role of the vascular wall in the genesis of atherosclerosis offers little information which may find application in the present experiment. The diabetic state has not been shown to influence the morphological structure of the vascular wall. Whether or not the physico-chemical properties of the collagen were altered by the diabetic state cannot be stated. Nevertheless, it is quite possible that the ability of the collagen to act as an adsorption medium for lipids and cholesterol has been altered by the diabetic condition.

It is, perhaps, a mistake to concentrate too much attention on the atherosclerotic reactions that were elicited in this experiment. The data, stated in a

simple form shows that the cholesterol-fed, diabetic animal had less lipid and cholesterol than the comparable non-diabetic, not only in the vascular system, but also in the parenchymal cells of the liver and adrenal. (The reticulo-endothelial cells of the liver, spleen and blood vessels are considered as part of the vascular system for the purpose of the present argument). This effect occurred in spite of comparable amounts of cholesterol existing in the serum of the diabetic and non-diabetic animals. The data does not allow us to say whether either the amount of fat in the aorta or the amount of fat in the liver and adrenal are causally related, or whether both are secondary to some third factor and are not causally related. If the latter hypothesis be true, then any explanation of the inhibition of experimental cholesterol atherosclerosis in the diabetic rabbit must also explain the inhibition of lipid deposition in the liver and adrenal. The mechanism of the inhibition of atherosclerosis would thus be placed on a somewhat different basis than heretofore, inasmuch as the relevant factors now become the blood, the cell membrane, and the internal metabolism of the cell. It remains possible, however, for the causal factor to influence the vasculature and the parenchymal cells by different mechanisms, and it is possible that two or more causal agents implicit in the diabetic state effect the phenomena which have been observed. The results of the experiment concerned with the regression of atherosclerosis under the influence of the diabetic state indicate by their negative nature, that the factor or factors concerned are operative in the deposition and accumulation of lipid material. They do not appear to operate in its mobilization from within or between cells. Any explanation of the experimental result must also take cognizance of, and be compatible with this fact.

One further experimental variable was gonadal atrophy among the diabetic animals. The literature relevant to the action of the sex hormones in experimental cholesterol atherosclerosis has been reviewed in the first part of this thesis. It is not possible to correlate those reports with the results of the present

experiment except to indicate that any effect induced by these hormones upon atherosclerosis appears to be directly related to their effect upon the quantity of cholesterol in the serum.

It is not possible to explain the present result. The only comparable experiments existing in the literature are those of Malisoff[66], of Hueper[67], of Eberhard[163] and of Page and Bernhard[147]. Of these reports only the first two, relating to the inhibitory action of potassium thiocyanate are worthy of serious consideration, and they are so fragmentary as to be inconclusive. Their work should be repeated.

The experiment does not throw much light upon the validity of the various theories of the genesis of atherosclerosis. It is quite incompatible with the senescent theory since the inhibitory action was expressed in the face of factors which are presumed to enhance tissue dehydration and senescence. It is incompatible with the toxic theory unless the diabetic state is presumed to "detoxify" the cholesterol administered, or unless the diabetic state is considered to elicit the alarm reaction of Selye in virtue of its toxicity and thereby inhibit experimental cholesterol atherosclerosis. It is incompatible with the anoxaemic theory of Hueper unless the formation of macromolecular films is prevented by the diabetic state. The theory of Winternitz is not supported since no relevant morphological changes were noted. Leary's lipophage theory is discounted by the absence of lipophages in the livers of two animals, each with aortic atherosclerosis, and by the results of the experiment employing thorotrast. There remains the imbibition theory which is not supported unless it is postulated that the factors which influence imbibition are altered by the diabetic state. The theory of imbibition and that of Hueper would seem to provide the best basis for further investigation.

It may be of some value to list some of the experimental concepts which this work develops. It is necessary that the work should be confirmed by another laboratory, and in a more complete and fully controlled study. It is advisable to

continue the regression experiment for a longer time than was possible in the present case. An experiment to test the effect of diabetes, partially or completely controlled by insulin, upon cholesterol feeding is necessary. The effect of types of experimental diabetes other than that induced by alloxan should be studied including the effect of a high sugar diet.

It would be interesting to test the action of alloxan diabetes on the atherosclerosis induced by intravenous cholesterol, by macromolecular substances and by agents which deposit calcium in vascular walls. The further study of surface active agents and perhaps of hyaluronidase in relation to diabetes and experimental atherosclerosis might provide useful information. The lipase group of enzymes are worthy of investigation.

The most useful approach to the problem would seem to lie in a systematic study of the physical and chemical properties of the serum of diabetic and non-diabetic animals in the presence and absence of hypercholesteraemia. Such differences as may be found should be confirmed in human material if possible and the isolation of the relevant variable or variables undertaken.

Experiments involving the ablation of various endocrine organs, or the use of hormonal and biological supplements might prove useful.

The bearing of the experimental result upon the problem of human arteriosclerosis is obscure. The postulates upon which the experiment was founded are valid to the extent that human diabetic patients suffer from an increased morbidity of and mortality from arteriosclerosis and occlusive vascular disease. That such persons have more than the expected degree of atherosclerosis is not adequately established but seems probable. The experimental result is apparently at variance with the postulates which it was designed to test. At any rate, if it is not opposed to observations which find that human diabetic patients do not actually suffer an increased degree and incidence of atherosclerosis, then it finds no confirmation in any observations which purport to find a decreased incidence and degree of atherosclerosis in human diabetics. It may be that the experiment was

not, in fact, designed to test the postulates which were derived from observation of human material, but that a study of experimental calcific vascular sclerosis and diabetes would have been more appropriate. However, one cannot escape the experimental conclusion that alloxan diabetes inhibits the development of experimental cholesterol atherosclerosis in the rabbit in the presence of hypercholesteraemia.

In addition to questioning the original hypothesis, one must also examine whether the experimental conditions were compatible with the corresponding conditions existing in man. Is experimental cholesterol atherosclerosis comparable to atherosclerosis in man? The answer was sought without success in the initial portion of this thesis. There is a high degree of similarity between the lesions of the two conditions, but differences which may be considered of greater or lesser importance exist. The influence of these differences is unknown at present. A somewhat similar situation exists with reference to human diabetes mellitus[106] and experimental alloxan diabetes[221-235]. The latter condition has not been discussed in the previous part of this paper because of a lack of adequate information. An exhaustive biochemical study of alloxan diabetes is necessary to elucidate this problem. At present there exists a high degree of similarity between the two conditions, but the available data is too meagre to allow valid conclusions.

It is apparent that the experimental result must be accepted without explanation and without the development of any analogue between it and the occurrence of atherosclerosis in man. The causal factor or factors of the effects observed reside in the diabetic state induced by alloxan. More than this cannot be stated.

SUMMARY - PART II

Data has been presented which demonstrates that alloxan diabetes inhibits the development of experimental cholesterol atherosclerosis in the rabbit. The effect was found to be independent of several of the experimental variables including the degree of hypercholesteraemia induced by feeding cholesterol. It was also found that alloxan diabetes could not be shown to hasten or alter the regression of the lesions of experimental cholesterol atherosclerosis. No explanation of these phenomena was derived from the available data.

CLAIM OF ORIGINAL WORK

The results reported in this experiment are, in the main, original. The finding of the inhibition of cholesterol atherosclerosis by alloxan diabetes, and of the failure of alloxan diabetes to influence the regression of cholesterol atherosclerosis has not been reported previously. The author may also claim the demonstration of glycogen vacuolation of the kidney, and of hydropic changes in the pancreas in alloxan diabetes as original observations. The observation of experimentally introduced thorium dioxide in endothelial cells and lipophages of the aorta of rabbits with experimental cholesterol atherosclerosis has not been reported previously.

CHOLESTEROL FEEDING SCHEDULE

Nos. 1 - 8.

Series 1

Day	Date	Day	Date	Day	Date	
0	Nov.30	30	Dec.30	60	Jan.29	Nov. - Feb. 1945
1	Dec. 1	31	31	61	30	
2	- -	32	- -	62	- -	Dose 0.5 gms. powdered
3	- -	33	Jan. 2	63	- -	cholesterol in No.00 P.D.
4	4	34	3	64	Feb. 2	gelatin capsules.
5	5	35	4	65	3	
6	6	36	5	66	4	
7	7	37	- -	67	5	
8	8	38	7	68	- -	Total time interval 91 days.
9	- -	39	8	69	7	
10	10	40	9	70	8	
11	11	41	10	71	9	
12	12	42	11	72	- -	Total number of doses 76.
13	13	43	12	73	11	
14	14	44	13	74	12	
15	15	45	14	75	13	
16	- -	46	15	76	14	Total amount of cholesterol
17	17	47	16	77	15	38.0 gms.
18	18	48	17	78	16	
19	19	49	18	79	- -	
20	20	50	19	80	18	
21	21	51	20	81	19	
22	22	52	21	82	20	
23	23	53	- -	83	21	
24	24	54	23	84	22	
25	- -	55	24	85	23	
26	- -	56	25	86	- -	
27	27	57	26	87	25	
28	28	58	27	88	26	
29	29	59	28	89	27	
				90	28	

Oct. 5/45 to Mar. 1/46 — Female — No. 1. Series 1.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Oct. 5/45	2.336	111	20	68	0
15	2.338	97	12	56	0
17	2.555	Alloxan administered			
19	2.610	83	16	64	0
24	2.737	440	4	40	0
Nov. 2	2.624	284	16	56	0
7	2.620	340	12	56	0
15	2.583	423	16	44	0
21	2.483	362	12	28	0
29	2.449	390	19	52	0
30		Cholesterol feeding begun			
Dec.13	2.352	560	24	48	0
19	2.410	512	14	46	0
27	2.254	518	28	72	0
Jan. 4/46	2.220	466	6	32	0
16	2.220	486	16	28	0
31	2.240	548	8	24	0
Feb.13	2.110	540	13	44	0
Mar. 1	1.980	635	36	128	0
		Killed			

Oct. 5/45 to Dec. 1/45 — Female — No. 2. Series 1.

Oct. 5/45	1.690	110	20	48	0
15	2.137	97	12	28	0
17	2.373	Alloxan administered			
19	2.323	224	20	56	2
24	2.388	318	8	36	2
Nov. 2	2.200	300	232	256	4
7	2.056	336	152	240	4
15	2.040	462	132	156	3
21	1.970	371	20	40	1
29	1.895	456	49	78	0
30		Cholesterol feeding begun			
Dec. 1		Died			

Oct. 5/45 to Nov. 1/45 — Male — No. 3. Series 1.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Oct. 5/45	2.470	114	12	32	0
15	2.500	84	12	36	0
17	2.744	Alloxan administered			
19	2.730	188	12	40	0
24	2.740	412	4	32	0
Nov. 1		Died			

Oct. 5/45 to Mar. 1/46 — Male — No. 4. Series 1.

Oct. 5/45	2.881	110	24	40	0
15	3.180	123	12	36	0
17	3.430	Alloxan administered			
19	3.269	366	24	56	2
24	3.270	304	12	48	2
Nov. 2	3.200	275	232	296	4
7	3.200	302	208	296	4
15	3.180	426	156	176	2
21	3.130	398	88	112	1
29	3.150	424	23	52	1
30		Cholesterol feeding begun			
Dec.13	3.075	470	68	112	1
19	2.995	444	24	47	1
27	2.998	502	56	90	1
Jan. 4/46	3.045	460	58	100	1
16	2.980	386	66	140	2
31	2.960	474	39	70	1
Feb.13	2.680	464	22	81	0
Mar. 1	2.640	554	51	116	0
		Killed			

Oct. 5/45 to Mar. 1/46 Female

No. 5.
Series 1.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Oct. 5/45	2.268	89	32	72	0
15	2.320	91	24	112	0
19	2.507	88	28	96	0
24	2.614	94	28	96	0
Nov. 2	2.970	86	24	68	0
7	3.120	106	16	64	0
15	3.385	115	20	32	0
21	3.400	87	12	28	0
29	3.540	94	14	32	0
30		Cholesterol feeding begun			
Dec.13	3.710	120	22	68	0
19	3.800	73	13	41	0
27	3.910	85	12	44	0
Jan. 4/46	3.955	89	17	66	0
16	4.060	78	21	44	0
31	4.130	107	27	98	0
Feb.13	4.140	102	38	156	0
Mar. 1	4.060	116	57	212	0
		Killed			

No. 6 omitted from experiment.

Oct. 5/45 to Mar. 1/46 — Female — No. 7. Series 1.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Oct. 5/45	2.370	94	16	40	0
15	2.510	90	16	68	0
19	2.637	103	16	72	0
24	2.649	96	16	60	0
Nov. 2	2.863	83	16	68	0
7	2.880	83	16	68	0
15	2.998	106	20	48	0
21	3.060	88	12	32	0
29	3.084	98	14	50	0
30		Cholesterol feeding begun			
Dec.13	3.404	96	10	47	0
19	3.410	74	20	88	0
27	3.470	83	14	68	0
Jan. 4/46	3.660	75	25	50	0
16	3.740	109	21	112	0
31	3.650	103	62	212	0
Feb.13	3.700	110	35	240	0
Mar. 1	3.356	119	99	304	0
		Killed			

Oct. 5/45 to Mar. 1/46 — Male — No. 8. Series 1.

Oct. 5/45	2.675	106	20	36	0
15	2.745	91	8	28	0
19	2.838	89	8	44	0
24	2.850	94	8	32	0
Nov. 2	2.773	83	12	36	0
7	2.760	81	12	42	0
15	2.800	97	4	20	0
21	2.782	96	12	20	0
29	2.840	120	26	50	0
30		Cholesterol feeding begun			
Dec.13	2.812	112	4	28	0
19	2.820	78	10	42	0
27	2.824	88	8	24	0
Jan. 4/46	2.825	86	6	20	0
16	2.870	84	25	32	0
31	2.850	126	22	128	0
Feb.13	2.750	104	50	304	0
Mar. 1	2.760	104	90	296	0
		Killed			

Nos. 9 to 17 inclusive were omitted from experiment.

CHOLESTEROL FEEDING SCHEDULE

Nos. 18 - 28.

Series 2

Day	Date	
0	Mar.20	Mar. - Apr. 1946
1	21	
2	22	Dose 0.25 gms. of cholesterol
3	23	in 7.5 cc. sunflower seed oil.
4	- -	Dissolved in boiling water
5	- -	bath and kept at room tempera-
6	- -	ture.
7	27	
8	28	
9	29	Total time interval 14 days.
10	30	
11	31	
12	Apr. 1	Total number of doses 11.
13	2	
		Total amount of cholesterol 2.75 gms.

Jan.17/46 to Apr. 1/46 — Male — No. 18. Series 2.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Jan.17	2.010	124	11	28	0
20		98	14	28	0
21	2.206	Alloxan administered			
24	2.125	76	8	28	0
Feb. 6	2.205	350	10	44	0
20	2.350	320	16	44	0
Mar. 6	2.510	440	13	42	0
20	2.510		25	56	0
		Cholesterol feeding begun			
Apr. 1		Died			

Jan.17/46 to Apr. 8/46 — Male — No. 19. Series 2.

Jan.17	2.210	122	7	40	0
20		83	10	18	0
21	2.304				
24	2.255	97	4	10	0
Feb. 6	2.330	93	6	32	0
20	2.620	89	8	40	0
Mar. 6	3.060	97	17	40	0
20	2.940		11	32	0
		Cholesterol feeding begun			
Apr. 3		101	8	26	0
		Cholesterol feeding stopped			
8		Died			

Jan.17/46 to Feb.20/46 — Female — No. 20. Series 2.

Jan.17	1.900	118	10	54	0
20		90	15	68	0
21	2.106				
24	2.200	101	9	24	0
Feb.20	2.000	132	18	64	0
		Killed			

Jan.17/46 to Mar.21/46 — Male — No. 21. Series 2.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Jan.17	2.180	128	9	36	0
20		83	5	16	0
21	2.231	Alloxan administered			
24	2.060	118	4	24	0
Feb. 6	1.985	372	6	20	0
20	2.100	342	9	40	0
Mar. 6	2.300	412	12	32	0
20	2.270		16	42	0
		Cholesterol feeding begun			
21		Killed			

Jan.17/46 to Mar.22/46 — Male — No. 22. Series 2.

Jan.17	1.700	86	6	24	0
20		86	7	12	0
21	1.822				
24	1.765	99	8	36	0
Feb. 6	2.025	111	6	36	0
20	2.140	99	8	36	0
Mar. 6	2.380	104	9	24	0
20	2.410		14	36	0
		Cholesterol feeding begun			
22		Died			

Jan.17/46 to Mar.16/46 — Male — No. 23. Series 2.

Jan.17	2.250	112	6	16	0
20	2.300	87	6	12	0
21	2.337	Alloxan administered			
24	2.225	356	6	12	0
Feb. 6	2.135	372	36	80	+
20	1.960	358	15	28	0
Mar. 6	1.680	404	8	28	0
16	1.250	Died			

Jan.17/46 to Jan.31/46 — Female — No. 24. Series 2.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Jan.17	1.950	103	13	46	0
20	2.120	90	8	27	0
21	2.190	Alloxan administered			
24	1.830	410	56	116	0
31	1.360	Died			

Jan.17/46 to Mar.24/46 — Female — No. 25. Series 2.

Jan.17	2.500	102	7	28	0
20	2.506	82	6	10	0
21	2.506				
24	2.450	110	8	22	0
Feb. 6	2.605	105	6	42	0
20	2.550	97	15	60	0
Mar. 6	2.560	98	11	48	0
20	2.770	Cholesterol feeding begun			
24		Died			

Jan.17/46 to Mar. 1/46 — Female — No. 26. Series 2.

Jan.17	1.900	117	11	65	0
20	1.875	96	9	32	0
21	1.845	Alloxan administered			
24	1.930	108	31	42	+
Feb. 6	1.525	374	480	860	++++
20	1.370	338	62	164	+
Mar. 2	1.200	Died			

Jan.17/46 to Jan.23/46 — Female — No. 27. Series 2.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0–4)
Jan.17	2.700	117	8	36	0
20	2.720	105	9	29	0
21	2.750	Alloxan administered			
23		Died			

Jan.17/46 to Mar.21/46 — Female — No. 28. Series 2.

Jan.17	1.800	99	10	50	0
20	1.850	82	12	26	0
21	1.980				
24	1.990	107	11	18	0
Feb. 6	2.200	110	8	72	0
20	2.340	102	16	58	0
Mar. 6	2.220	111	22	72	0
20	2.460		20	58	0
21		Cholesterol feeding begun Died			

Nos. 29, 30 and 31 were omitted from experiment.

CHOLESTEROL FEEDING SCHEDULE

Nos. 32 - 42.
Series 3

Day	Date	Day	Date	Day	Date
0	Apr.25	30	May 25	60	- -
1	26	31	26	61	June 25
2	- -	32	- -	62	26
3	- -	33	28	63	- -
4	- -	34	29	64	28
5	30	35	- -	65	29
6	May 1	36	31	66	30
7	2	37	June 1	67	- -
8	3	38	2	68	July 2
9	4	39	- -	69	3
10	5	40	4	70	- -
11	6	41	5	71	5
12	7	42	- -	72	6
13	8	43	7	73	7
14	9	44	8	74	- -
15	10	45	9	75	9
16	- -	46	- -	76	10
17	- -	47	11	77	- -
18	13	48	12	78	12
19	14	49	13	79	- -
20	15	50	14	80	14
21	16	51	15	81	- -
22	- -	52	- -	82	16
23	18	53	17	83	17
24	19	54	18	84	- -
25	- -	55	19	85	19
26	21	56	- -	86	- -
27	22	57	21	87	21
28	- -	58	22	88	- -
29	24	59	23	89	23
				90	24

Apr. - July 1946

Dose 0.25 gms. of cholesterol in 7.5 cc. of sunflower seed oil. Dissolved at 100°C. and maintained at room temperature.

Fed by 14F catheter.

Total time interval 91 days.

Total number of doses 65.

Total amount of cholesterol 16.25 gms.

Mar. 7/46 to Mar.26/46 — Male — No. 32. Series 3.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Mar. 7	3.100	112	7	16	0
13	3.200	110	6	18	0
14		Alloxan administered			
26		Died			

Mar. 7/46 to Mar.13/46 — Male — No. 33. Series 3.

Mar. 7	2.230	104	12	25	0
13	2.250	111	6	36	0
		Died			

Mar. 7/46 to May 20/46 — Male — No. 34. Series 3.

Mar. 7	2.800	104	9	20	0
13	2.820	97	6	24	0
14		Alloxan administered			
16	3.040	486	10	50	0
28	2.850	306	7	24	0
Apr.10	2.950	322			
24	2.830	342	15	28	0
25		Cholesterol feeding begun			
May 8	2.854	348	24	56	0
20		Died			
	2.820		16	92	0

Mar. 7/46 to July 25/46 — Male — No. 35. Series 3.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Mar. 7	3.010	101	16	28	0
13	3.130	106	11	72	0
14		Alloxan administered			
16	3.350	494	25	80	0
28	3.110	364	38	62	0
Apr.10	3.030	402	10	26	0
24	2.980	338	8	44	0
25		Cholesterol feeding begun			
May 8	2.990	418	42	76	0
22	2.810	496	50	108	0
June 5	2.780	586	42	60	0
19	2.880	470	48	72	0
July 3	2.740	490	62	100	+
10	2.760	670	160	184	++
24	2.610	600	130	270	+
25		Killed			

No. 36 omitted from experiment.

Mar. 7/46 to June 5/46 — Male — No. 37. Series 3.

