THE ACCUMULATION OF COLLOIDAL SUBSTANCES IN THE LESIONS OF EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS

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INTRODUCTION

"Wie schwer sind nicht die Mittel zu erwerben
Durch die Man zu den Quellen steigt"

Faust, Goethe.

The study of atherosclerosis has been pursued by men in many parts of the world with Churchillian tenacity. The literature published on the facts and theories about this disease, can only be described as colossal. It is a faithful record of the confusion and disappointments accompanying the struggle to conquer the disease which respects no race and attacks the age when respect and reverence are due. No wonder then that experimentation in this field is viewed with some trepidation and much determination!

The experiment described here was undertaken as a result of a recent report on the accumulation of colloidal Thorium Dioxide in experimental cholesterol atherosclerosis. In a fully developed atheromatous plaque, the substance was demonstrated both in the intimal lining and the foam cells of the lesion. Normal endothelium did not accumulate thorium dioxide at the dose administered in the experiment. The next

question obviously is: When, in the course of experimental atherosclerosis, does the endothelium acquire the property of accumulating particulate matter in its cytoplasm?

Would normal endothelium pick up the macromolecular substance if enough of it were injected - is there a threshold where the intimal lining becomes permeable to, or becomes phagocytic towards, macromolecular substances? Other factors could be observed in the course of the experiment. The influence of hemodynamics in determining the localization of particulate matter in the altered endothelium could be studied. It is hoped that the findings of the experiment, as reported here, will be of some value in the struggle to master this plague of the human race.

PART I.

THE DEFINITION OF ATHEROSCLEROSIS.

An ever increasing number of afflictions of the human body, when specifically named, bring to the mind a clear and definite notion of the nature of the disease involved. But a condition of the arteries, known to the layman as "hardening of the arteries" and to the more medically minded as "arteriosclerosis" or "atherosclerosis" has defied definition universally acceptable. How is it defined?

Anyone seeking the meaning of arteriosclerosis in the medical dictionary(1) will find the following - "a condition of thickening of the coats of the arteries with inflammatory changes, degenerative or productive". Such a definition is both uninformative and inaccurate. A variety of definitions may be found in text books. Bell(2) dispatches arteriosclerosis summarily in these terma - "All forms of arterial disease, except those that are frankly inflammatory in character, are commonly called arteriosclerosis". Anderson(3) does not attempt an exact definition, giving instead a general description of what it should include. He recognizes three alterations of arteriosclerosis: (1) atherosclerosis, consisting of "intimal fibrosis and lipoidosis", (2) medial calcinosis (Mönckeberg's sclerosis), and (3) arteriolar sclerosis. Boyd(4) agrees with those to whom arteriosclerosis signifies "an omnibus term which

includes a variety of conditions not necessarily related to one another". Like Anderson, he distinguishes three forms according to their microscopic appearance, and distribution. This distinction is a step toward clarification, since the forms mentioned differ morphologically and a common etiology cannot be claimed or denied at present.

Turning to the more advanced contributions of investigators limited to the field of arteriosclerosis, it is found that the definition of this term remains in the form of a general description of a "constellation of processes"(5) which of necessity excludes the still unknown etiology and approaches pathogenesis through the morphological evolution of the disease. Among the few who attempt a strict definition, Moschcowitz(6) succeeds only in presenting a too inclusive and inaccurately localized description. The section of his definition describing hyperplasia of one or more of the structural elements (of the arteries) as a "primary reaction" is insufficiently localized, since it is known that, in the early stages, the lesion is of the intimal and medial layers.

As previously indicated, the terms arteriosclerosis and atherosclerosis are frequently employed synonymously. It is here considered preferable to maintain the definition of arteriosclerosis as given by Aschoff(7) - "To sum up, we understand by arteriosclerosis a chronic disturbance of the vessels which manifests itself by deposits of the most varied kinds in the vascular walls and which becomes irreversible on reaching its climax in vessels impaired by changes attending the process of aging with resulting deformation of the lumen and brittleness of the vascular walls". Atherosclerosis, on the other hand, could more

adequately be defined by MacCallum[8] (although this author used the term arteriosclerosis himself) "a fairly sharply defined disease of the arterial wall most easily comprehended as the result of the abnormal localization of lipid in the intima with consequent in wandering of phagocytes to engulf the fat and new formation of much dense tissue".