Mar. 7	1.920	100	7	16	0
13	1.970	103	6	44	0
16	2.020	90	10	32	0
28	2.200	87	10	26	0
Apr.10	2.370	92	8	44	0
24	2.420	94	6	28	0
25		Cholesterol feeding begun			
May 8	2.370	113	34	50	0
22	2.310	114	40	78	0
June 5	1.860	Died			
			96	204	0

Mar. 7/46 to July 25/46 — Male — No. 38. Series 3.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Mar. 7	1.750	117	10	12	0
13	1.850	113	12	68	0
16	1.920	109	24	64	0
28	2.080	101	10	40	0
Apr.10	2.270	91	10	28	0
24	2.560	105	6	29	0
25		Cholesterol feeding begun			
May 8	2.690	112	16	44	0
22	2.720	105	16	44	0
June 5	2.990	106	10	32	0
19	2.980	112	10	16	0
July 3	3.090	119	8	16	0
10	3.140	113	12	27	0
24	3.200	102	10	32	0
25		Killed			

Mar. 7/46 to July 25/46 — Male — No. 39. Series 3.

Mar. 7	2.000	122	16	28	0
13	2.070	114	6	50	0
14		Alloxan administered			
16	2.520	134	26	80	0
28	2.530	98	9	39	0
Apr.10	2.700	84	12	24	0
24	3.080	111	6	29	0
25		Cholesterol feeding begun			
May 8	3.070	97	28	44	0
22	3.110	109	18	48	0
June 5	3.270	106	16	32	0
19	3.390	103	10	24	0
July 3	3.450	107	12	16	0
10	3.410	126	10	20	0
24	3.510	110	10	46	0
25		Killed			

Mar. 7/46 to July 25/46 — Male — No. 40. Series 3.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Mar. 7	2.110	107	9	28	0
13	2.200	115	6	44	0
14		Alloxan administered			
16	2.390	590	16	64	0
28	2.090	490	30	108	+
Apr.10	2.160	426	50	116	++
24	2.120	552	9	28	0
25		Cholesterol feeding begun			
Mar. 8	2.150	600	194	220	+++
22	2.050	518	98	124	++
June 5	1.960	538	6	30	+-
19	1.920	584	82	88	+
July 3	2.070	660	46	50	++
10	2.050	630	230	244	++++
24	1.890	660	34	104	0
25		Killed			

Mar. 7/46 to July 25/46 — Male — No. 41. Series 3.

Mar. 7	2.450	107	15	30	0
13	2.460	113	9	44	0
14		Alloxan administered			
16	2.750	432	19	72	0
28	2.490	318	7	56	0
Apr.10	2.650	318	6	16	0
24	2.740	316	6	20	0
25		Cholesterol feeding begun			
May 8	2.740	420	14	20	0
22	2.870	438	16	64	0
June 5	2.860	366	16	24	0
19	2.980	400	9	13	0
July 3	2.950	380	9	32	0
10	2.900	470	20	28	0
24	2.920	388	12	52	0
25		Killed			

Mar. 7/46
to
May 8/46

Male

No. 42.
Series 3.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Mar. 7	2.040	115	14	30	0
13	1.990	107	11	66	0
16	2.040	108	16	60	0
28	2.010	106	10	42	0
Apr.10	2.060	88	6	20	0
24	2.080	110	6	16	0
25		Cholesterol feeding begun			
May 8	2.080		45	100	0
		Died			

CHOLESTEROL FEEDING SCHEDULE

Nos. 43 - 53
Series 4.

Day	Date	Day	Date	Day	Date	
0	July 2	30	Aug. 1	60	Aug. 31	July - Sept. 1946
1	- -	31	2	61	Sept. 1	
2	4	32	3	62	2	Dose July 2 - 26,
3	5	33	4	63	- -	
4	6	34	5	64	4	0.25 gms. of cholesterol
5	- -	35	6	65	- -	in 7.5 cc. of sunflower seed
6	8	36	- -	66	6	oil.
7	9	37	- -	67	7	
8	- -	38	9	68	8	
9	11	39	10	69	9	Dose July 27 - Sept. 29,
10	- -	40	11	70	10	
11	13	41	12	71	- -	0.5 gms. in 15 cc.
12	- -	42	- -	72	12	
13	15	43	- -	73	13	Dissolved at 100°C. and
14	16	44	15	74	14	maintained at room temperature.
15	- -	45	16	75	15	
16	18	46	17	76	16	Fed by 14F. catheter.
17	- -	47	18	77	17	
18	20	48	19	78	18	
19	- -	49	20	79	19	Total time interval 90 days.
20	22	50	- -	80	20	
21	23	51	22	81	21	
22	- -	52	23	82	22	Total number of doses 69.
23	- -	53	24	83	- -	
24	- -	54	25	84	24	
25	27	55	26	85	25	Total amount of cholesterol
26	28	56	- -	86	26	31.0 gms.
27	29	57	28	87	27	
28	30	58	29	88	28	
29	31	59	30	89	29	

Apr.17/46 to Aug. 1/46

Male

No. 43.
Series 4.

Date		Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Apr.	17	2.000	102	10	20	0
May	1	2.480	110	6	20	0
	2		Alloxan administered			
	4	2.630	372	9	38	0
	15	2.590	224	8	42	0
	29	2.920	292	9	16	0
June	15	2.750	328	6	46	0
July	2		Cholesterol feeding begun			
	17	3.150	522	10	36	0
	30		Died			

No. 44 omitted from experiment.

Apr.17/46 to Sept.30/46 — Male — No. 45. Series 4.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Apr. 17	1.900	104	6	20	0
May 1	1.970	111	10	14	0
15	2.250	99	10	24	0
29	2.580	96	9	28	0
June 15	3.010	140	6	24	0
27	3.260	129	13	22	0
July 2		Cholesterol feeding begun			
17	3.670	121	10	20	0
Aug. 13	4.170	122	8	20	0
27	4.360	104	8	20	0
Sept.11	4.540	108	15	36	0
23	4.640	103	10	16	0
30	4.660	124 Killed	14	24	0

Apr.17/46 to Sept.30/46 — Male — No. 46. Series 4.

Apr. 17	1.600	103	8	22	0
May 1	1.880	102	12	28	0
15	2.400	93	10	18	0
29	2.780	93	8	30	0
June 15	3.160	142	7	20	0
27	3.090	119	10	38	0
July 2		Cholesterol feeding begun			
17	3.010	115	20	60	0
Aug. 13	3.430	118	16	42	0
27	3.620	101	26	50	0
Sept.11	3.760	112	27	60	0
23	3.730	115	33	72	0
30	3.750	123 Killed	39	64	0

Apr.17/46 to Aug.27/46 — Male — No. 47. Series 4.

Date		Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Apr.	17	1.430	118	10	24	0
May	1	1.650	115	8	20	0
	15	2.160	89	6	20	0
	29	2.600	92	12	28	0
June	15	3.070	147	10	26	0
	27	3.110	118	16	48	0
July	2		Cholesterol feeding begun			
	17	3.010	118	13	26	0
Aug.	13	2.670	122	16	30	0
	27	1.880	142	80	82	0
			Killed			

Apr.17/46 to July 3/46 — Male — No. 48. Series 4.

Apr.	17	1.570	109	10	16	0
May	1	1.470	111	10	30	0
	15	1.830	99	6	18	0
	29	1.870	93	18	22	0
June	15	2.030	136	6	20	0
	27	2.150	118	8	16	0
July	2		Cholesterol feeding begun			
	3		Killed			

Apr.17/46 to Sept.1/46 — Male — No. 49. Series 4.

Apr.	17	2.240	116	11	30	0
May	1	2.410	121	12	16	0
	2		Alloxan administered			
	4	2.490	426	13	44	0
	15	2.450	410	14	26	0
	29	2.550	404	16	20	0
June	15	2.660	484	9	16	0
	27	2.720	520	19	24	0
July	2		Cholesterol feeding begun			
	13		Cholesterol feeding stopped			
Aug.	13	2.210	650	13	32	0
	27	2.110	390	14	20	0
Sept.	1		Killed			

No. 50 omitted from experiment.

Apr. 17/46 to Aug. 8/46 — Male — No. 51. Series 4.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Apr. 17	2.220	118	4	16	0
May 1	2.180	103	4	12	0
2		Alloxan administered			
4	2.220	300	6	32	0
15	2.220	334	16	36	0
29	2.230	412	104	116	+
June 15	2.220	470	72	88	++
27	2.200	430	9	24	+
July 2		Cholesterol feeding begun			
17	1.780	605	36	236	++
Aug. 8		Died			

Apr. 17/46 to Sept.30/46 — Male — No. 52. Series 4.

Apr. 17	1.830	124	6	12	0
May 1	2.220	125	10	42	0
2		Alloxan administered			
4	2.260	534	12	44	0
15	2.310	348	14	48	0
29	2.330	330	9	20	0
June 15	2.520	340	10	20	0
27	2.530	420	18	32	0
July 2		Cholesterol feeding begun			
17	2.580	600	17	62	0
Aug. 13	2.470	496	18	56	0
27	2.530	510	52?	68?	+
Sept.11	2.400	428	100?	106?	+++
23	2.240	590	94	112	+++
30	2.240	460	58	62	++
		Killed			

Apr. 17/46 to Sept.30/46

Male

No. 53.
Series 4.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0–4)
Apr. 17	1.870	135	6	12	0
May 1	2.090	116	6	10	0
2		Alloxan administered			
4	2.190	488	10	38	0
15	2.010	444	86	160	+
29	1.910	446	18	22	0
June 15	1.940	406	6	16	0
27	2.090	452	8	20	0
July 2		Cholesterol feeding begun			
17	1.960	650	15	36	0
Aug. 13	2.260	410	14	32	0
27	2.440	420	52	120	++++
Sept.11	2.320	408	12	30	0
23	2.300	340	18	22	0
30	2.500	420	24	36	0
		Killed			

CHOLESTEROL FEEDING SCHEDULE

Nos. 54 - 63.
Series 5.

Day	Date	Day	Date	Day	Date
0	Oct.28	30	Nov.27	60	Dec.27
1	29	31	28	61	28
2	30	32	29	62	29
3	31	33	30	63	30
4	Nov. 1	34	Dec. 1	64	31
5	2	35	2	65	Jan. 1
6	3	36	3	66	2
7	4	37	4	67	3
8	5	38	5	68	4
9	6	39	6	69	5
10	7	40	- -	70	6
11	8	41	8	71	7
12	9	42	9	72	8
13	10	43	10	73	9
14	11	44	11	74	10
15	12	45	12	75	11
16	13	46	13	76	12
17	14	47	14	77	13
18	15	48	15	78	14
19	16	49	16	79	15
20	17	50	- -	80	16
21	18	51	18	81	17
22	19	52	19	82	18
23	20	53	20	83	- -
24	21	54	21	84	20
25	22	55	- -	85	21
26	23	56	- -	86	22
27	24	57	- -	87	23
28	25	58	25	88	24
29	- -	59	26		

Oct. 1946 - Jan. 1947.

Dose Oct. 28 - Nov. 28,

0.75 gms. of cholesterol in 1.5 cc. of corn oil.

Dose Nov. 29 - Jan. 24,

0.5 gms. in 10 cc. of corn oil.

Dissolved at 100°C. and reheated to solution before feeding until Dec. 20. Thereafter dissolved and maintained at 60°C.

Fed by 14F. catheter.

Total time interval 89 days.

Total number of doses 82.

Total amount of cholesterol 48.5 gms.

Aug.21/46 to Jan.25/47 — Male — No. 54. Series 5

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Aug. 21/46	3.420	82	9	30	0
26	3.560	84	8	18	0
27	Alloxan administered				
Sept. 6	3.860	122	6	12	0
19	4.040	119	10	20	0
Oct. 16	4.450	92	11	12	0
28	Cholesterol feeding begun				
Nov. 11	4.520	108	45	86	±
24	4.450	107	73	192	++
Dec. 12	?	130	56	80	+
Jan. 8/47	4.820	105	46	132	0
24	4.990	118	124	336	++
25	Killed				

Aug.21/46 to Jan.25/47 — Male — No. 55. Series 5.

Aug. 21/46	2.820	99	10	22	0
26	2.900	92	10	22	0
27	Alloxan administered				
29	3.100	364	10	18	0
Sept. 6	3.000	300	6	20	0
19	3.330	320	11	16	0
Oct. 16	3.480	380	9	15	0
28	Cholesterol feeding begun				
Nov. 11	3.430	592	68	236	+
24	3.380	440	58	224	+++
Dec. 12	3.410	420	74	200	++
Jan. 8/47	3.550	454	124	404	+
24	3.450	428	260	584	+++
25	Killed				

Aug.21/46 to Oct. 5/46

Male

No. 56.
Series 5

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Aug. 21	2.790	79	10	22	0
26	2.860	83	9	12	0
27		Alloxan administered			
29	3.000	390	8	20	0
Sept. 6	2.660	372	16	27	0
19	2.500	428	36	60	++
Oct. 5		Died			

Aug.21/46 to Jan.25/47

Male

No. 57.
Series 5.

Aug. 21	3.060	90	10	20	0
26	3.160	100	10	20	0
Sept. 6	3.420	116	10	20	0
19	3.700	126	12	32	0
Oct. 16	4.380	96	13	15	0
28		Cholesterol feeding begun			
Nov. 11	4.530	108	11	30	0
24	4.610	107	18	212	±
Dec. 12	4.570	121	8	32	0
Jan. 8/47	4.910	121	16	36	0
24	5.250	133	22	56	0
25		Killed			

No. 58 omitted from experiment.

Aug. 21/46 to Jan. 25/47 — Male — No. 59. Series 5.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Aug. 21/46	2.540	80	12	24	0
26	2.640	111	9	16	0
Sept. 6	2.900	104	6	18	0
19	3.160	128	6	10	0
Oct. 16	3.400	107	11	12	0
28		Cholesterol feeding begun			
Nov. 11	3.410	96	129	208	++
24	3.520	146	12	68	+
Dec. 12	3.540	131	8?	152	+
Jan. 8/47	3.460	113	154	364	++
24	3.680	118	82	196	+
25		Killed			

Aug. 21/46 to Jan. 25/47 — Male — No. 60. Series 5.

Aug. 21/46	3.030	82	12	42	0
26	3.200	105	11	16	0
27		Alloxan administered			
29	3.520	300	12	16	0
Sept. 6	3.400	162	8	34	0
19	3.580	136	8	10	0
Oct. 16	3.940	102	10	30	0
28		Cholesterol feeding begun			
Nov. 11	4.020	110	50	98	+
24	4.080	135	106	224	++
Dec. 12	4.140	116	36	104	0
Jan. 8/47	4.210	117	34	116	0
24	4.510	113	124	168	++
25		Killed			

Aug. 21/46 to Sept.17/46 — Male — No. 61 Series 5

Aug. 21	2.820	76	8	26	0
26	2.820	107	8	18	0
27		Alloxan administered			
29	21780	512	13	16	0
Sept. 6	2.450	350	8	20	0
17		Killed			

Aug. 21/46 to Jan. 25/47 — Male — No. 62. Series 5

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0–4)
Aug. 21/46	2.540	91	17	36	0
26	2.660	129	8	12	0
Sept. 6	2.900	101	6	26	0
19	3.020	113	10	16	0
Oct. 16	3.290	94	10	18	0
28		Cholesterol feeding begun			
Nov. 11	2.910	101	120	828	+++
24	2.550	122	36	160	+
Dec. 12	2.960	122	50	184	+
Jan. 8/47	3.060	?	168	304	++
24	3.130	118	200	400	++
25		Killed			

Aug. 21/46 to Nov. 5/46 — Sex ? — No. 63. Series 5.

Aug. 21	3.030	88	8	24	0
26	3.000	104	6	16	0
Sept. 6	3.180	100	6	10	0
19	3.410	131	6	20	0
Oct. 16	3.710	106	8	12	0
28		Cholesterol feeding begun			
Nov. 5		Died			

CHOLESTEROL FEEDING SCHEDULE

Nos. 64 - 75.
Series 6.

Day	Date	Day	Date	Day	Date
0	Dec. 3	30	Jan. 2	60	Feb. 1
1	4	31	3	61	2
2	5	32	4	62	3
3	- -	33	5	63	4
4	7	34	6	64	5
5	8	35	7	65	6
6	9	36	8	66	7
7	10	37	9	67	8
8	- -	38	10	68	9
9	12	39	11	69	10
10	13	40	12	70	11
11	14	41	13	71	12
12	15	42	14	72	13
13	- -	43	15	73	14
14	17	44	16	74	15
15	18	45	17	75	16
16	19	46	- -	76	17
17	20	47	19	77	18
18	- -	48	20	78	19
19	- -	49	21	79	20
20	- -	50	22	80	21
21	24	51	23	81	22
22	25	52	- -	82	23
23	26	53	25	83	24
24	27	54	26	84	25
25	- -	55	27	85	26
26	29	56	28	86	27
27	30	57	29	87	28
28	31	58	30	88	Mar. 1
29	Jan. 1	59	31	89	2

Dec. - Mar. 1947

Dose Dec. 3 - Jan. 2,

0.75 gms. of cholesterol in 15 cc. of corn oil.

Dose Jan. 3 - Mar. 2,

0.5 gms. of cholesterol in 10 cc. of corn oil.

Oil dissolved and heated to 100°C.

Fed by 14F. catheter.

Total time interval 90 days.

Total number of doses 81.

Total amount of cholesterol 46.25 gms.

Oct. 17/46 to Mar. 3/47 — Male — No. 64. Series 6.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Oct.17/46	2.820	101	17	30	0
23	2.870	115	9	20	0
24		Alloxan administered			
Nov.11	2.980	109	17	24?	0
Dec. 1	3.040	138	6	20	0
3		Cholesterol feeding begun			
18	2.930	112	112	260	++
Jan.12/47	3.950	120	120	652	+
22	3.130	120	110	576	+
Feb. 8	3.210	102	172	580	++
23	3.220	102	90	224	++
Mar. 2	3.230	119	120	198	+++
3		Killed			

Oct. 17/46 to Dec. 13/46 — Sex ? — No. 65. Series 6.

Oct. 17	3.260	125	14	30	0
23	3.360	120	9	12	0
Nov. 11	3.330	108	10	20	0
Dec. 1	3.360	?	6	38	0
3		Cholesterol feeding begun			
13		Died			

Oct. 17/46 to Mar. 3/47 — Female — No. 66. Series 6.

Oct.17/46	3.640	114	14	46	0
23	3.680	125	9	14	0
Nov.11	3.840	110	10	24	0
Dec. 1	3.900	?	6	20	0
3		Cholesterol feeding begun			
18	4.020	107	86	96?	0
Jan.12/47	4.170	107	102	264	+
22	4.330	145	194	380	++
Feb. 8	4.470	108	274	712	++
23	4.640	100	212	440	++
Mar. 2	4.140	122	211	424	+++
3		Killed			

Oct.17/46 to Jan.16/47 — Male — No. 67. Series 6.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Oct.17/46	3.000	102	16	18	0
23	3.150	127	10	12	0
24		Alloxan administered			
26	3.130	346	9	28	0
Nov.11	3.010	380	10	32	0
Dec. 1	2.950	308	12	36	0
3		Cholesterol feeding begun			
18	2.970	402	56	128	++
Jan.12/47	2.940	396	180	400	++
16	2.970	Died			

Oct.17/46 to Nov.14/46 — Sex ? — No. 68. Series 6.

Oct.17/46	3.020	120	13	32	0
23	2.990	120	9	12	0
24		Alloxan administered			
26	3.250	464	18	44	0
Nov.11	2.560	452	11	20	0
14		Died			

Oct.17/46 to Mar. 3/47 — Male — No. 69. Series 6.

Oct.17/46	2.700	129	15	26	0
23	2.720	126	18	20?	0
24		Alloxan administered			
26	2.630	446	42	68	0
Nov.11	2.650	486	10	18	0
Dec. 1	2.580	374	8	16	0
3		Cholesterol feeding begun			
18	2.530	420	196	348	++++
Jan.12/47	2.270	432	202	528	++++
22	2.310	498	294	556	++++
Feb. 8	2.100	376	340	720	++++
23	1.880	130	196	440	+++
Mar. 2	1.840	254	108	162	+++
3		Killed			

Oct.17/46 to Mar. 3/47

Female

No. 70.
Series 6.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Oct.17/46	3.000	91	12	26	0
23	2.930	116	15	20	0
24		Alloxan administered			
26	2.940	386	17	40	0
Nov.11	2.700	370	8	20	0
Dec. 1	2.730	320	6	21	0
3		Cholesterol feeding begun			
18	2.520	272	85	96	+
Jan.12/47	2.640	382	110	336	+
22	2.740	390	106	336	+
Feb. 8	2.620	372	28?	122?	+
23	2.170	364	39	50	+++
Mar. 2	2.600	378	25	154	+++
3		Killed			

Oct.17/46 to Jan.30/47

Male

No. 71.
Series 6.

Oct.17/46	3.360	104	15	18	0
23	3.250	121	9	26	0
24		Alloxan administered			
26	3.330	346	21	30?	0
Nov.11	3.330	154	15	28	0
Dec. 1	3.320	176	10	26	0
3		Cholesterol feeding begun			
18	3,460	146	39	100	+
Jan.12/47	3.490	143	160	360	+
22	3.550	240	194	604	++
30		Killed			

Oct.17/46 to Mar. 3/47

Male

No. 72.
Series 6.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Oct.17/46	3.010	110	16	22	0
23	2.960	125	10	24	0
24		Alloxan administered			
26	3.190	228	10	40	0
Nov.11	3.250	136	10	32	0
Dec. 1	3.430	122	6	20	0
3		Cholesterol feeding begun			
18	3.520	82	44	102	+
Jan.12/47	3.800	115	86	160	+
22	3.870	124	100	348	+
Feb. 8	3.880	110	184	504	+
23	3.820	100	112	256	+
Mar. 2	3.780	104	?	?	++
3		Killed			

No. 73 omitted from experiment.