In the present review, the terms as used in the literature referred to will be retained, but consideration of the disease will be more limited to what MacCallum has called arteriosclerosis, but has here been taken as a definition of atherosclerosis.

HISTORY[9]

Any review of arteriosclerosis must of necessity take into account its history because in the earnest, if sometimes confused, search of the men in the early centuries lies the seed of our knowledge on the subject today. From the history also it can be seen that although great strides have been made in the histology, pathology, and clinico-pathological relation, the same cannot be said of the etiology and pathogenesis. About the latter two, many theories have enriched the literature. Their very multiplicity indicates the answer has not yet been found.

Arteriosclerosis was first reported by the anatomists of the 16th century. We find no reference made to this affliction in the writings of the ancients, although we know that it did occur among them, through the studies of Marc Armand Ruffer (1911), who succeeded in dissecting, fixing and sectioning arteries of Egyptian mummies from as far back as 1580 B.C. He concluded that arteriosclerosis was as common among the ancients as it is in modern times. It was not, however, until the 16th century that anatomists began noting "bony" areas in the aorta. Vesalius (1514-64) showed familiarity with aortic aneurysm. Although by 1600 physicians were generally aware of "ossification" of arteries, not much attention was attracted by the associated softening, and the lesion was

generally regarded as a natural phenomenon of old age. Most references to the subject were purely descriptive. The 17th century contributed little further. Wepfer (1620-95) described the arteriosclerotic aneurysms at the base of the brain and had his own aortic lesions described at necropsy by Brunner. With the dawn of the 18th century appeared a number of treatises on the physiology and pathology of the vascular system. Vieussens (1715), Lancisi (1707) and others following roused speculation on the nature of arteriosclerosis. Briefly mentioned may be such theories as Crell, who maintained that incrustations universally spoken of as osseous were not bony but tophaceous, derived from pus. Along with Haller and Meckel he has the honour of directing attention to the softening process in arterial degeneration. Morgagni (1761) associated pain in the chest and "ossification" of the coronary arteries.

The early part of the 19th century made great strides in pathological anatomy. Bichat approached the problem from the histologists point of view. Dissecting the aortic coats, he placed the initial lesion, "ossification", in the intima. Hodgson (1815) considered atheromatous changes the result of inflammation, with its soft content as pus. He concluded that arteriosclerosis was a true disease and not a natural phenomenon of aging. To Loebstein of Strasbourg (1833) goes the honour of coining the word arteriosclerosis. Further understanding was forecast by the observations of Jean Cruvelhier who described the extreme sclerotic change in the arterioles in bodies where larger arteries were almost intact.

An attempt to explain the pathogenesis was made by Karl Rokitansky. He believed arteriosclerosis resulted from an excessive deposition on the intima of material from the blood mass and although he did not understand the nature of the blood disturbance causing the deposition, he felt it could not be attributed to age alone. With the introduction of the microscope and microtome, cellular pathology came to the fore. Risse showed that intimal lesions were under, not on top of, the endothelium. This convinced Virchow that it was not a question of surface deposit as Rokitansky had thought, but an imbibition of the intima with swelling and degeneration, followed by proliferation of the connective tissue cells, and softening of the ground substance. His later views, as found in the writings of Rindfleisch, were that the initial lesion was represented as a chronic inflammatory overgrowth of connective tissue with resulting thickening, fatty degeneration and impregnation with a calcium substance. This was distinguished from simple fatty degeneration of the intima and still another condition described as a ring-like petrifaction of the media.

Meanwhile, in England, George Johnson described arteriosclerosis of the kidney. He did not, however, recognize the thickening as degenerative in nature, but believed it to be a simple thickening of the muscular coat. Gull and Sutton showed the arteriolar thickening to be a "hyaline fibroid" change. They considered this as the "primary and essential condition of the morbid state called chronic Bright's disease with contracted kidney".

Huebner and Döhle on the continent and Welch in England separated syphilitic arteritis from arteriosclerosis. Benda (1903) stressed the relation of syphilis to aortic aneurysm.