Oct.17/46 to Mar. 3/47

Female

No. 74.
Series 6.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Oct.17/46	3.230	113	15	44	0
23	3.260	126	10	20	0
Nov.11	3.350	120	10	26	0
Dec. 1	3.620	122	10	42	0
3		Cholesterol feeding begun			
18	3.900	124	60	90	+
Jan.12/47	4.030	122	208	348	++
22	4.260	145	126	464	++
Feb. 8	4.300	108	288	608	++
23	4.370	108	82	524	++
Mar. 2	4.440	120	200	368	+++
3		Killed			

Oct.17/46 to Mar. 3/47

Female

No. 75.
Series 6

Oct.17/46	3.120	108	11	40	0
23	3.080	127	17	20?	0
24		Alloxan administered			
26	3.290	190	29	42	0
Nov.11	3.510	128	10	28	0
Dec. 1	3.550	130	6	24	0
3		Cholesterol feeding begun			
18	3.560	83	58	124	+
Jan.12/47	3.850	116	36	328	++
22	3.970	143	114	424	+
Feb. 8	3.960	110	194	748	++
23	4.100	105	144	408	++
Mar. 2	3.980	121	147	516	+++
3		Killed			

CHOLESTEROL FEEDING SCHEDULE

Nos. 76 - 85.
Series 7

Day	Date	Day	Date	Day	Date
0	Jan. 9	30	Feb. 8	60	Mar.10
1	10	31	9	61	- -
2	11	32	10	62	12
3	12	33	11	63	13
4	13	34	12	64	14
5	14	35	13	65	15
6	15	36	14	66	16
7	16	37	15	67	17
8	17	38	16	68	18
9	- -	39	17	69	19
10	19	40	18	70	20
11	20	41	19	71	21
12	21	42	20	72	22
13	22	43	- -	73	23
14	23	44	22	74	24
15	- -	45	23	75	25
16	25	46	24		
17	26	47	25		
18	27	48	26		
19	28	49	27		
20	29	50	28		
21	30	51	Mar. 1		
22	31	52	- -		
23	Feb. 1	53	3		
24	2	54	4		
25	3	55	5		
26	4	56	6		
27	5	57	7		
28	6	58	8		
29	7	59	9		

Jan. - Mar. 1947.

Dose Jan. 9 - Feb. 7,

0.75 gms. of cholesterol in 15 cc. of corn oil.

Dose Feb. 8 - 13,

0.5 gms. of cholesterol in 10 cc. of corn oil.

Dose Feb. 14 - Mar. 25,

0.75 gms. of cholesterol in 15 cc. of corn oil.

Solution dissolved and heated to 100°C.

Fed by 14F. catheter.

Total time interval 76 days.

Total number of doses 71.

Total amount of cholesterol 51.75 gms.

Nov.13/46 to Mar. 8/47

Sex ?

No. 76.
Series 7.

Date	Weight kgm.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Nov.13/46	2.460	115	10	26	0
17	2.550	136	6	12	0
18		Alloxan administered			
Dec. 5	3.170	138	6	21	0
Jan. 8/47	3.720	91	12	36	0
9		Cholesterol feeding begun			
23	4.040	101	50	216	+
Feb. 9	4.320	167	40	236	+
23	4.260	114	52	172	+
Mar. 8		Died			

Nov.13/46 to Mar.26/47

Male

No. 77.
Series 7.

Nov.13/46	2.630	111	20	30	0
17	2.600	136	10	20	0
18		Alloxan administered			
20		392	21	26	0
Dec. 5	3.200	124	6	18	0
Jan. 8/47	3.660	128	?	?	0
9		Cholesterol feeding begun			
23	3.750	120	70	312	0
Feb. 9	3.940	132	159	512	++
23	4.050	113	112	592	++
Mar.13	4.230	111	142	266	++
26		Killed			

Nov.13/46 to Mar.26/47

Male

No. 78.
Series 7.

Nov.13/46	2.610	123	13	20	0
17	2.730	123	6	16	0
Dec. 5	2.410	126	6	36	0
Jan. 8/47	3.500	114	9	16	0
9		Cholesterol feeding begun			
23	4.020	108	124	572	0
Feb. 9	4.340	107	85	596	++
23	4.020	126	128	440	+++
Mar.13	4.200	110	258	500	++
26		Killed			

Nov.13/46 to Mar.24/47 — Male — No. 79. Series 7.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Nov.13/46	2.350	120	14	16	0
17	2.300	136	6	32	0
18		Alloxan administered			
20		356	20	24	0
Dec. 5	2.790	144	6	24	0
Jan. 8/47	3.120	133	14	40	0
9		Cholesterol feeding begun			
23	2.980	131	126	272	++
Feb. 9	3.140	168	156	576	+++
23	3.270	119	222	816	++
Mar.13	3.250	128			++
24		Killed	279	656	(post-mortem)

Nov.13/46 to Jan.23/47 — Male — No. 80. Series 7.

Nov.13/46	2.550	129	19	24	0
17	2.420	132	6	12	0
18		Alloxan administered			
20		440	13	16	0
Dec. 5	2.900	120	6	42	0
Jan. 8/47	3.420	172	9	26	0
9		Cholesterol feeding begun			
23	2.180	370	278	618	(post-mortem)
		Killed			

Nov.13/46 to Mar.26/47 — Male — No. 81. Series 7.

Nov.13/46	2.270	113	10	20	0
17	2.200	154	6	18	0
18		Alloxan administered			
20		552	15	20	0
Dec. 5	2.490	300	12	28	0
Jan. 8/47	2.470	288	12	40	0
9		Cholesterol feeding begun			
23	2.840	454	68	288	++++
Feb. 9	2.530	546	194	368	++++
23	2.640	458	270	800	++++
Mar.13	2.840	130	310	510	++++
25		Died			

Nov.13/46 to Feb. 3/47 — Sex ? — No. 82. Series 7

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Nov.13/46	2.690	148	12	20	0
17	2.630	144	6	10	0
Dec. 5	3.230	120	6	32	0
Jan. 8/47	3.850	143	13	36	0
9		Cholesterol feeding begun			
23	4.210	110	24	108	+
Feb. 3		Died			

Nov.13/46 to Dec. 3/46 — Sex ? — No. 83. Series 7.

Nov.13/46	2.960	116	11	22	0
17	2.910	139	6	14	0
Dec. 3		Died			

Nov.13/46 to Mar.26/47 — Male — No. 84. Series 7.

Nov.13/46	2.630	131	12	14	0
17	2.750	131	6	14	0
Dec. 5	3.210	105	6	16	0
Jan. 8/47	3.720	126	6	28	0
9		Cholesterol feeding begun			
23	3.900	118	77	200	0
Feb. 9	3.730	116	129	468	++
23	3.880	122	86	448	++
Mar.13	3.950	109	53.9?	216?	+
26	4.090	Killed			

Nov.13/46 to Mar.13/47

Male

No. 85.
Series 7.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Nov.13/46	2.620	122	10	30	0
17	2.630	138	10	18	0
18		Alloxan administered			
20		520	12	16?	0
Dec. 5	3.360	154	6	20	0
Jan. 8/47	3.700	130			0
9		Cholesterol feeding begun			
23	3.920	129	56	180	0
Feb. 9	3.970	238	138	412	+
23	3.910	119	238	556	++
Mar.13	3.950	117	164	376	+
21	4.000	Died			

CHOLESTEROL FEEDING SCHEDULE

Nos. 86 - 98.
Series 8

Day	Date	Day	Date	Day	Date
0	Apr. 3	30	May 3	60	June 2
1	4	31	4	61	3
2	5	32	5	62	4
3	6	33	6	63	5
4	7	34	- -	64	6
5	8	35	- -	65	7
6	9	36	9	66	8
7	10	37	10	67	9
8	11	38	11	68	10
9	12	39	12	69	11
10	13	40	13	70	12
11	14	41	14	71	13
12	15	42	15	72	14
13	16	43	16	73	15
14	17	44	17	74	16
15	18	45	18	75	17
16	19	46	19	76	18
17	20	47	20	77	19
18	21	48	21	78	20
19	22	49	22	79	21
20	23	50	23	80	22
21	24	51	24	81	23
22	25	52	25		
23	26	53	26		
24	27	54	27		
25	28	55	28		
26	29	56	29		
27	30	57	30		
28	May 1	58	31		
29	2	59	June 1		

Apr. - June 1947

Dose 0.75 gms. of cholesterol in 15 cc. of corn oil. Dissolved and maintained at 60°C.

Fed by 14F. catheter.

Total time interval 82 days.

Total number of doses 80.

Total amount of cholesterol 60. gms.

Feb. 2/47 to Feb.10/47 — Sex ? — No. 86. Series 8.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Feb. 2	2.970	119	6	36	0
9			6	16	0
10		Alloxan administered			
		Died			
		380 (post-mortem)			

Feb. 2/47 to May 16/47 — Male — No. 87. Series 8.

Feb. 2	2.560	123	8	36	0
9			6	34	0
10		Alloxan administered			
23	2.920	340	18	78	0
Mar.13	3.040	372	12	14	0
31	3.120	386	20	46	0
Apr. 3		Cholesterol feeding begun			
16	3.140	380	85	324	±
May 3	2.750	187	119	328	+
9		Died			

Feb. 2/47 to June 23/47 — Male — No. 88. Series 8

Feb. 2	1.970	116	8	42	0
9			16	20	0
10		Alloxan administered			
12		440	22	24	0
23	2.520	202	6	54	0
Mar.13	2.830	360	15	18	0
31	3.070	372	19	29	0
Apr. 3		Cholesterol feeding begun			
16	3.110	418	330	784	++++
May 3	3.070	422	368	840	+++
19	2.960	492	778	1548	++++
June 4	3.060	442	820	1556	++++
18	3.150	516	718	1692	++++
23		Killed			

Feb. 2/47 to May 7/47 — Sex ? — No. 89. Series 8.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Feb. 2	2.230	139	6	46	0
9			9	20	0
10		Alloxan administered			
12		444	8	16	0
23	2.330	442	45	116	+
Mar.13	2.310	458	10	26	0
31	2.050	388	16	44	0
Apr. 3		Cholesterol feeding begun			
16	1.880	518	272	624	++++
May 3	1.960	328	86	228	+
7		Died			

Feb. 2/47 to June 23/47 — Female — No. 90. Series 8.

Feb. 2	2.560	132	6	28	0
9			18	19	0
10		Alloxan administered			
12		410	10	24	0
23	3.240	118	13	72	0
Mar.13	3.800	132	21	42?	0
31	3.850	122	19	29	0
Apr. 3		Cholesterol feeding begun			
16	4.210	102	140	684	+
May 3	4.360	111	226	796	++
19	4.640	134	348	864	++
June 4	4.940	110	378	796	+++
18	5.040	110	302	764	++
23	5.090	Killed			

Feb. 2/47 to Feb. 11/47 — Sex ? — No. 91. Series 8.

Feb. 2	2.760	113	6	32	0
9			18	19	0
10		Alloxan administered			
11		Killed			

Feb. 2/47 to June 23/47 — Male — No. 92. Series 8

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Feb. 2	2.550	121	6	32	0
9			9	16	0
10		Alloxan administered			
12		554	12	18	0
23	2.860	352	14	42	0
Mar.13	3.200	320	21	40	0
31	3.100	371	21	25	0
Apr. 3		Cholesterol feeding begun			
16	3.320	451	38	176	0
May 3	3.350	438	222	491	++++
19	3.280	518	508	860	++++
June 4	2.990	220	684	1268	++++
18	2.840	392	420	1400	++++
23	2.190	Died			

Feb. 2/47 to Apr. 8/47 — Male — No. 93. Series 8

Feb. 2	2.170	110	22	88	0
9			13	26	0
10		Alloxan administered			
12		462	22	40	0
23	2.810	460	8	78	0
Mar.13	2.920	410	19	31	0
31	2.300	115	17	21	0
Apr. 3		Cholesterol feeding begun			
8	2.360	Died			

Feb. 2/47 to Feb. 11/47 — Sex ? — No. 94. Series 8

Feb. 2	2.520	113	12	32	0
9			8	10	0
10		Alloxan administered			
11		Killed			

Mar. 19/47 to Apr. 6/47 — Sex ? — No. 95. Series 8.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Mar.19	2.840	125	10	36	0
31	3.310	106	22	67	0
Apr. 3		Cholesterol feeding begun			
6		Killed			

Mar. 19/47 to June 23/47 — Female — No. 96. Series 8.

Mar.19	3.800	123	6	18	0
31	4.050	112	23	30	0
Apr. 3		Cholesterol feeding begun			
16	4.300	136	41	222	0
May 3	4.700	113	142	496	+
19	4.540	132	230	754	++
June 4	4.440	125	287	684	+++
18	4.200	138	262	1188	++
23	4.200	Killed			

Mar. 19/47 to May 2/47 — Female — No. 97. Series 8.

Mar.19	3.310	106.5	6	14	0
31	3.550		11.5	55	0
Apr.16	3.870	126	119	448	0
May 2		Killed			

Mar. 19/47 to June 23/47 — Female — No. 98. Series 8.

Mar.19	3.350	123	6	10	0
31	3.730	97.5	10	43	0
Apr. 3		Cholesterol feeding begun			
16	3.300	122.5	109	484	0
May 3	3.850	109	155	562	+
19	3.980	148.5	323	878	++
June 4	4.290	106.5	306	812	+++
18	4.580	115.5	252	830	++
23		Killed			

CHOLESTEROL FEEDING SCHEDULE

Nos. 99 - 110.
Series 9

Day	Date	Day	Date	Day	Date
0	May 5	31	June 5	60	July 4
1	6	32	6	61	5
2	7	33	7	62	6
3	8	34	8	63	7
4	9	35	9	64	8
5	10	36	10	65	9
6	11	37	11	66	10
7	12	38	12	67	11
8	13	39	13	68	12
9	14	40	14	69	13
10	15	41	15	70	14
11	16	42	16	71	15
12	17	43	17	72	16
13	18	44	18	73	17
14	19	45	19	74	18
15	20	46	20	75	19
16	21	47	21	76	20
17	22	48	22	77	21
18	23	49	23	78	22
19	24	50	24	79	23
20	25	51	25	80	- -
21	26	52	26	81	25
22	27	53	27	82	26
23	28	54	28	83	27
24	29	55	29	84	28
25	30	56	30	85	29
26	31	57	- -	86	30
27	June 1	58	July 2	87	31
28	2	59	3	88	Aug. 1
29	3				
30	4				

May - Aug. 1947.

Dose 0.75 gms. of cholesterol in 15 cc. of corn oil.
Dissolved and maintained at 60°C.

Fed by 14F. catheter.

Total time interval 89 days.

Total number of doses 87.

Total amount of cholesterol 65.25 gms.

Mar. 31/47 to Aug. 2/47 — Female — No. 99. Series 9.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Mar. 31	1.960	86	33.5	78	0
Apr. 2	1.910	115.5	43	77.8	0
3		Alloxan administered			
5		364	10	68	0
16	2.390	380	12	44	0
May 4	2.690	283	18	47	0
5		Cholesterol feeding begun			
19	2.780	420	172	389	+
June 4	2.840	378	239	658	++
18	3.010	444	186	552	+
July 3	3.140	376	98	284	+
15	3.200	420			+
21			62	124	0
Aug. 1	3.310	426	168	366	++
2		Killed			

Mar.31/47 to Apr.18/47 — Sex ? — No. 100. Series 9.

Mar. 31	1.890	86.5	10.5	34	0
Apr. 2	1.950	121.5	11	34	0
3		Alloxan administered			
5		528	27	64	+
16	1.800	460	183	258	++++
18		Died			

Mar.31/47 to Apr.27/47 — Sex ? — No. 101. Series 9.

Mar. 31	2.600	91	16	27	0
Apr. 2	2.680	125	13	24	0
3		Alloxan administered			
5		422			
16	2.460	280	8.9	42	0
27		Died			

Mar.31/47 to Apr.27/47 — Sex ? — No. 102. Series 9.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Mar. 31	2.030	90	17	64	0
Apr. 2	2.100	122	11	35.8	0
3		Alloxan administered			
5		546	56	88	+
10		Died			..

Mar.31/47 to Apr.18/47 — Sex ? — No. 103. Series 9.

Mar. 31	2.210	118.5	13	34	0
Apr. 2	2.270	121.5	9	40	0
3		Alloxan administered			
5		556	17	68	+
16	2.290	442	162	240	++++
18		Died			....

Mar. 31/47 to May 16/47 — Female — No. 104. Series 9

Mar. 31	2.110	124.5	25.5	56	0
Apr. 2	1.960	115.5	24	43.8	0
5		176	7	58	0
16	2.200	414	190	312	+++
May 4	1.900	518	21	42	0
5		Cholesterol feeding begun			
16		Died			

Mar.31/47 to June 2/47

Female

No. 105.
Series 9.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Mar. 31	1.770	123	16	42	0
Apr. 2	1.860	122	15	26	0
3		Alloxan administered			
5		502	19	64	0
16	2.080	640	64	126	+
May 4	1.780	510	12.9	53	0
5		Cholesterol feeding begun			
19	1.530	640	370	734	++++
June 2		Died			. . . .

Mar.31/47 to Apr.17/47

Sex ?

No. 106.
Series 9.

Mar. 31	2.360	111	19	58	0
Apr. 2	2.380	127.5	9	24	0
3		Alloxan administered			
5		402	10	56	0
16	2.510	404	116.5	198	++++
17		Died			. .

May 1/47 to June 27/47

Male

No. 107. (control)
Series 9.

May 1	2.800	93.5	15	29	0
4	2.840	139	14.7	40	0
19	2.830	121	238	686	+
June 4	2.350	101	370	756	+++
18	2.370	126.5	327	822	+++
27		Died			. . .

May 1/47 to Aug. 2/47

Male

No. 108. (control)
Series 9.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
May 1	2.720	98.5	14.5	39.8	0
4	2.800	142	14	43	0
5		Cholesterol feeding begun			
19	3.060	145	73.5	330	0
June 4	3.380	117	63	226	0
18	3.760	120	70	254	+
July 3	3.800	111	79	334	0
15	3.970	120			0
21			37	144	0
Aug. 1	4.170	116	47	218	0
2		Killed			

May 1/47 to Aug. 2/47

Male

No. 109. (control)
Series 9.

May 1	2.940	120	21	58	0
4	3.170	147	26	52	0
5		Cholesterol feeding begun			
19	3.380	128.5	14.8	584	+
June 4	3.620	123	131	540	+
18	3.800	129	173	682	++
July 3	4.000	120	124	454	0
15	4.100	131			+
21			157	496	+
Aug. 1	4.360	120	105	340	+
2		Killed			

May 1/47 to Aug.2/47

Male

No. 110 (control)
Series 9.

May 1	3.610	108	9.9	42	0
4	3.500	148	13	46	0
5		Cholesterol feeding begun			
19	3.500	124	146	548	+
June 4	3.940	113	162	666	+
18	4.180	125	93	760	++
July 3	4.220	109	143	695	0
15	4.440	116			+
21			199	766	+
Aug. 1	4.510	121	246	670	++
2		Killed			

CHOLESTEROL FEEDING SCHEDULE

Nos. 111 - 123.
Series 10.

Day	Date	Day	Date	Day	Date	
0	June 27	19	July 16	37	Aug. 3	June - Aug. 1947.
1	28	20	17	38	4	
2	29	21	18	39	5	Dose 0.75 gms. of cholesterol
3	30	22	19	40	6	in 15 cc. of corn oil.
4	- -	23	20	41	7	Dissolved and maintained at
5	July 2	24	21	42	8	60°C.
6	3	25	22	43	9	
7	4	26	23	44	10	Fed by 14F. catheter.
8	5	27	- -	45	11	
9	6	28	25	46	12	Total time interval 55 days.
10	7	29	26	47	13	
11	8	30	27	48	14	Total number of doses 53.
12	9	31	28	49	15	
13	10	32	29	50	16	Total amount of cholesterol
14	11	33	30	51	17	39.75 gms.
15	12	34	31	52	18	
16	13	35	Aug. 1	53	19	
17	14	36	2	54	20	
18	15					

May 7/47 to June 15/47

Sex ?

No. 111
Series 10.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
May 7	2.810	129	20	54	0
12	2.760	104	9.9	48	0
		Alloxan administered			
14	3.020	162	29	64	0
28	3.080	522	24	52	0
June 15		Died			

May 7/47 to May 17/47

Sex ?

No. 112
Series 10

May 7	2.790	199	19	61	0
12	2.780	116	16	34	0
		Alloxan administered			
14		195	22.4	72	0
17		Died			

May 7/47 to Aug. 7/47

Sex ?

No. 113
Series 10.

May 7	2.820	190	12	47	0
12	2.570	123	20	76	0
		Alloxan administered			
14		112	20.7	66	0
28	2.840	406	16.5	40	0
June 26	2.980	480	6	20	0
27		Cholesterol feeding begun			
July 11	2.790	594	50	140	+
28	2.400	130	45	134	0
Aug. 7		Died			

May 7/47 to July 20/47 — Sex ? — No. 114. Series 10.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
May 7/47	2.850	134.5	14	40	0
12	2.780	134	7	26	0
		Alloxan administered			
14		290	16.3	37	0
28	3.120	.29	6	14	0
June 26	3.570	109	6	18	0
27		Cholesterol feeding begun			
July 11	3.810	138	18	66	0
20		Killed			

May 7/47 to July 12/47 — Sex ? — No. 115 Series 10.

May	7/47	2.910	143	16.5	76.6	0
	12	2.860	104	8	22	0
			Alloxan administered			
	14		320	22	54	0
	28	2.730	150	9	12	0
June	26	2.920	225	6	32	0
	27		Cholesterol feeding begun			
July	11	2.450	262	93	266	++
	12		Died			....

May 7/47 to Aug. 20/47 — Male — No. 116 Series 10.

May	7/47	2.560	125	15	47	0
	12	2.430	109.5	11	54	0
			Alloxan administered			
	14		396	16.5	48	0
	28	2.640	432	8.5	22	0
June	26	2.220	456	10	33	0
	27		Cholesterol feeding begun			
July	11	2.080	650	270	490	++++
	28	2.100	490	169	440	+++
Aug.	12	2.070	420	316	610	++++
	20		Died			....
			390	328	540	(post-mortem)

May 7/47 to Aug.18/47 — Male — No. 117. Series 10.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
May 7/47	2.800	126	11.5	40	0
12	2.700	109	6	24	0
		Alloxan administered			
14		201	15	51.4	0
28	2.860	412	10	17	0
June 26	2.370	342	13	26	0
27		Cholesterol feeding begun			
July 11	2.190	540	?	?	+++
28	1.930	356	75	146	+
Aug. 12	1.880	470	240	472	++++
18		Died			

May 7/47 to May 31/47 — Female — No. 118. Series 10.

May 7/47	2.810	120	15	51	0
12	2.760	132	13	52	0
		Alloxan administered			
14		292	34	62	0
28	2.790	652	170	276	++++
31		Died			

May 7/47 to Aug.21/47 — Male — No. 119. Series 10.

May 7/47		123	10.5	39.4	0
12	2.450	106	15	33	0
		Alloxan administered			
14		239	15	22	0
28	2.760	111	6	14	0
June 26	3.320	97.5	6	20	0
27		Cholesterol feeding begun			
July 11	3.300	115	24	110	0
28	3.530	116	75	82	0
Aug. 12	3.560	118	38	72	0
20	3.630		15	102	+
21		Killed			

June 26/47 to Aug. 21/47

Female

No. 120.
Series 10.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
June 26	4.580	116	7	18	0
27		Cholesterol feeding begun			
July 11	4.900	122	19	96	0
28	5.000	114	80	210	+
Aug. 12	5.250	123	86	348	0
20	5.250		126	378	+
21		Killed			

CHOLESTEROL FEEDING SCHEDULE

No. 121
Series 10.

Day	Date	Day	Date	Day	Date
0	June 27	30	July 27	60	Aug.26
1	28	31	28	61	27
2	29	32	29	62	28
3	30	33	30	63	29
4	- -	34	31	64	- -
5	July 2	35	Aug. 1	65	31
6	3	36	2	66	Sept.1
7	4	37	3	67	2
8	5	38	4	68	3
9	6	39	5	69	4
10	7	40	6	70	5
11	8	41	7	71	6
12	9	42	8	72	7
13	10	43	9	73	8
14	11	44	10	74	9
15	12	45	11	75	10
16	13	46	12	76	11
17	14	47	13	77	12
18	15	48	14	78	13
19	16	49	15	79	- -
20	17	50	16	80	15
21	18	51	17	81	16
22	19	52	18	82	17
23	20	53	19	83	18
24	21	54	20	84	19
25	22	55	- -	85	20
26	23	56	- -	86	21
27	- -	57	23	87	22
28	25	58	24	88	23
29	26	59	25		

June - Sept. 1947.

Dose 0.75 gms. of cholesterol in 15 cc. of corn oil. Dissolved and maintained at 60°C.

Fed by 14F. catheter.

Total time interval 89 days.

Total number of doses 83.

Total amount of cholesterol 62.25 gms.

June 26/47 to Nov. 24/47 — Female — No. 121. Series 10.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
June 26		114	15	40	0
27		Cholesterol feeding begun			
July 11		126	17	76	0
28		104	73	200	++
Aug. 12	4.050	119	97	272	0
20	4.120		122	338	++
Sept.11			178	482	+
23		138	166	330	+
24		Alloxan administered			
25	4.340	150	189	375	+
27		500	215	348	+++
29		79	290	408	+++
Oct. 1		376	242	606	++
2		364	228	390	++
6		480	270	600	+++
19	3.250	450	257	400	++++
Nov. 11	2.460	340	6	16	0
24	2.060	Died			

June 26/47 to Aug. 21/47 — Female — No. 122. Series 10.

June 26	3.500	138	14	38	0
27		Cholesterol feeding begun			
July 11	4.030	132	47	178	0
28	4.210	142	100	282	++
Aug. 12	4.540	111	166	488	0
20	4.590		197	592	++
21		Killed			

June 26/47 to Aug. 21/47 — Female — No. 123. Series 10.

June 26	3.020	126.5	8	26	0
27		Cholesterol feeding begun			
July 11	3.360	127	79	336	+
28	3.480	128	140	450	++
Aug. 12	3.730	135	156	484	+
20	3.730		170	650	++
21		Killed			

CHOLESTEROL FEEDING SCHEDULE

Nos. 124 - 132.
Series 11A.

Day	Date	Day	Date	Day	Date
0	July 2	31	Aug. 2	62	Sept.2
1	3	32	3	63	3
2	4	33	4	64	4
3	5	34	5	65	5
4	6	35	6	66	6
5	7	36	7	67	7
6	- -	37	8	68	8
7	9	38	9	69	9
8	10	39	10	70	10
9	11	40	11	71	11
10	12	41	12	72	12
11	13	42	13	73	13
12	14	43	14	74	- -
13	15	44	15	75	15
14	16	45	16	76	16
15	17	46	17	77	17
16	18	47	18	78	18
17	19	48	19	79	19
18	20	49	20	80	20
19	21	50	- -	81	21
20	22	51	- -	82	22
21	23	52	23	83	23
22	- -	53	24	84	24
23	25	54	25	85	25
24	26	55	26	86	- -
25	27	56	27	87	- -
26	28	57	28	88	28
27	29	58	29	89	29
28	30	59	- -		
29	31	60	31		
30	Aug. 1	61	Sept.1		

July - Sept. 1947

Dose 0.75 gms. of cholesterol in 15 cc. of corn oil. Dissolved and maintained at 60°C.

Fed by 14F. catheter.

Total time interval 90 days.

Total number of doses 82.

Total amount of cholesterol 61.5 gms.

June 30/47 to Nov. 16/47 — Female — No. 124. Series 11A.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
June 30	3.090	124	12	38	0
July 2		Cholesterol feeding begun			
21			106	360	+
Aug. 11			106	390	+
Sept.11			153	550	+
30	4.600	128	102	182	+
		Alloxan administered			
Oct. 6		615	36	100	++
19	3.950	460	6	40	0
Nov. 11	3.890	496	10	20	0
16		Died			

June 30/47 to July 4/47 — Sex ? — No. 125. Series 11A.

June 30	2.780	106	21	61	0
July 2		Cholesterol feeding begun			
4		Killed			

June 30/47 to Oct. 20/47 — Female — No. 126. Series 11A.

June 30	2.960	101	10	40	0
July 2		Cholesterol feeding begun			
21			167	680	++
Aug. 11			134	480	++
Sept.11			263	580	++
30	4.270	131	240	576	+++
		Alloxan administered			
Oct. 6		500	220	364	++++
19	3.110	440	270	820	++
20		Died			

June 30/47 to July 14/47

Sex ?

No. 127.
Series 11A.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
June 30	2.560	100	21	40	0
July 2		Cholesterol feeding begun			
14		Died			

June 30/47 to Jan. 17/48

Female

No. 128.
Series 11A.

June 30/47	2.740	96	24	83	0
July 2		Cholesterol feeding begun			
21			210	770	+
Aug. 11			185	520	+
Sept.11			225	510	++
30	3.080	114	270	510	+++
		Alloxan administered			
Oct. 6		456	152	130	++
19	2.820	470	40	88	0
Nov. 11	3.030	420	6	42	0
Dec. 1	2.670	426	12	26	0
28	2.540	490	6	40	0
Jan. 17/48	2.140	Died			

June 30/47 to Nov. 3/47

Female

No. 129.
Series 11A.

June 30	2.710	95	36	90	0
July 2		Cholesterol feeding begun			
21			190	780	++
Aug. 11			204	650	+
Sept.11			215	546	++
Oct. 6		117	122	260	0
19	4.390	152	60	210	0
Nov. 3	4.510	?	20	150	0
		Killed			

June 30/47 to Nov. 3/47 — Female — No. 130. Series 11A.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
June 30	3.020	118	15	45	0
July 2		Cholesterol feeding begun			
21			85	348	+
Aug. 11			122	304	+
Sept.11			205	468	++
Oct. 6		150	95	244	0
19	4.600	146	28	40	0
Nov. 3	5.090	?	10	20	0
		Killed			

June 30/47 to Jan. 18/48 — Female — No. 131. Series 11A.

June 30/47	3.000	105	7	42	0
July 2		Cholesterol feeding begun			
21			68	250	+
Aug. 11			99	320	++
Sept.11					+
Oct. 6	4.450	144	52	192	0
19	4.600	138	30	88	0
Nov. 11	5.240	151	6	12	0
Dec. 1	5.350	109	25	54	0
28	5.850	130	10	22	0
Jan. 18/48	6.100	127	10	84	0
		Killed			

June 30/47 to July 4/47 — Sex ? — No. 132. Series 11A.

June 30/47	3.010	107	11	41	0
July 2		Cholesterol feeding begun			
4		Killed			

CHOLESTEROL FEEDING SCHEDULE

Nos. 133 - 141.
Series 11A

Day	Date	Day	Date	Day	Date	
0	July 3	30	Aug. 2	60	Sept.1	July - Sept. 1947
1	4	31	3	61	2	
2	5	32	4	62	3	Dose 0.75 gms. of cholesterol
3	6	33	5	63	4	in 15 cc. of corn oil.
4	7	34	6	64	5	Dissolved and maintained at
5	- -	35	7	65	6	60°C.
6	9	36	8	66	7	
7	10	37	9	67	8	Fed by 14F. catheter.
8	11	38	10	68	9	
9	12	39	11	69	10	Total time interval 90 days.
10	13	40	12	70	11	
11	14	41	13	71	12	Total number of doses 82.
12	15	42	14	72	13	
13	16	43	15	73	- -	Total amount of cholesterol
14	17	44	16	74	15	61.5 gms.
15	18	45	17	75	16	
16	19	46	18	76	17	
17	20	47	19	77	18	
18	21	48	20	78	19	
19	22	49	- -	79	20	
20	23	50	- -	80	21	
21	- -	51	23	81	22	
22	25	52	24	82	23	
23	26	53	25	83	24	
24	27	54	26	84	25	
25	28	55	27	85	- -	
26	29	56	28	86	- -	
27	30	57	29	87	28	
28	31	58	- -	88	29	
29	Aug. 1	59	31	89	30	

July 2/47 to Dec.17/47

Male

No. 133.
Series 11A.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
July 2	2.750	122	6	48	0
3		Cholesterol feeding begun			
21			122	470	+
Aug.11			334	716	++
Sept.11			307	592	+++
Oct. 1	3860	120	320	800	++
		Alloxan administered			
7		134	235	425	++
8	3.210	136			0
19	3.700	500	390	820	+++
Nov.11	2.990	436	54	100	0
Dec. 1	3.400	570	6	54	0
17	2.260	Killed			

July 2/47 to Oct. 5/47

Female

No. 134.
Series 11A.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
July 2	2.760	123	6	50	0
3		Cholesterol feeding begun			
21			110	446	+
Aug. 11			74	266	+
Sept.11			129	342	+
Oct. 1	4.500	93	132	280	+
		Alloxan administered			
5		Died			

July 2/47 to July 4/47

Sex ?

No. 135.
Series 11A.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
July 2	2.860	124	6	18	0
3		Cholesterol feeding begun			
4		Killed			

July 2/47 to Nov. 3/47 — Female — No. 136. Series 11A.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0–4)
July 2	3.010	120	8	32	0
3		Cholesterol feeding begun			
21			110	500	+
Aug. 11			193	540	+
Sept.11			232	632	+
Oct. 1	4.660	101	176	424	++
		Alloxan administered			
7		530	340	640	+++
19	4.140	520	240	610	+++
Nov. 3	2.910	Died			

July 2/47 to Jan.18/48 — Male — No. 137. Series 11A.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0–4)
July 2/47	2.880	116	9	55	0
3		Cholesterol feeding begun			
21			92	388	+
Aug. 11			114	320	++
Sept.11			116	430	+
Oct. 7	3.200	113	152	420	++
19	3.510	160	158	468	0
Nov. 11	4.060	138	6	14	0
Dec. 1	4.080	108	6	12	0
28	4.370	114	6	12	0
Jan. 18/48	4.370	124	38	106	0
		Killed			

July 2/47 to July 4/47 — Sex ? — No. 138. Series 11A.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0–4)
July 2	2.840	108	6	38	0
3		Cholesterol feeding begun			
4		Killed			

July 2/47 to Aug.20/47

Sex ?

No. 139.
Series 11A.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
July 2	2.220	133	12	52	0
3		Cholesterol feeding begun			
21			58	246	+
Aug. 11			81	204	0
20		Killed			

July 2/47 to July 15/47

Sex ?

No. 140.
Series 11A.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
July 2	2.300	109	14	24	0
3		Cholesterol feeding begun			
15		Died			

July 2/47 to Nov.16/47

Male

No. 141.
Series 11A

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
July 2	2.560	109	6	18	0
3		Cholesterol feeding begun			
21			107	384	+
Aug. 11			60	102	0
Sept.11			130	560	+
Oct. 7		113	15?	50?	0
19	3.480	130	8	22	0
Nov. 11	3.840	121	6	14	0
16		Killed			

[illegible]TEROL FEEDING SCHEDULE

Nos. 142 - 150.
Series 11B.

Day	Date	Day	Date	Day	Date
0	July 4	30	Aug. 3	60	Sept.2
1	5	31	4	61	3
2	6	32	5	62	4
3	7	33	6	63	5
4	- -	34	7	64	6
5	9	35	8	65	7
6	10	36	9	66	8
7	11	37	10	67	9
8	12	38	11	68	10
9	13	39	12	69	11
10	14	40	13	70	12
11	15	41	14	71	13
12	16	42	15	72	- -
13	17	43	16	73	15
14	18	44	17	74	16
15	19	45	18	75	17
16	20	46	19	76	18
17	21	47	20	77	19
18	22	48	- -	78	20
19	23	49	- -	79	21
20	- -	50	23	80	22
21	25	51	24	81	23
22	26	52	25	82	24
23	27	53	26	83	25
24	28	54	27	84	- -
25	29	55	28	85	- -
26	30	56	29	86	28
27	31	57	- -	87	29
28	Aug. 1	58	31	88	30
29	2	59	Sept.1	89	Oct. 1

July - Oct. 1947

Dose 0.75 gms. of cholesterol in 15 cc. of corn oil. Dissolved and maintained at 60°C.

Fed by 14F. catheter.

Total time interval 90 days.

Total number of doses 82.

Total amount of cholesterol 61.5 gms.

July 4/47 to Jan.18/48 — Female — No. 142. Series 11B.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
July 4/47	2.890	111	7	36	0
		Cholesterol feeding begun			
22			167	474	++
Aug. 11			450	650	++
Sept.11			288	666	++
Oct. 2	4.130	83	250	576	+++
		Alloxan administered			. .
8		610	330	800	++++
19	3.360	560	175	320	+++
Nov. 11	3.570	514	6	18	0
Dec. 1	3.540	430	6	36	0
28	2.920	590	6	12	0
Jan.18/48	3.010	632			
		Killed			

July 4/47 to Dec.17/47 — Male — No. 143. Series 11B.

July 4	2.970	107	6	46	0
		Cholesterol feeding begun			
22			130	474	++
Aug. 11			185	630	++
Sept.11			272	654	+++
Oct. 2	3.750	97	220	630	++
		Alloxan administered			. .
8		284	310	750	++
19	3.780	170	140	300	++
Nov. 11	3.800	165	18	36	0
Dec. 1	3.800	97	6	20	0
17	3.710	Killed			

July 4/47 to Oct.10/47 — Female — No. 144. Series 11B.

July 4	2.880	105	6	45	0
		Cholesterol feeding begun			
22			70	260	+
Aug. 11			185	504	+
Sept.11			195	610	+
Oct. 2	3.660	88	212	490	++
		Alloxan administered			. .
8		570	210	490	+++
10		Died			. . .

July 4/47 to Jan.18/48 — Male — No. 145. Series 11B.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
July 4/47	2.830	103	6	59	0
		Cholesterol feeding begun			
22			110	426	+
Aug. 11			125	360	0
Sept.11			264	640	+
Oct. 8	3.710	126	170	360	0
19	3.950	140	122	320	0
Nov. 11	4.080	130	28	36	0
Dec. 1	4.150	105	15	48	0
28	4.330	118	10	32	0
Jan. 18/48	4.270	103	-	-	0
		Killed			

July 4/47 to Jan.18/48 — Male — No. 146. Series 11B.

July 4/47	2.750	103	6	23	0
		Cholesterol feeding begun			
22			26	156	0
Aug. 11			65	280	+
Sept.11			255	840	++
Oct. 8		120	148	600	++
Nov. 11	4.480	132	6	12	0
Dec. 1	4.600	99	6	12	0
28	4.790	99	6	80	0
Jan. 18/48	4.740	103			
		Killed			

July 4/47 to July 13/47 — Sex ? — No. 147. Series 11B.

July 4	2.800	113	7	51	0
		Cholesterol feeding begun			
13		Died			

July 4/47 to Jan.18/48 — Female — No. 148. Series 11B.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
July 4/47	2.630	106	10	45	0
		Cholesterol feeding begun			
22			110	350	++
Aug. 11			193	550	++
Sept.11			260	582	++
Oct. 8	4.610	138	215	540	++
19	4.880	126	97	280	0
Nov. 11	5.100	131	6	12	0
Dec. 1	5.150	125	28	35	0
28	5.400	107	6	12	0
Jan. 18/48	5.300	118			
		Killed			

July 4/47 to July 7/47 — Sex ? — No. 149. Series 11B.

July 4	2.870	126	6	59	0
		Cholesterol feeding begun			
7		Killed			

July 4/47 to Aug.19/47 — Sex ? — No. 150. Series 11B.

July 4	2.440	123	6	30	0
		Cholesterol feeding begun			
22			115	426	++
Aug. 11			175	500	+
19		Died			

CHOLESTEROL FEEDING SCHEDULE

Nos. 151 - 157.
Series 11B.

Day	Date	Day	Date	Day	Date
0	July 5	30	Aug. 4	60	Sept.3
1	6	31	5	61	4
2	7	32	6	62	5
3	- -	33	7	63	6
4	9	34	8	64	7
5	10	35	9	65	8
6	11	36	10	66	9
7	12	37	11	67	10
8	13	38	12	68	11
9	14	39	13	69	12
10	15	40	14	70	13
11	16	41	15	71	- -
12	17	42	16	72	15
13	18	43	17	73	16
14	19	44	18	74	17
15	20	45	19	75	18
16	21	46	20	76	19
17	22	47	- -	77	20
18	23	48	- -	78	21
19	- -	49	23	79	22
20	25	50	24	80	23
21	26	51	25	81	24
22	27	52	26	82	25
23	28	53	27	83	- -
24	29	54	28	84	- -
25	30	55	29	85	28
26	31	56	- -	86	29
27	Aug. 1	57	31	87	30
28	2	58	Sept.1	88	Oct. 1
29	3	59	2	89	2

July - Oct. 1947

Dose 0.75gms. of cholesterol in 15 cc. of corn oil. Dissolved and maintained at 60°C.

Fed by 14F. catheter.

Total time interval 90 days.

Total number of doses 82.

Total amount of cholesterol 61.5 gms.

July 5/47 to Sept.20/47 — Sex ? — No. 151. Series 11B.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
July 5	1.590	127			0
		Cholesterol feeding begun			
22			43	230	0
Aug. 11			170	496	++
Sept.11			158	504	+
20		Killed			

July 5/47 to Sept.10/47 — Sex ? — No. 152. Series 11B.

July 5	2.900	116	6	30	0
		Cholesterol feeding begun			
22			100	394	++
Aug. 11			122	416	+
Sept.10		Died			

July 5/47 to Aug. 24/47 — Sex ? — No. 153. Series 11B.

July 5	2.030	108	6	18	0
		Cholesterol feeding begun			
22			35	190	+
Aug. 11			100	352	+
24		Died			

July 5/47 to Jan. 18/48 — Female — No. 154. Series 11B.

Date	Weight kgms	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
July 5/47	2.420	104	6	13	0
		Cholesterol feeding begun			
22			20	112	0
Aug. 11			105	310	+
Sept.11			155	524	+
Oct. 8	3.600	132	6?	18?	0
19	3.840	135	6	12	0
Nov. 11	4.300	158	6	12	0
Dec. 1	4.090	109	6	30	0
28	4.110	111	15	24	0
Jan. 18/48	4.060	115			
		Killed			

July 5/47 to July 11/47 — Sex ? — No. 155. Series 11B.

July 5	1.630	101	6	18	0
		Cholesterol feeding begun			
11		Killed			

July 5/47 to Sept.15/47 — Sex ? — No. 156. Series 11B.

July 5	2.980	106	6	20	0
		Cholesterol feeding begun			
22			42	240	0
Aug. 11			66	170	0
Sept.11			75	268	+
15		Died			

July 5/47 to Aug. 10/47 — Male — No. 157. Series 11B.

July 5	3.650	104	6	14	0
		Cholesterol feeding begun			
22			174	670	++
Aug. 10			226	562	++
		Killed			

CHOLESTEROL FEEDING SCHEDULE

Nos. 158 - 169.
Series 12.

Day	Date	Day	Date	Day	Date
0	Sept.15	30	Oct.15	60	Nov.14
1	16	31	16	61	15
2	17	32	17	62	16
3	18	33	18	63	17
4	19	34	19	64	18
5	20	35	20	65	19
6	21	36	21	66	20
7	22	37	22	67	21
8	23	38	23	68	22
9	24	39	24	69	- -
10	25	40	25	70	24
11	- -	41	26	71	25
12	- -	42	27	72	26
13	28	43	28	73	27
14	29	44	29	74	28
15	30	45	30	75	29
16	Oct. 1	46	31	76	- -
17	2	47	Nov. 1	77	Dec. 1
18	3	48	2	78	2
19	4	49	3	79	3
20	5	50	4	80	4
21	6	51	5	81	5
22	7	52	- -	82	6
23	8	53	7	83	- -
24	9	54	8	84	8
25	10	55	9	85	9
26	11	56	10	86	10
27	12	57	11	87	11
28	13	58	12		
29	14	59	13		

Sept. - Dec. 1947

Dose 0.75 gms. of cholesterol in 15 cc. of corn oil. Dissolved and maintained at 60°C.

Total time interval 88 days.

Total number of doses 82.

Total amount of cholesterol 61.5 gms.

Sept.14/47 to Feb. 14/48 — Female — No. 158. Series 12.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Sept.14/47	2.600	130	12	62	0
15		Cholesterol feeding begun			
Oct. 15			165	512	++
Nov. 10			81	438	++
Dec. 1			238	630	++++
12	3.890	131	202	560	++..
23	3.380	118	240	580	0
Jan. 22/48	3.380	89	113	470	0
Feb. 14	3.110	110	72	194	0
		Killed			

Sept.14/47 to Nov. 6/47 — Sex ? — No. 159. Series 12.

Sept.14/47	2.460	130	16	62	0
15		Cholesterol feeding begun			
Oct. 15			196	544	++
Nov. 6		Died			....

Sept.14/47 to Jan. 24/48 — Female — No. 160. Series 12.

Sept.14/47	2.600	128	8	24	0
15		Cholesterol feeding begun			
Oct. 15			202	562	+
Nov. 10			225	560	++
Dec. 1			255	452	+++
12	3.960	128	282	760	++..
13		Alloxan administered			.
23	4.110	490	287	780	0
Jan. 22/48	4.260	340	36	225	0
24		Died			

Sept.14/47 to Sept.15/47 — Sex ? — No. 161. Series 12.

Sept.14/47	2.540	102	8	30	0
15		Cholesterol feeding begun			
		Died			

Sept.14/47 to Dec. 17/47 — Sex ? — No. 162. Series 12.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0–4)
Sept.14/47	2.660	111	6	56	0
15		Cholesterol feeding begun			
Oct. 15			204	396	+
Nov. 10			215	240	++
Dec. 1			200	580	+++
12	3.830	133	247	720	+
17		Killed			

Sept.14/47 to Feb. 28/48 — Male — No. 163. Series 12.

Sept.14/47	2.500	113	10	20	0
15		Cholesterol feeding begun			
Oct. 15			118	280	0
Nov. 10			150	290	++
Dec. 1			120	440	++
12	4.050	142	220	560	+
13		Alloxan administered			
23	4.100	176	190	546	0
Jan. 22/48	4.320	102	40	230	0
Feb. 15	4.240	142	42	96	0
27	4.220	130	6	20	0
28		Killed			

Sept.14/47 to Feb. 28/48 — Male — No. 164. Series 12.

Sept.14/47	2.400	105	10	30	0
15		Cholesterol feeding begun			
Oct. 15			72	120	0
Nov. 10			84	272	+
Dec. 1			36	110	++
12	4.050	142	202	510	++
13		Alloxan administered			
23	4.060	270	65	468	0
Jan. 22/48	4.220	?	50	190	0
Feb. 15	4.100	132	30	64	0
27	3.940	158	6	12	0
28		Killed			

Sept.14/47 to Feb. 29/48 — Female — No. 165. Series 12.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Sept.14/47	3.100	95	10	36	0
15		Cholesterol feeding begun			
Oct. 15			242	720	+
Nov. 10			?	480	++
Dec. 1			116	430	+
12	4.560	167	170	482	++
13		Alloxan administered			..
23	4.100	376	?	?	0
Jan. 22/48	3.950	330	45	140	0
Feb. 15	3.560	276	42	136	0
27	3.570	280	6	12	0
28		Alloxan administered			
29		Died			

Sept.14/47 to Dec. 17/47 — Male — No. 166. Series 12.

Sept.14/47	2.780	116	10	38	0
15		Cholesterol feeding begun			
Oct. 15			123	264	0
Nov. 10			148	576	+
Dec. 1			?	?	+
12	4.040	136	202	600	+
13		Alloxan administered			.
17		Died.			

Sept.14/47 to Sept.21/47 — Sex ? — No. 167. Series 12

Sept.14/47	3.370	127	8	40	0
15		Cholesterol feeding begun			
21		Died			

Sept.14/47 to Oct. 20/47 — Sex ?

No. 168.
Series 12.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Sept.14/47	2.890	110	8	20	0
15		Cholesterol feeding begun			
Oct. 20		Died			

Sept.14/47 to Sept.22/47 — Sex ?

No. 169.
Series 12.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Sept.14/47	3.120	107	14	32	0
15		Cholesterol feeding begun			
22		Died			

CHOLESTEROL FEEDING SCHEDULE

Nos. 170 - 181.
Series 13

Day	Date	Day	Date	
0	Oct.29	30	Nov.28	Oct. - Dec. 1947.
1	30	31	29	
2	31	32	- -	Dose 0.75 gms. of cholesterol
3	Nov. 1	33	Dec. 1	in 15 cc. of corn oil.
4	2	34	2	Dissolved and maintained at
5	3	35	3	60°C.
6	4	36	4	
7	5	37	5	Total time interval 59 days.
8	- -	38	6	
9	7	39	- -	Total number of doses 54.
10	8	40	8	
11	9	41	9	Total amount of cholesterol
12	10	42	10	40.5 gms.
13	11	43	11	
14	12	44	12	
15	13	45	13	
16	14	46	14	
17	15	47	15	
18	16	48	16	
19	17	49	17	
20	18	50	18	
21	19	51	19	
22	20	52	20	
23	21	53	21	
24	22	54	22	
25	- -	55	23	
26	24	56	24	
27	25	57	- -	
28	26	58	26	
29	27			

Oct.28/47 to Feb. 8/48 — Male — No. 170. Series 13.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Oct.28/47	3.040	102	6	30	0
29		Cholesterol feeding begun			
Dec. 1			170	370	+
27	3.860	126	74	410	++
28		Alloxan administered			
Jan.23/48	3.820	106	28	130	0
Feb. 7	4.100	140	25	100	0
8		Alloxan administered 10 hours before death.			
		Killed			

Oct.28/47 to Dec.18/47 — Sex ? — No. 171. Series 13.

Oct.28/47	3.470	97	18	50	0
29		Cholesterol feeding begun			
Dec. 1			170	354	++
18		Died			

Oct.28/47 to Feb. 8/48 — Female — No. 172. Series 13.

Oct.28/47	2.760	106	6	20	0
29		Cholesterol feeding begun			
Dec. 1			218	280	+
27	4.130	118	195	660	+
28		Alloxan administered			
Jan.22/48	3.960	330	21	100	0
Feb. 7	4.200	480	11	82	0
8		Alloxan administered 10 hours before death.			
		Killed			

Oct.28/47 to Feb. 8/48 — Female — No. 173. Series 13.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Oct.28/47	2.640	97	36	85	0
29		Cholesterol feeding begun			
Dec. 1			115	424	+
28	3.780	147	262	764	++
Jan.22/48	3.110	115	242	1112?	0
Feb. 7	3.330	128	90	380	0
8		Alloxan administered 10 hours before death.			
		Killed			

Oct.28/47 to Dec.16/47 — Sex ? — No. 174. Series 13.

Oct.28/47	3.550	102	28	56	0
29		Cholesterol feeding begun			
Dec. 1			58	290	+
16		Died			

Oct.28/47 to Jan. 3/48 — Male — No. 175. Series 13.

Oct.28/47	2.640	120	6	12	0
29		Cholesterol feeding begun			
Dec. 1			120	244	+
28	3.700	120	206	416	++
Jan. 3/48		Died			

Oct.28/47 to Feb. 8/48 — Male — No. 176. Series 13.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Oct.28/47	2.700	96	6	16	0
29		Cholesterol feeding begun			
Dec. 1			48	172	+
28	3.320	126	70	374	++
Jan.22/48	3.430	115	15	80	0
Feb. 7	3.330	?	21	80	0
8		Alloxan administered 10 hours before death.			
		Killed			

Oct.28/47 to Jan. 2/48 — Sex ? — No. 177. Series 13.

Oct.28/47	2.900	117	6	20	0
29		Cholesterol feeding begun			
Dec. 1			98	230	++
28	3.880	133	35	360	++
		Alloxan administered			
Jan. 2/48		Died			

Oct.28/47 to Feb. 8/48 — Female — No. 178. Series 13

Oct.28/47	2.450	121	6	22	0
29		Cholesterol feeding begun			
Dec. 1			145	300	+
28	3.580	138	113	464	++
		Alloxan administered			
Jan.22/48	3.490	440	25	116	0
Feb. 7	3.600	540	35	80	0
8		Alloxan administered 10 hours before death.			
		Killed			

Oct.28/47 to Feb. 8/48

Female

No. 179.
Series 13

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Oct.28/47	3.200	105	54	80	0
29		Cholesterol feeding begun			
Dec. 1			140	436	++
28	4.200	127	242	840	++
Jan.22/48	4.440	109	25	120	0
Feb. 7	4.260	147	42	170	0
8		Alloxan administered 10 hours before death.			
		Killed			

Oct.28/47 to Feb. 8/48

Female

No. 180.
Series 13.

Oct.28/47	2.270	117	6	12	0
29		Cholesterol feeding begun			
Dec. 1			164	480	++
28	3.580	136	44	150	++
Jan.22/48	4.070	?	15	88	0
Feb. 7	4.270	144	21	100	0
8		Alloxan administered 10 hours before death.			
		Killed			

Oct.28/47 to Dec.31/47

Sex ?

No. 181.
Series 13.

Oct.28/47	3.370	107	6	12	0
29		Cholesterol feeding begun			
Dec. 1			50	216	+
28	4.550	128	175	442	++
		Alloxan administered			
31		Died			

CHOLESTEROL FEEDING SCHEDULE

Nos. 182 - 204.
Series 14.

Day	Date	Day	Date	Day	Date	
0	Dec.26	29	Jan.24	57	Feb.21	Dec. 1947 - Mar. 1948.
1	27	30	- -	58	22	
2	28	31	26	59	23	Dose 0.75 gms. of cholesterol
3	29	32	27	60	24	in 15 cc. of corn oil.
4	30	33	28	61	25	Dissolved and maintained at
5	31	34	29	62	26	60°C.
6	- -	35	30	63	27	
7	Jan. 2	36	31	64	28	Total time interval 82 days.
8	3	37	Feb. 1	65	29	
9	4	38	2	66	Mar. 1	Total number of doses 69.
10	5	39	3	67	2	
11	6	40	4	68	3	Total amount of cholesterol
12	7	41	5	69	4	51.75 gms.
13	8	42	6	70	5	
14	9	43	- -	71	- -	
15	10	44	- -	72	- -	
16	11	45	- -	73	8	
17	12	46	10	74	9	
18	13	47	11	75	10	
19	14	48	12	76	11	
20	15	49	13	77	- -	
21	16	50	14	78	13	
22	17	51	15	79	- -	
23	- -	52	16	80	- -	
24	19	53	17	81	- -	
25	20	54	18			
26	- -	55	19			
27	22	56	20			
28	23					

Nov.12/47 to Dec.29/47 — Sex ? — No. 182. Series 14.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Nov.12	2.490	110	6	12	0
13		Alloxan administered			
Dec. 1		162			
22	3.680	154	10	12	0
26		Cholesterol feeding begun			
29		Died			

Nov.12/47 to Nov.13/47 — Sex ? — No. 183. Series 14.

Nov.12	2.530	108	6	16	0
13		Alloxan administered			
		Died			

Nov.12/47 to Feb.16/48 — Male — No. 184. Series 14.

Nov.12/47	2.660	114	6	12	0
13		Alloxan administered			
Dec. 1		380			
22	3.240	340	6	12	0
26		Cholesterol feeding begun			
Jan.25/48	3.600		18	36	0
Feb.16	3.580	126	18	96	0
		Killed			

Nov.12/47 to Nov.20/47 — Sex ? — No. 185. Series 14.

Nov.12	2.820	103	10	12	0
13		Alloxan administered			
20		Died			

Nov.12/47 to Mar.16/48 — Male — No. 186. Series 14.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Nov.12/47	2.510	99	6	22	0
13		Alloxan administered			
Dec. 1		346			
22	2.940	120	18	42	0
26		Cholesterol feeding begun			
Jan.25/48	3.110		600	1800?	++
Feb.18		168	1100	2100	+..
25			520	1944	++
Mar.12	3.110	152	820	3360	++++
15		Thorotrast injected			.....
16		Killed			

Nov.12/47 to Feb.14/48 — Sex ? — No. 187. Series 14.

Nov.12/47	2.610	113	6	12	0
13		Alloxan administered			
Dec. 1		386			
22	2.270	270	6	22	0
26		Cholesterol feeding begun			
Jan.25/48	2.510		445	1200	++
Feb.14		Died			.....

Nov.12/47 to Nov.12/47 — Sex ? — No. 188. Series 14.

Nov.12	2.030	121	12	36	0
13		Alloxan administered			
22		Died			

Nov.12/47 to Nov.21/47 — Sex ? — No. 189. Series 14.

Nov.12	2.530	108	6	36	0
13		Alloxan administered			
21		Died			

Nov.12/47 to Dec.29/47 — Sex ? — No. 190. Series 14.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0–4)
Nov.12	2.310	113	6	20	0
13		Alloxan administered			
Dec. 1		436			
22	1.930	482	270	550	+++
26		Cholesterol feeding begun			
29		Died			

Nov.12/47 to Nov.29/47 — Sex ? — No. 191. Series 14.

Nov.12	2.500	185	6	20	0
13		Alloxan administered			
29		Died			

Nov.12/47 to Mar.16/48 — Male — No. 192. Series 14.

Nov.12/47	2.710	120	6	30	0
13		Alloxan administered			
Dec. 1		366			
22	2.960	330	8	16	0
26		Cholesterol feeding begun			
Jan.25/48	3.590		506	2600?	0
Feb.18		496	560	3360	++++
Mar.12	3.360	365	764	1912	++++
14		Thorotrast injected			
16		Killed			

Nov.12/47 to Dec.28/47 — Sex ? — No. 193. Series 14

Nov.12	3.220	123	6	12	0
13		Alloxan administered			
Dec. 1		130			
26		Cholesterol feeding begun			
28		Died			

Nov.12/47 to Mar.16/48 — Male — No. 194. Series 14.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Nov.12/47	2.660	107	6	12	0
13		Alloxan administered			
Dec. 1		118			
22	3.570	246	10	12	0
26		Cholesterol feeding begun			
Jan.25/48	3.880		200	900	+
Feb.18		150	52	380	+
Mar.12	4.500	140	36	136	++
15		Thorotrast injected			. .
16		Died			

Nov.12/47 to Feb.16/48 — Male — No. 195. Series 14.

Nov.12/47	2.400	118	10	12	0
13		Alloxan administered			
Dec. 1		520			
22	2.230	350	6	12	0
26		Cholesterol feeding begun			
Jan.25/48	2.500		435	1440	++++
Feb.16	2.050				. . . .
		Died			

Nov.12/47 to Nov.17/47 — Sex ? — No. 196. Series 14.

Nov.12	2.810	160	15	40	0
13		Alloxan administered			
17		Died			

Nov.12/47 to Dec.30/47 — Sex ? — No. 197. Series 14.

Nov.12	2.400	107	40	80	0
13		Alloxan administered			
Dec. 1		276			
22	2.560	130	8	12	0
26		Cholesterol feeding begun			
30		Died			

Dec.22/47 to Jan. 2/48 — Sex ? — No. 198. Series 14

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Dec.22/47	2.700	130	20	24	0
26		Cholesterol feeding begun			
Jan. 2/48		Died			

Dec.22/47 to Feb.16/48 — Male — No. 199. Series 14.

Dec.22/47	2.280	132	8	20	0
26		Cholesterol feeding begun			
Jan.25/48	2.690		75	220	+
Feb.18	2.630	140	?	?	++
		Killed			

Dec.22/47 to Mar.16/48 — Male — No. 200. Series 14.

Dec.22/47	2.060	126	11	28	0
26		Cholesterol feeding begun			
Jan.25/48	2.380		510	820	+
Feb.18		158	404	800	++
Mar.12	2.830	150	450	882	++
		Thorotrast injected			
16		Killed			

Dec.22/47 to Feb.19/48 — Male — No. 201. Series 14.

Dec.22/47	2.410	142	8	36	0
26		Cholesterol feeding begun			
Jan.25/48	3.010		275	950	+
Feb.18		165	960	4200	+++
19	2.070				
		Died			

Dec.22/47 to Mar.16/48 — Male — No. 202. Series 14.

Date	Weight kgms.	Blood Sugar mgms.%	Serum Cholesterol Free mgms.%	Serum Cholesterol Total mgms.%	Lipaemia (0-4)
Dec.22/47	2.490	142	10	16	0
26		Cholesterol feeding begun			
Jan.25/48	3.480		228	670	+
Feb.18		144	300	235	0
Mar.12	4.100	124	180	416	++
14		Thorotrast injected			
16		Killed			

Dec.22/47 to Mar.15/48 — Male — No. 203. Series 14.

Dec.22/47	2.340	144	11	12	0
26		Cholesterol feeding begun			
Jan.25/48	3.410		320	780	++
Feb.18		127	180	550	+
Mar.12	4.170	136	260	624	++
14		Thorotrast injected			
15		Died			

Dec.22/47 to Dec.29/47 — Sex ? — No. 204. Series 14.

Dec.22	2.040	127	6	12	0
26		Cholesterol feeding begun			
29		Killed			

Figure 1

Diagram showing the gradation of lesions as 0, 1, 2, 3 and 4+ degrees of atherosclerosis.

No. 81, diabetic
No. 85, semi-diabetic
No. 84, control
No. 77, alloxan resistant
No. 79, alloxan resistant

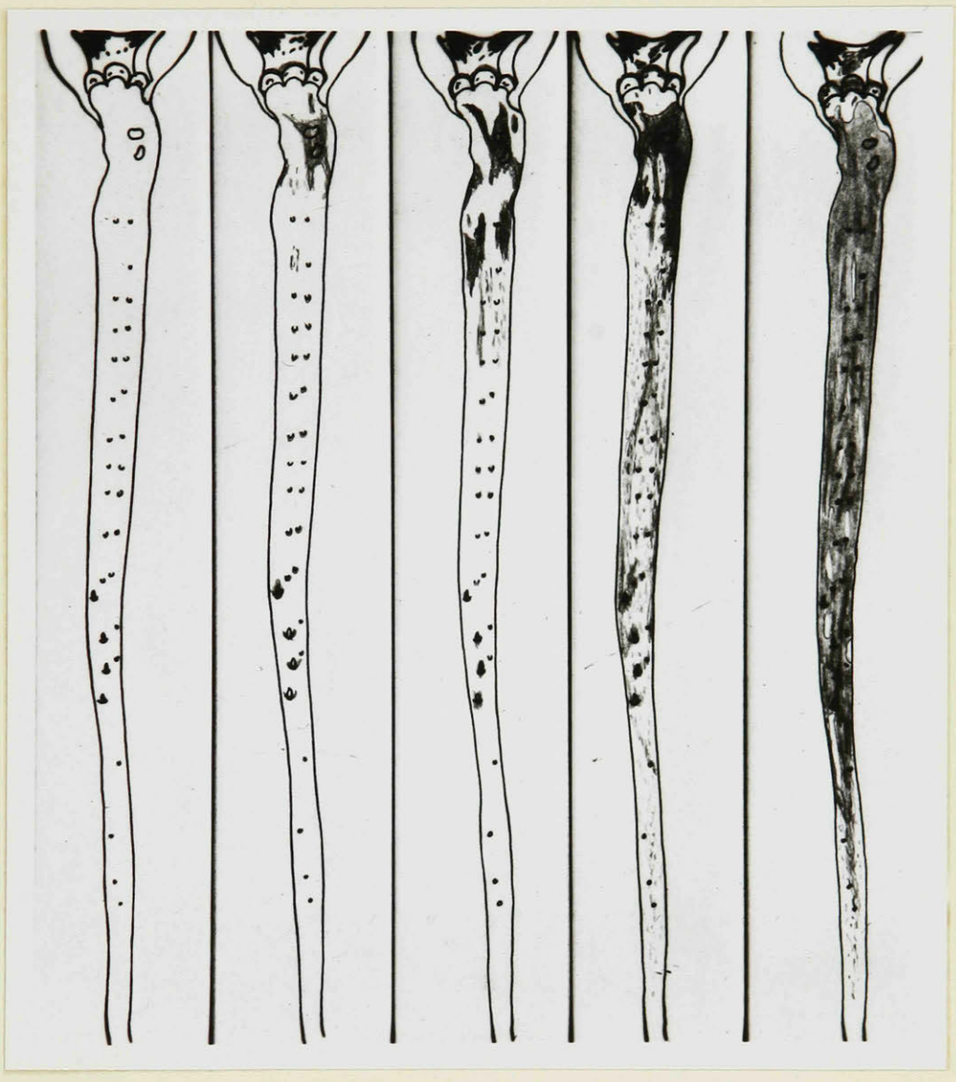
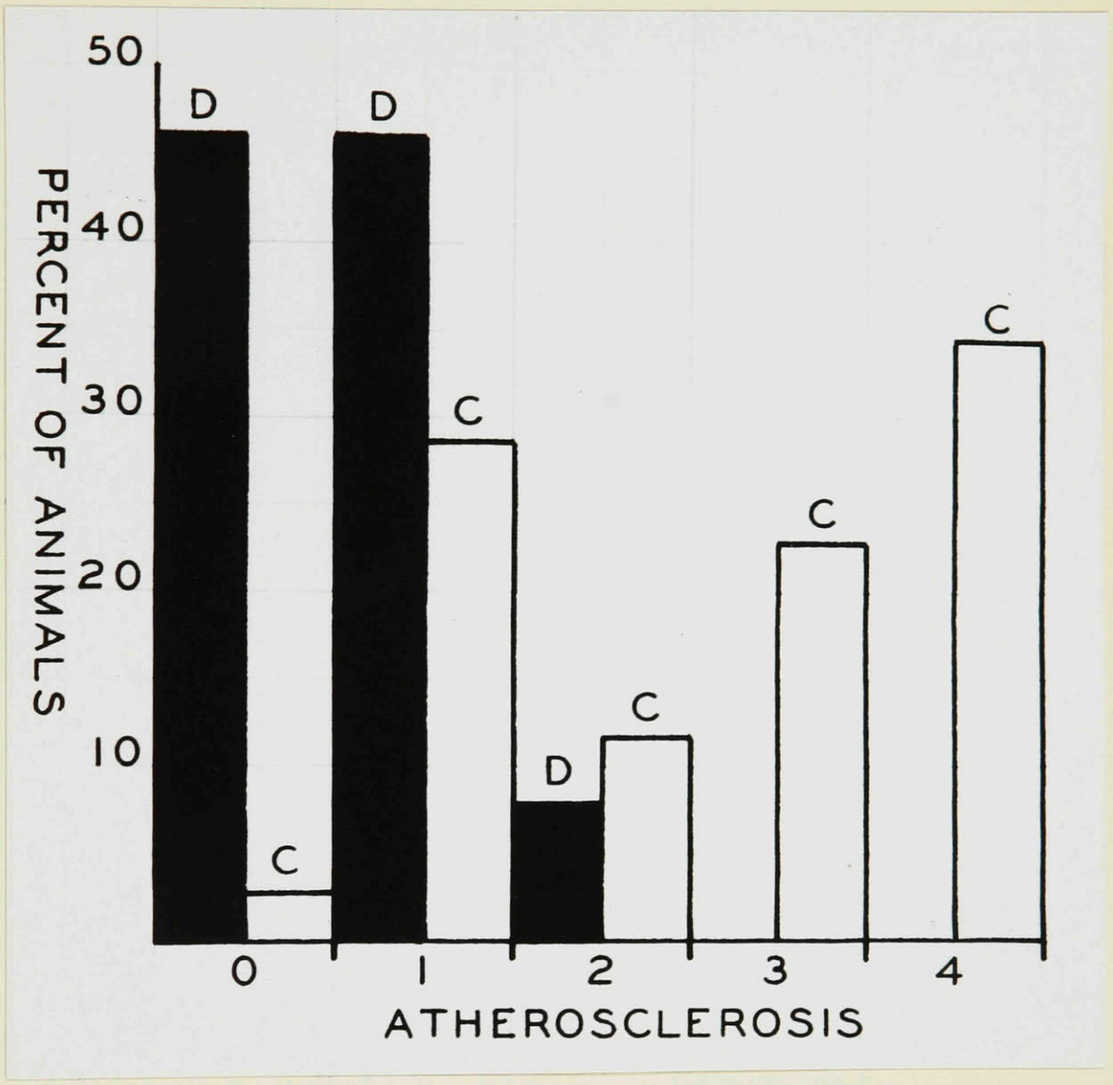


Figure 2

Chart showing distribution of aortic atherosclerosis among rabbits rendered diabetic and then fed cholesterol.

Series 3 and 4 are omitted.

C = control D = diabetic


50
40
30
20
10
PERCENT OF ANIMALS
D
C
D
C
D
C
C
C
0
1
2
3
4
ATHEROSCLEROSIS

2

Figure 3

Series 1 - 38 grams of dry cholesterol fed during 91 days.

No. 1, diabetic
No. 4, diabetic
No. 5, control
No. 7, control
No. 8, control

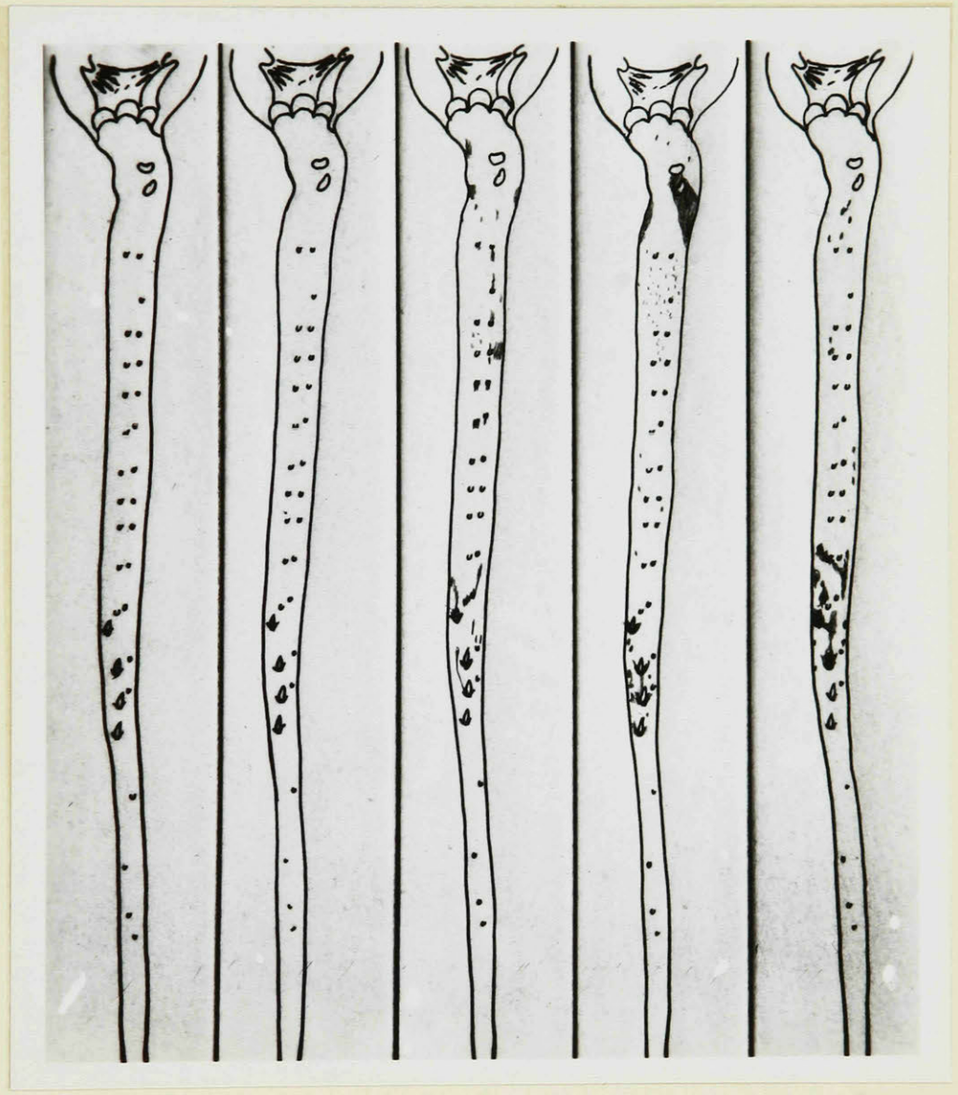


Figure 4

Series 5 - 48.5 grams of cholesterol fed during 89 days.

No. 55, diabetic
No. 57, control
No. 59, control
No. 62, control
No. 54, alloxan resistant
No. 60, alloxan resistant

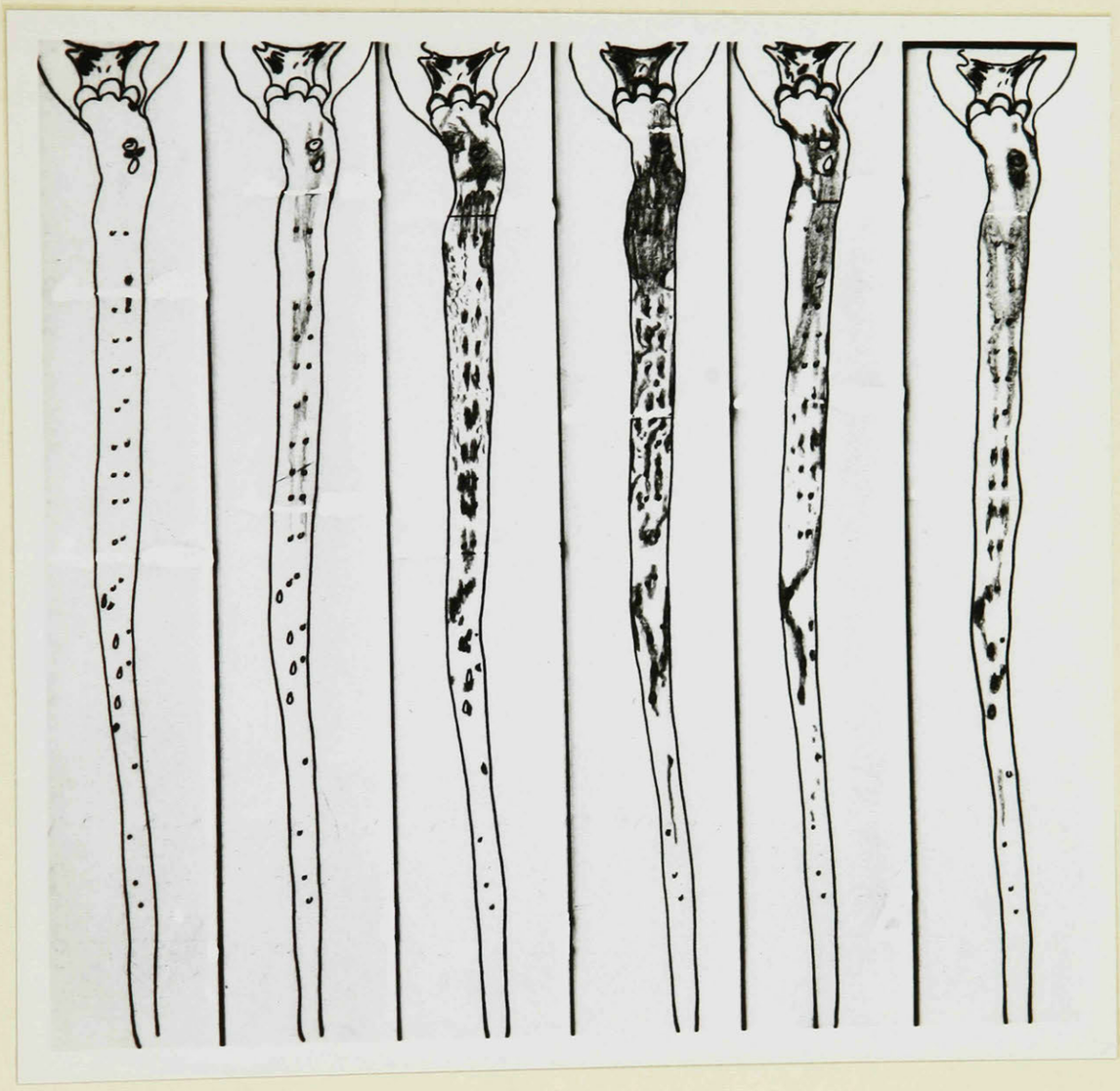


Figure 5 Series 6 - 46.25 grams of cholesterol fed during 90 days.

No. 69, diabetic
No. 70, diabetic
No. 66, control
No. 74, control
No. 64, alloxan resistant
No. 72, alloxan resistant
No. 75, alloxan resistant

Figure 6

Series 7 - 51.75 grams of cholesterol fed during 76 days.

No. 81, diabetic
No. 78, control
No. 84, control
No. 77, alloxan resistant
No. 79, alloxan resistant
No. 85, semi-diabetic

Figure 7

Series 8 - 60 grams of cholesterol fed during 82 days.

No. 88, diabetic
No. 92, diabetic
No. 96, control
No. 98, control
No. 90, alloxan resistant

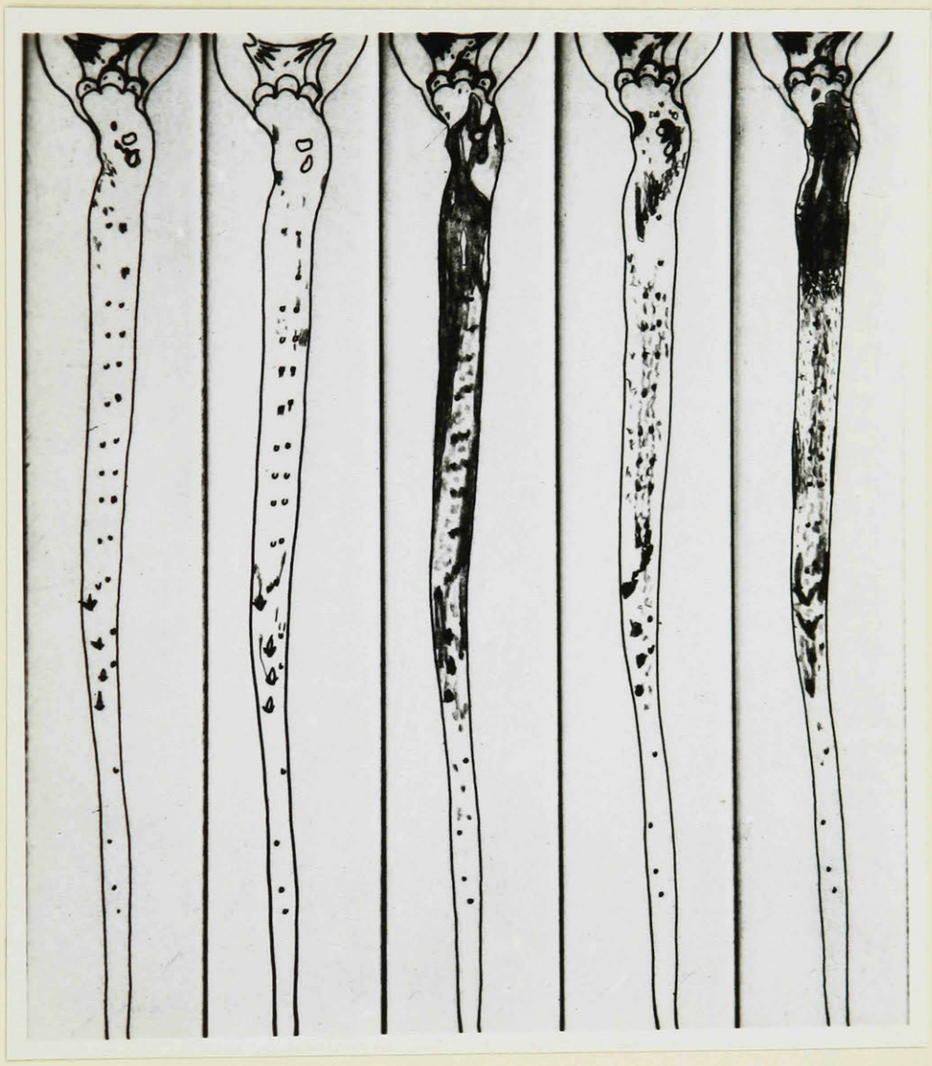


Figure 8 Series 9 - 65.25 grams of cholesterol fed during 89 days.

No. 99, diabetic
No. 108, control
No. 109, control
No. 110, control

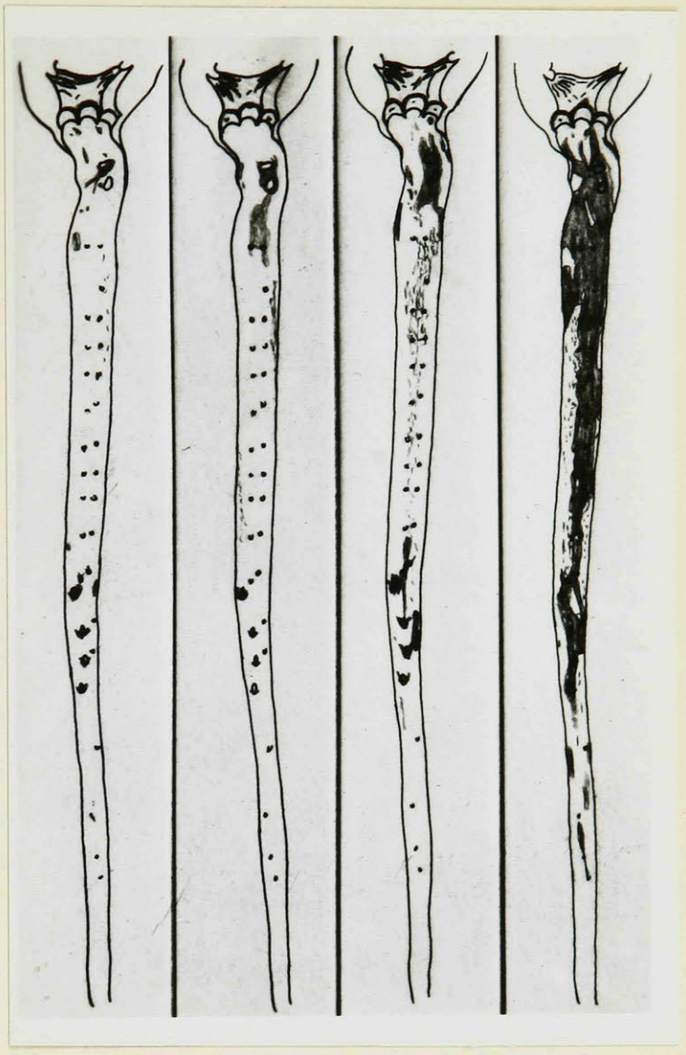
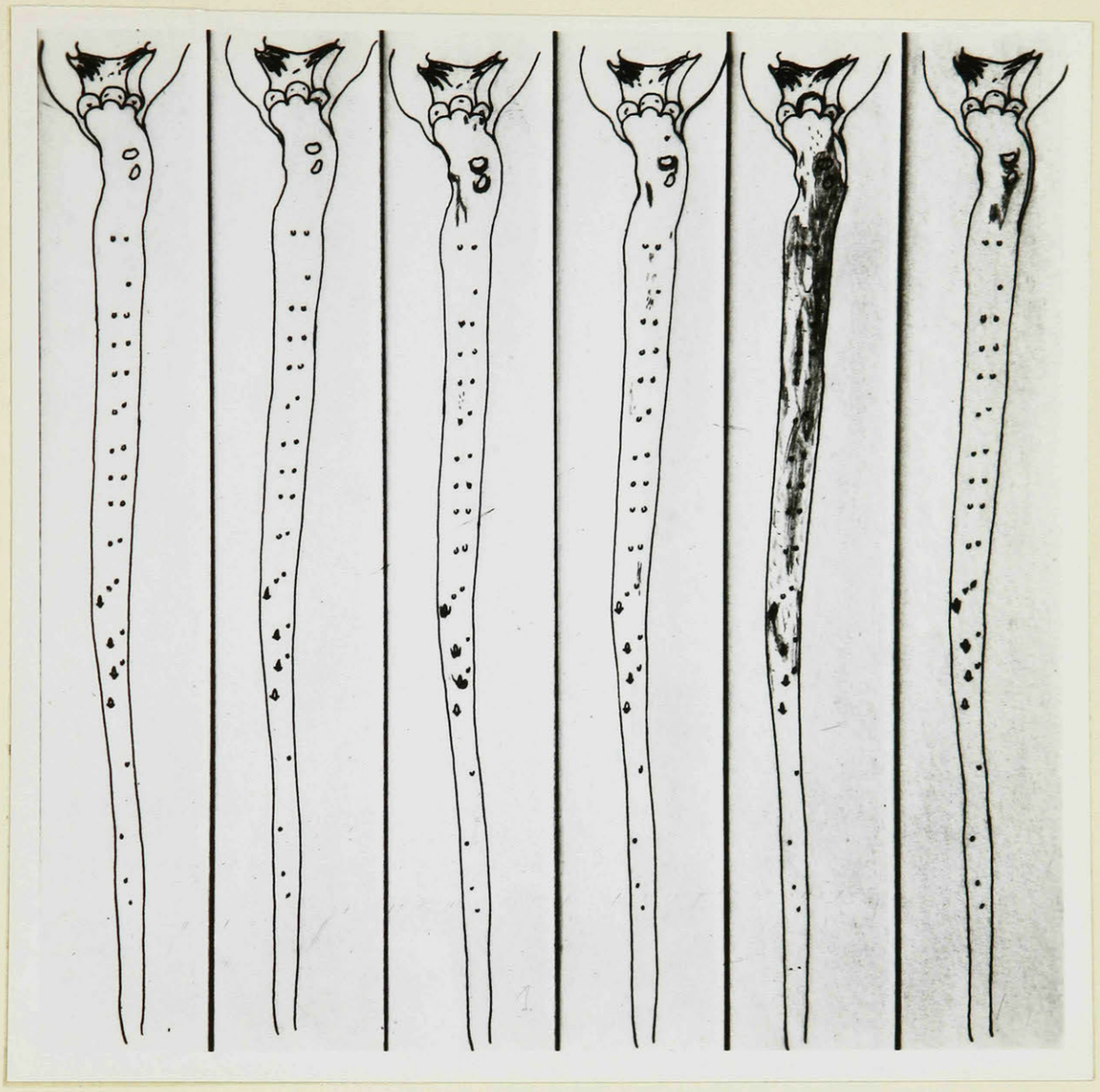


Figure 9 Series 10 - 39.25 grams of cholesterol fed during 53 days.

No. 116, diabetic
No. 117, diabetic
No. 120, control
No. 122, control
No. 123, control
No. 119, alloxan resistant



9

Figure 10

Series 14 (A) - 39 grams of cholesterol fed during 52 days.

No. 195, diabetic
No. 199, control
No. 201, control
No. 184, alloxan resistant

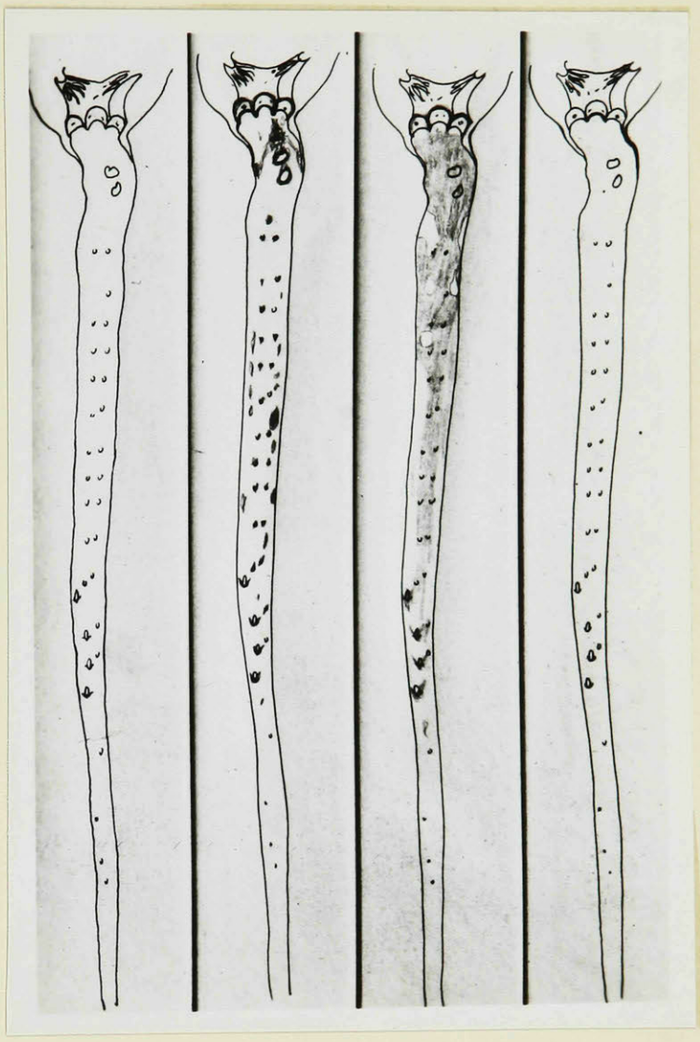


Figure 11 Series 14 (B) - 51.75 grams of cholesterol fed during 82 days.

No. 192, diabetic
No. 200, control
No. 202, control
No. 203, control
No. 186, alloxan resistant
No. 194, alloxan resistant

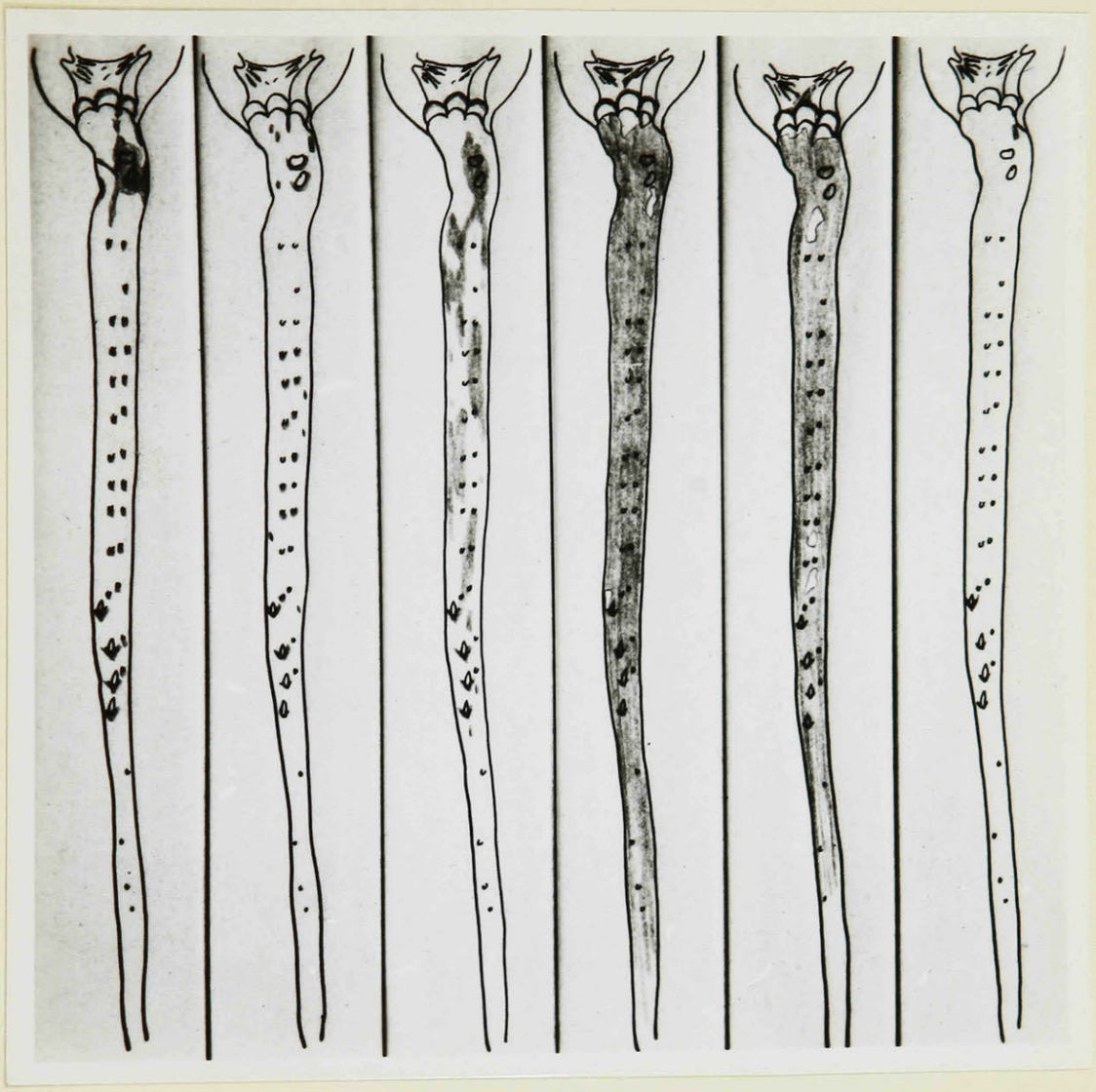


Figure 12 Chart of distribution of atherosclerosis of aorta in Series 11, 12 and 13 comprising aortae from animals fed cholesterol, then rendered diabetic and allowed to live without cholesterol feeding for from 5 to 16 weeks.

C = control D = diabetic

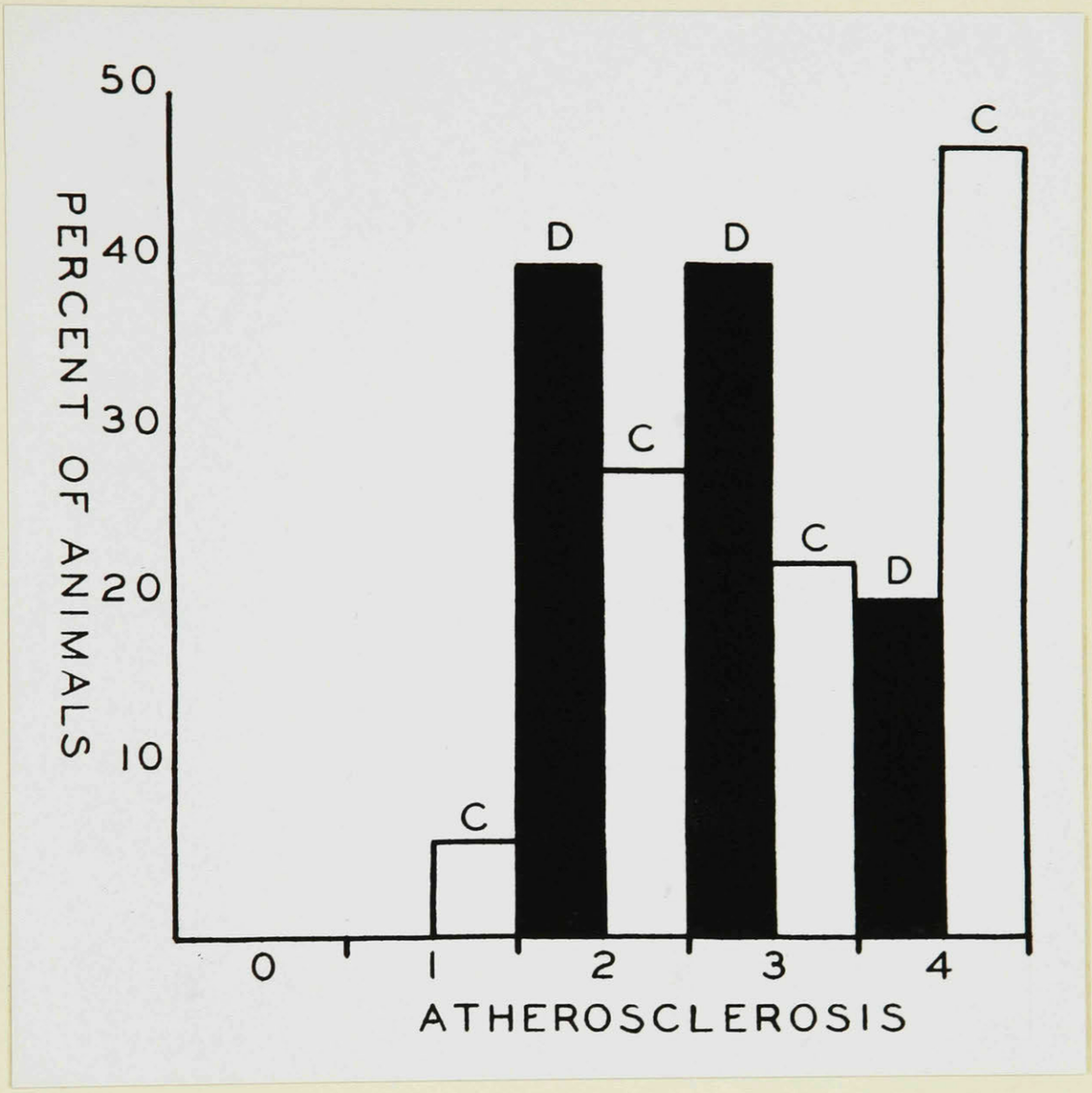

50
40
30
20
10
PERCENT OF ANIMALS
C
D
C
D
C
D
C
0
1
2
3
4
ATHEROSCLEROSIS

Figure 13

61.5 grams of cholesterol fed during 90 and 88 days. Regression period, 1 week.

No. 134, diabetic
No. 144, diabetic
No. 166, diabetic
No. 162, control

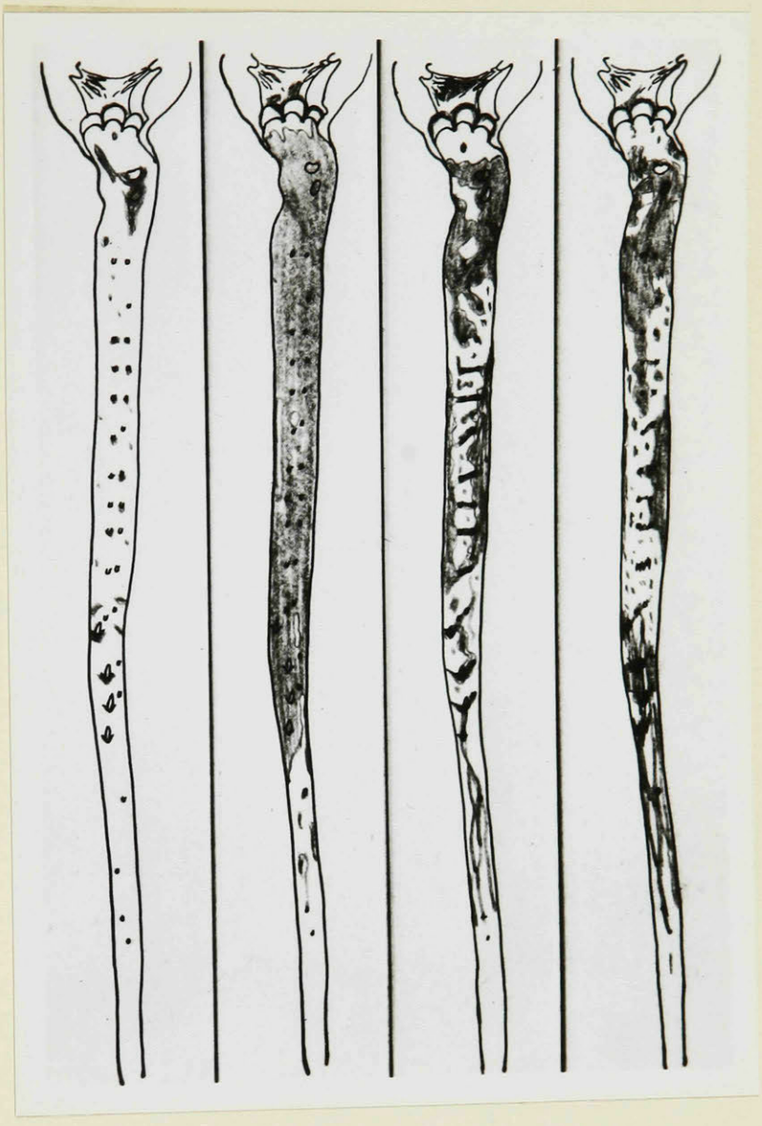


Figure 14

61.5 grams of cholesterol fed during 90 days.

Regression period, 3 weeks.

No. 126, diabetic

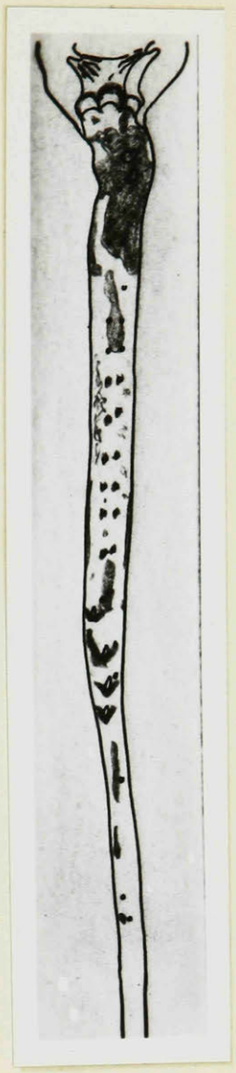


Figure 15

61.5 grams of cholesterol fed during 90 days.

Regression period 5 weeks.

No. 136, diabetic
No. 129, control
No. 130, control

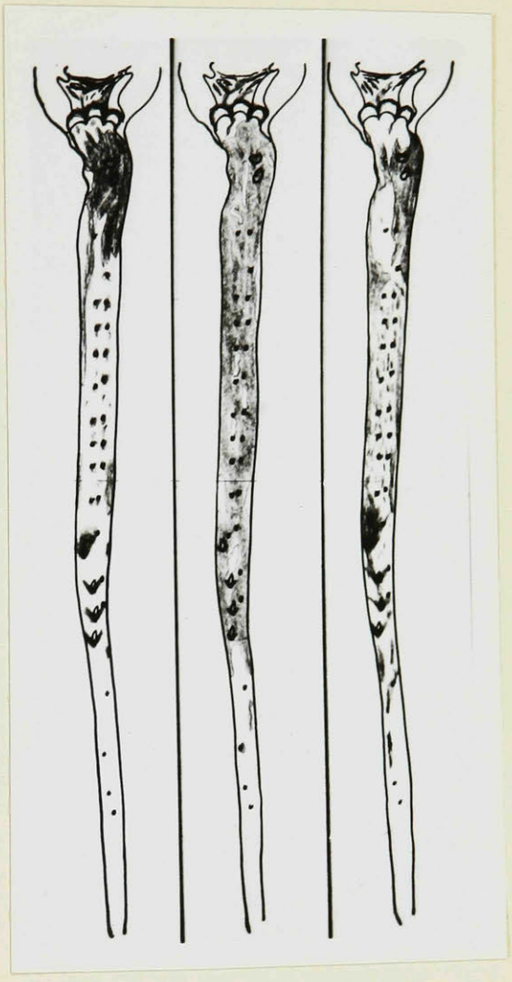


Figure 16

61.5 grams of cholesterol fed during 90 days.

Regression period, 7 weeks.

No. 124, diabetic
No. 141, control

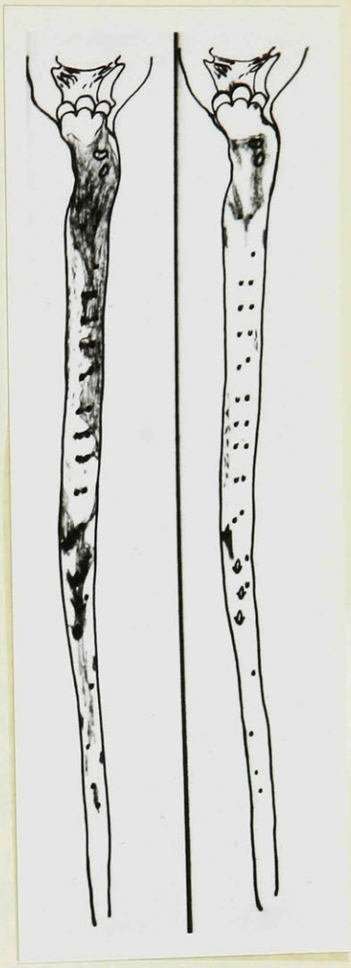


Figure 17

62.25 and 61.5 grams of cholesterol fed during 89 and 88 days respectively. Regression period, 9 weeks.

No. 121, diabetic

No. 158, control

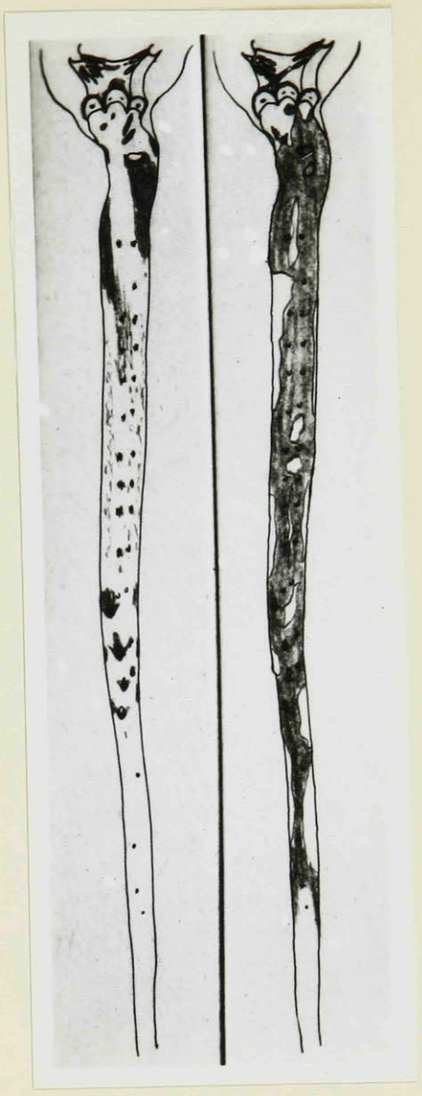


Figure 18

61.5 grams of cholesterol fed for 88 and 90 days. Regression period, 11 weeks.

No. 165, mildly diabetic,
No. 133, diabetic,
No. 163, alloxan resistant,
No. 164, alloxan resistant,
No. 143, alloxan resistant.

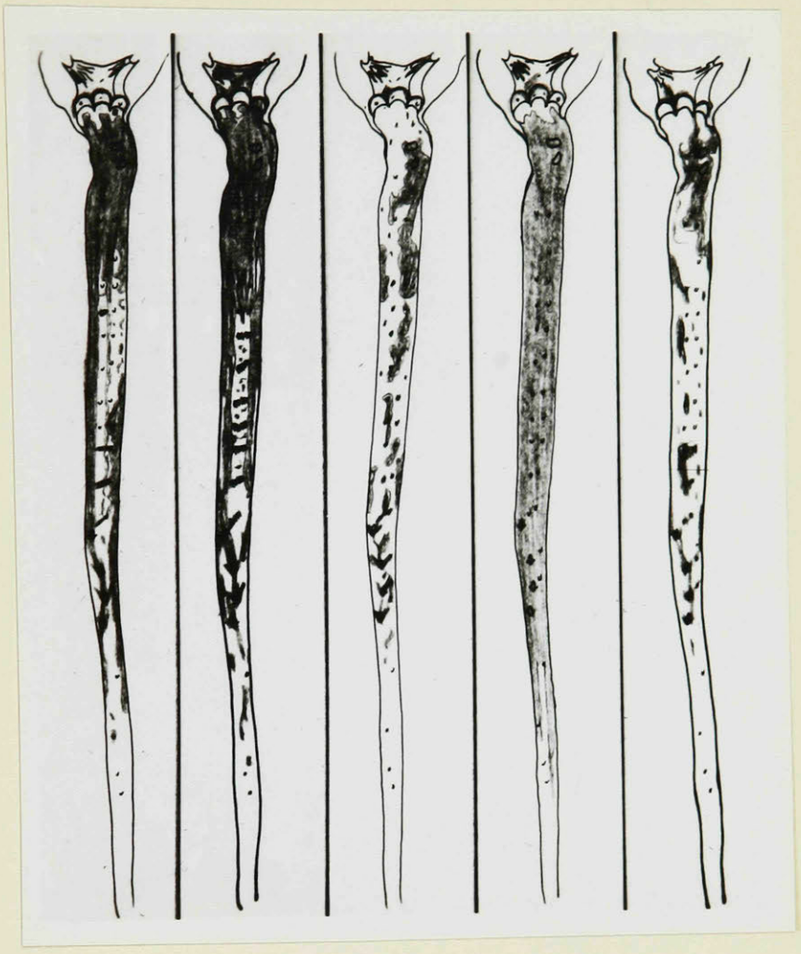


Figure 19

61.5 grams of cholesterol fed during 90 days.

Regression period, 16 weeks.

No. 128, diabetic
No. 142, diabetic
No. 148, control
No. 154, control
No. 146, control
No. 145, control
No. 137, control
No. 131, control

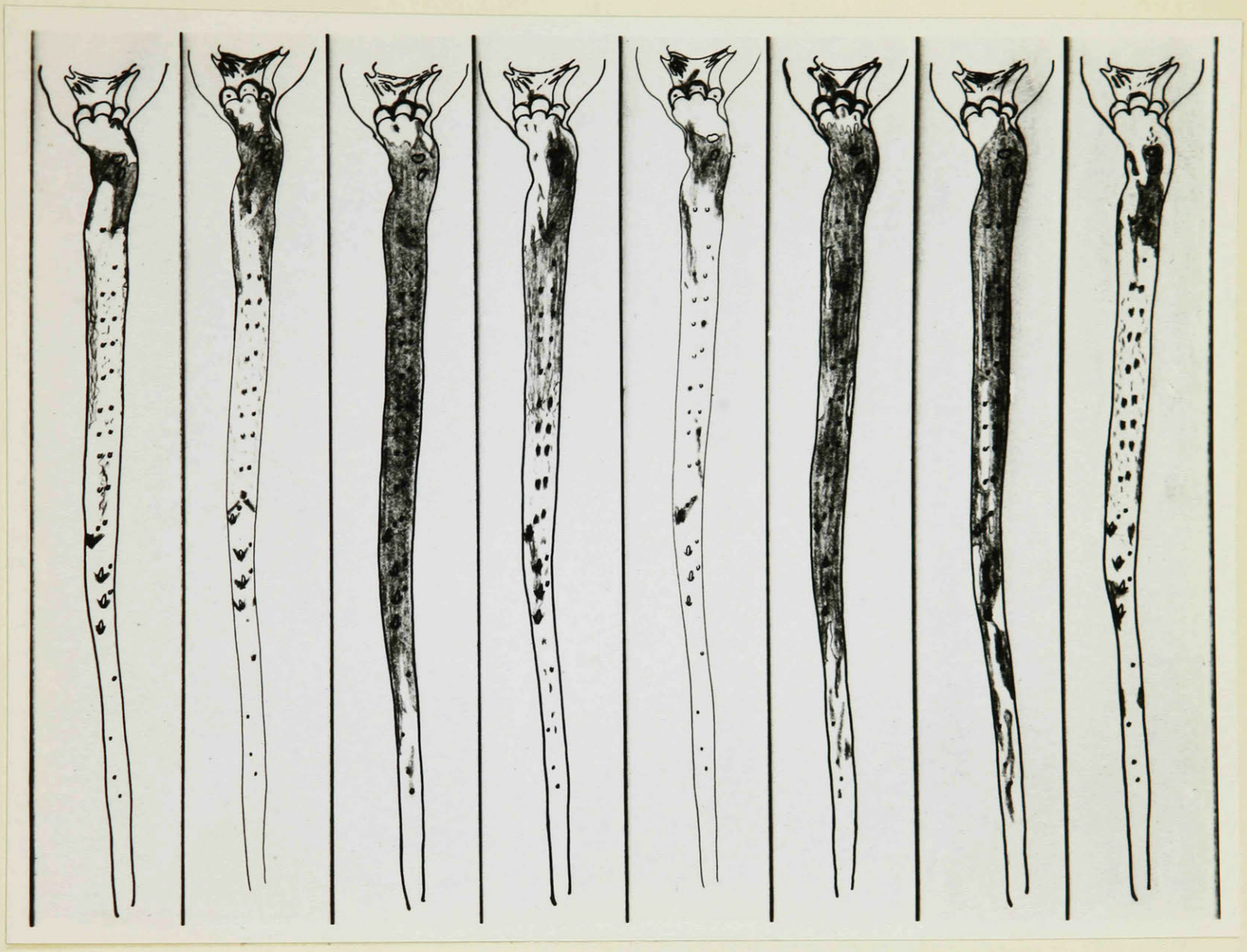


Figure 20

40.5 grams of cholesterol fed during 59 days.

Regression period, 6 weeks.

No. 172, diabetic
No. 178, diabetic
No. 173, control
No. 176, control
No. 179, control
No. 180, control
No. 170, alloxan resistant

Figure 21

Graph of experimental results obtained with rabbit #88, Series 8.

There is a + degree of aortic atherosclerosis.

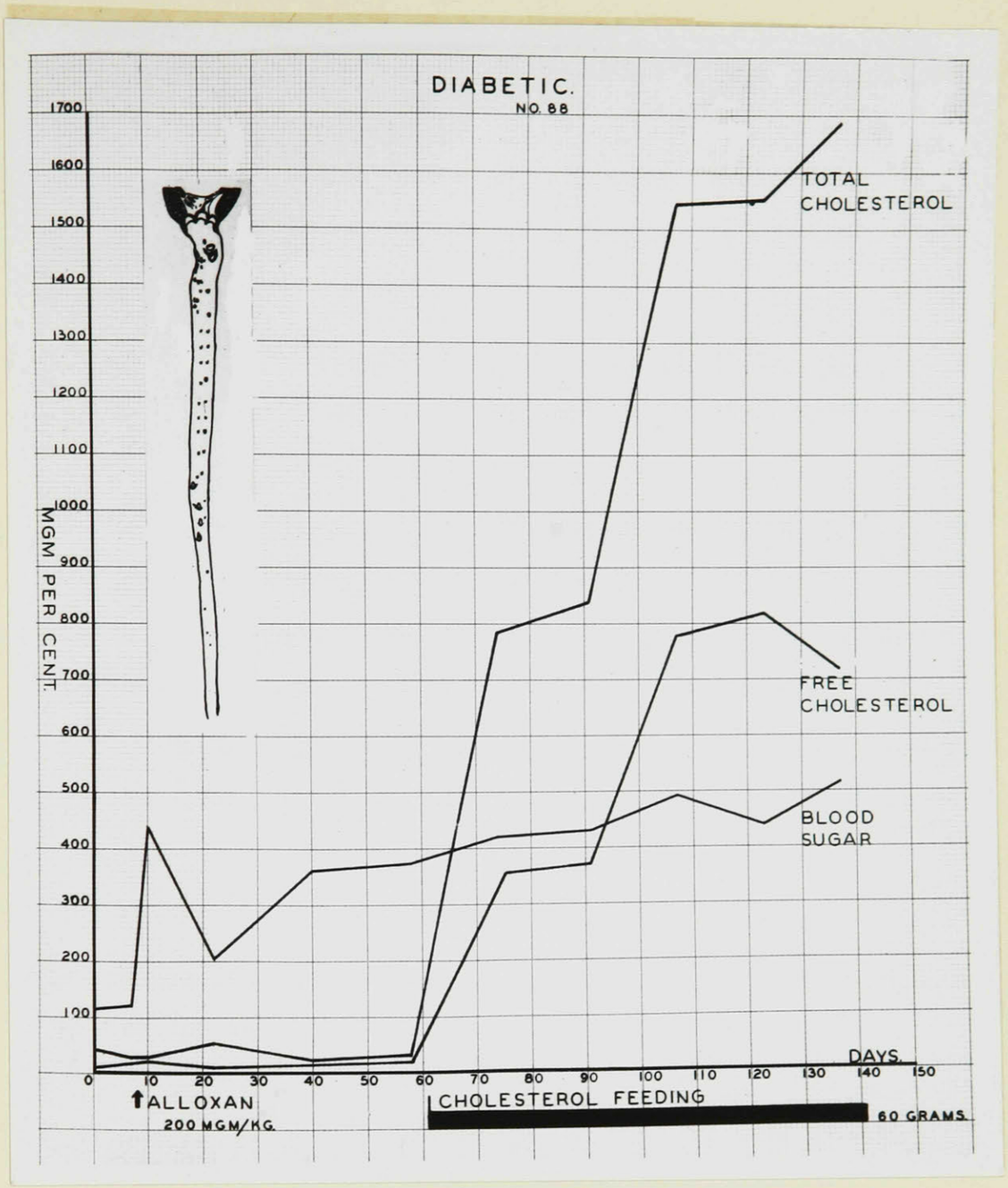

DIABETIC.
NO. 88
TOTAL CHOLESTEROL
FREE CHOLESTEROL
BLOOD SUGAR
MGM. PER CENT.
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
DAYS.
ALLOXAN
200 MGM/KG.
CHOLESTEROL FEEDING
60 GRAMS.

Figure 22

Graph of experimental results obtained with rabbit #96, Series 8.

There is a ++++ degree of aortic atherosclerosis.

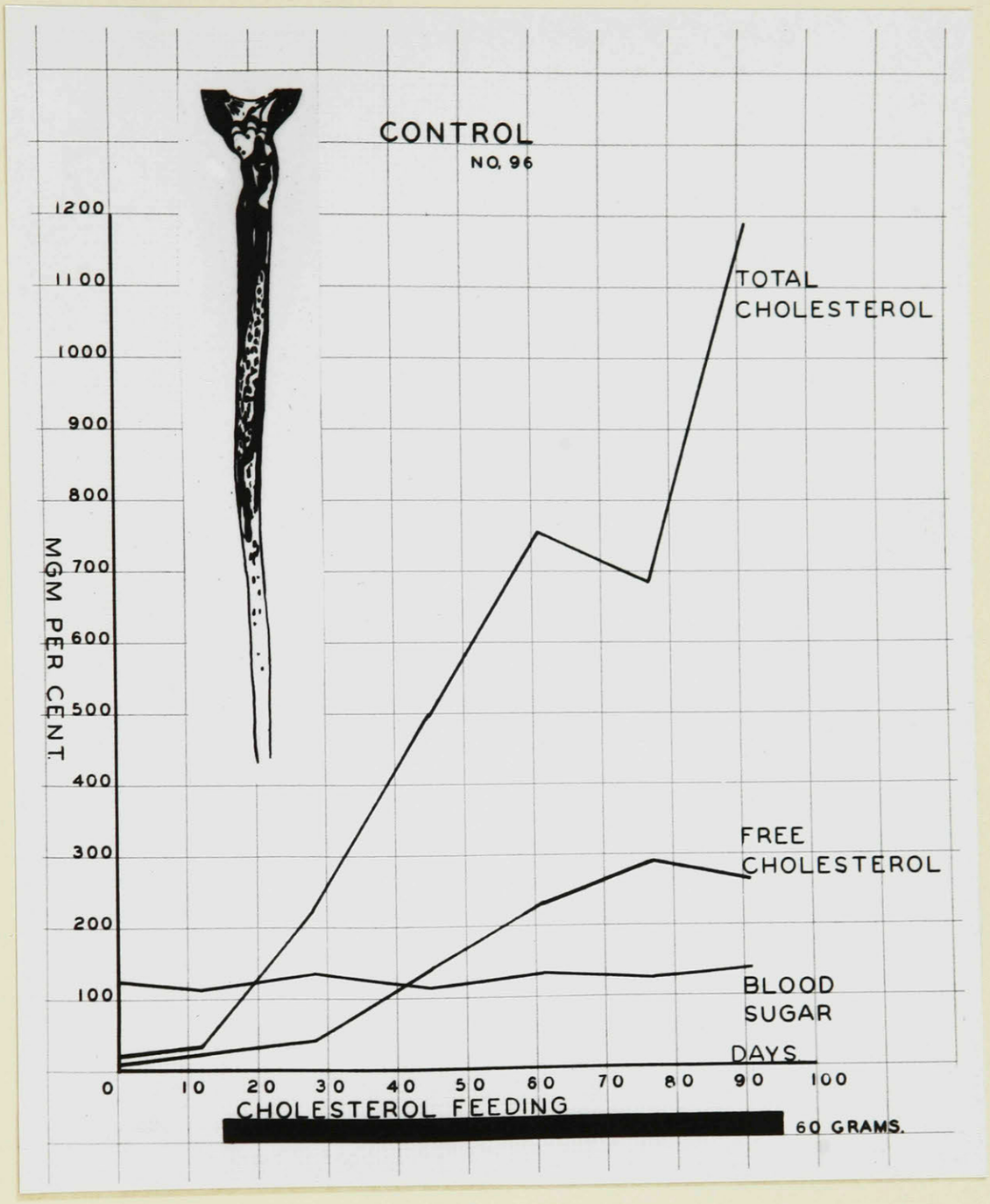

CONTROL
NO. 96
1200
1100
1000
900
800
700
600
500
400
300
200
100
MGM PER CENT.
TOTAL
CHOLESTEROL
FREE
CHOLESTEROL
BLOOD
SUGAR
DAYS
0
10
20
30
40
50
60
70
80
90
100
CHOLESTEROL FEEDING
60 GRAMS.

Figure 23

Graph of experimental results obtained with rabbit #92, Series 8.

There is a + degree of aortic atherosclerosis.

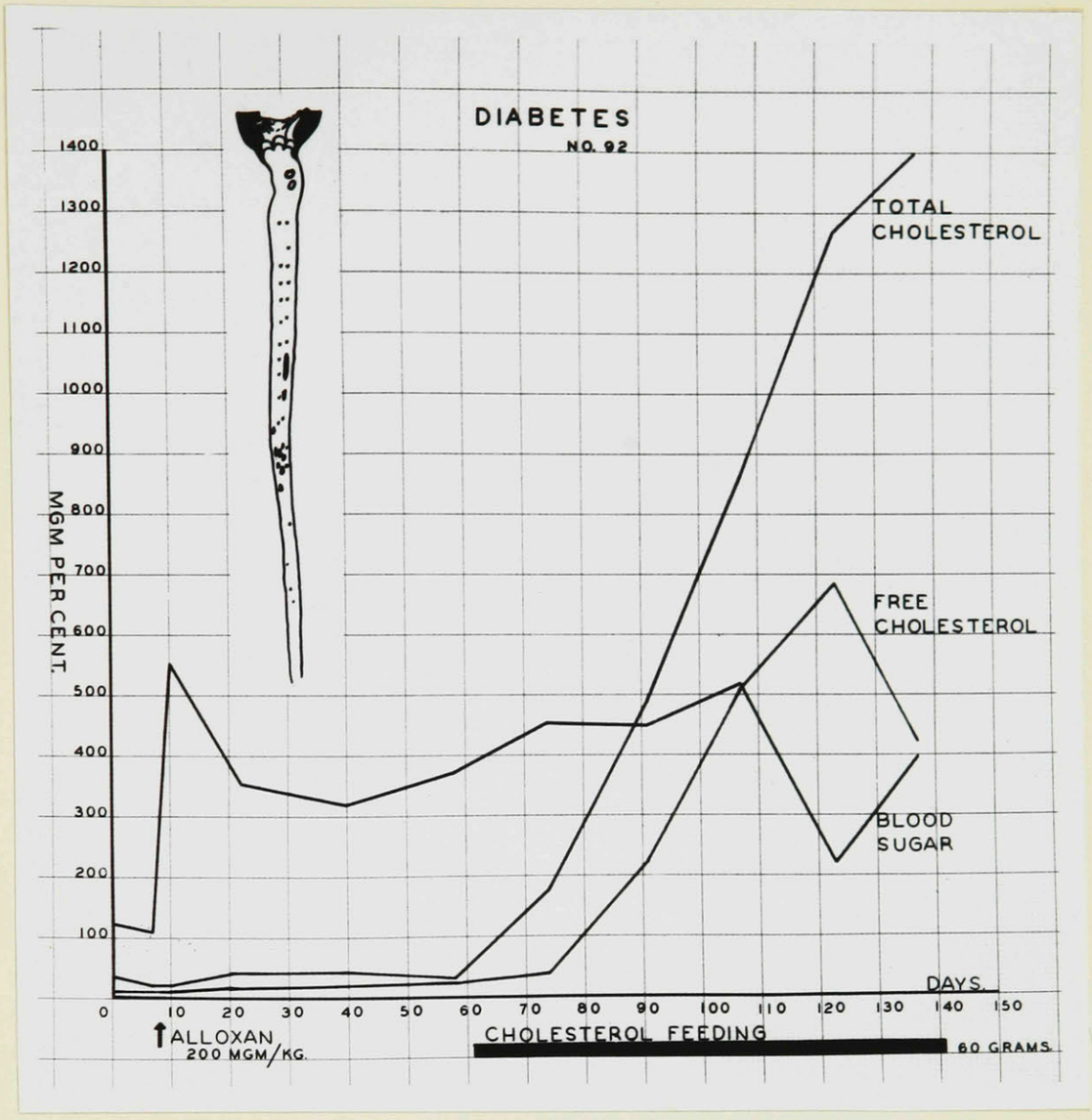
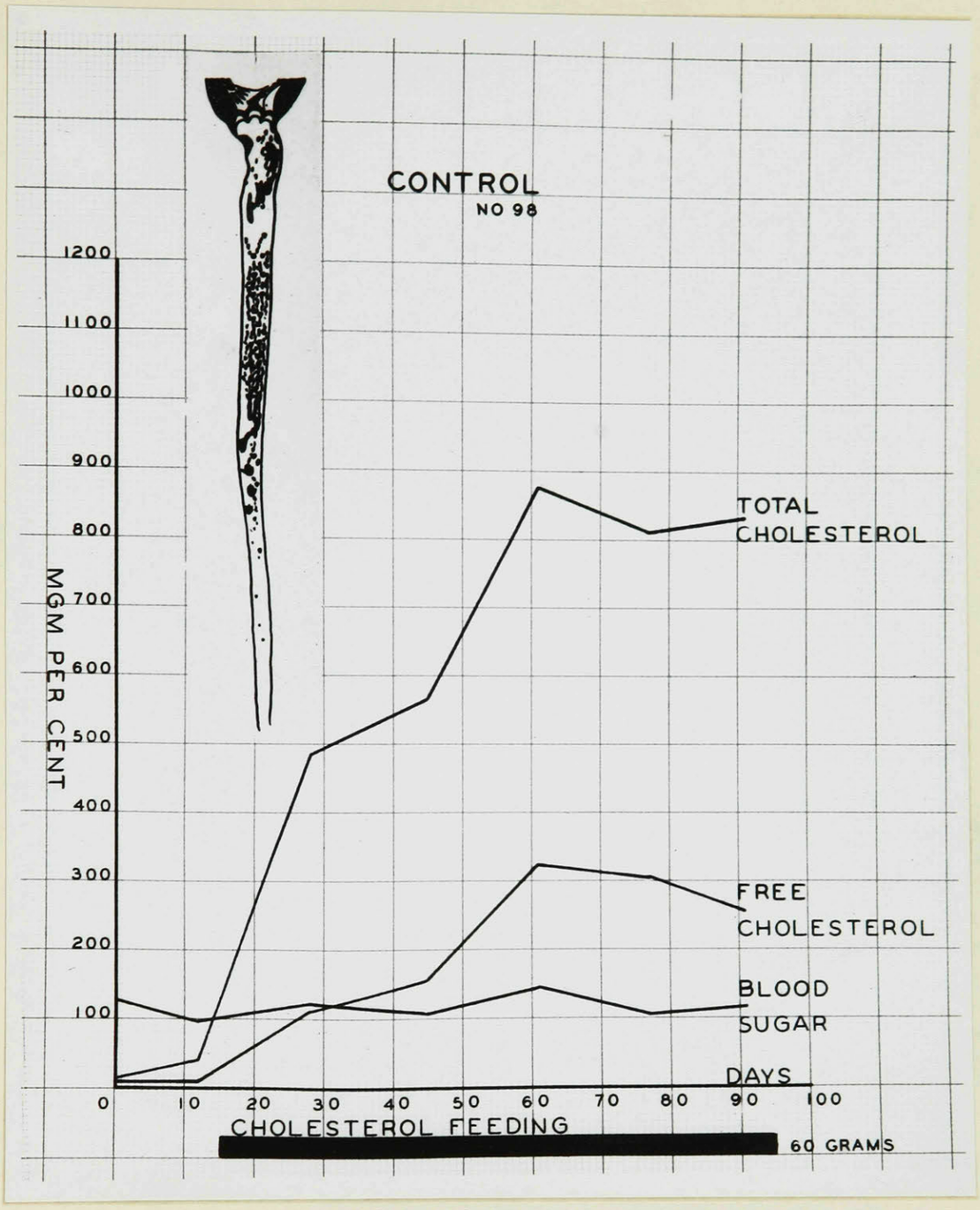

DIABETES
NO. 92
TOTAL CHOLESTEROL
FREE CHOLESTEROL
BLOOD SUGAR
MGM PER CENT.
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
DAYS.
ALLOXAN
200 MGM/KG.
CHOLESTEROL FEEDING
60 GRAMS.

Figure 24

Graph of experimental results obtained with rabbit #98, Series 8.

There is a ++ to +++ degree of aortic atherosclerosis.


CONTROL
NO 98
1200
1100
1000
900
800
700
600
500
400
300
200
100
MGM PER CENT
TOTAL
CHOLESTEROL
FREE
CHOLESTEROL
BLOOD
SUGAR
DAYS
0
10
20
30
40
50
60
70
80
90
100
CHOLESTEROL FEEDING
60 GRAMS

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