It was now evident that several types of chronic arterial damage existed. Further investigations into the pathogenesis of atheroma, calcification and related damage of the arterial trunks were made by Thoma and Jores whose theories are now but of historical interest. Marchand proposed the term atherosclerosis as describing the changes most conspicuous in arteriosclerosis. Oskar Klotz, in 1911, ably reviewed the pathology of this disease and stressed the pleural etiology, naming infections, poisons, work, regional strain and old age.

While the anatomical lesions had been noted and studied since the 16th century, clinical investigation into the functional morbidity of the arterial system lagged behind, and it was not until the early 19th century that clinical investigation was begun. Measurements of the blood pressure were gradually perfected. The relation of hypertension, Bright's disease, cardiac hypertrophy and arteriosclerosis were studied. Mahomed, Albutt, and Huchard are prominent figures in this field.

The early 20th century saw the dawn of the experimental production of arteriosclerosis in animals(10). The first attempts appear to have been made by producing mechanical injury to arteries, resulting only in inflammatory changes (Malkoff, Jores, Lange and others). Permeability of the arterial wall and hypertrophy of the intima were studied by Petroff, Glasunow, Anitschkoww and Okuneff. Loeb, Adler and Zinserling studied

spontaneous arterial lesions in animals. Adrenalin sclerosis in rabbits was first produced by Josué (1903). Ignatowski, in 1908, produced experimental cholesterol atherosclerosis by feeding animal proteins to rabbits. He attributed the lesions to the protein in the diet. Atherosclerotic lesions produced in rabbits by intravenous injections of staphylococci and alcohol by Saltykow (1908) and those produced by infectious agents, toxic and mechanical, by Klotz (1908) were not confirmed by subsequent workers. On the other hand, Farr, Stuckey and Wesselkin were able to reproduce the lesions first found by Ignatowski, and the latter two demonstrated decisively that alimentary atherosclerosis was due to the fatty content of the food. Anitschkow and Chalaton then produced a typical picture of the disease by the feeding of pure cholesterol.

Thus the foundation was layed upon which numerous modern investigators, both clinical and experimental, attempted to build a knowledge of arteriosclerosis, and thence pave the way towards its prevention and cure.

THE ETIOLOGY AND PATHOGENESIS OF ARTERIOSCLEROSIS

Numerous indeed are the factors incriminated in the production of arteriosclerosis. A century ago, speculations on the etiology and pathogenesis appeared in the works of the pathologists. Rokitansky[11] believed atheromatous plaques were due to excessive deposits on the intima due to the "pre-existence of a peculiar crasis of the blood". According to Aschoff[12], Virchow considered the humoral dyscrasia as a possible etiological factor, but favoured a "mechanical etiology". In his book "Die Cellularpathologie", Virchow[13] does not treat the theories of etiology and pathogenesis attributed to him. After showing that the atheromatous lesion is part of the intima itself, he dispatches with the theory introduced by Rokitansky in the following terms: "Auf dieser weise wird die, auch von Rokitansky längere Zeit vertheidigte Ansicht widerlegt, dass es sich ursprünglich um eine Auflagerung auf die Fläche der interen Haut handele". He describes two processes: one, a simple fatty degeneration or metamorphosis of the aorta - these are very superficial in the intima and appear as white or yellowish-white streaks. They are not to be confused with what he calls "Endarterites Chronica deformans nodosa", which he believes originates as an inflammatory process "Der an sich passive Charakter des fettigen Endstadiums (Ausganges) ändert nichts an dem activen, irritativen Aufangstadium[13a]. This active,

irritative stage of the initial atheromatous lesions he compared to an active inflammatory process. "...sodann eine zweite Reihe von Vorgängen, wo wir vor der Fettmetamorphose ein Stadium der Reizung untersheiden können, welches übereinstimmt mit dem Stadium des Schwellung Vergrösserung Trübung, das wir an anderen entzündeten Stellen sehen(136)".

It may be pointed out that although Virchow's theory of the inflammatory etiology of arteriosclerosis is no longer universally acceptable, it is, nevertheless, possible that certain infections may play a role in the production of the disease in a limited number of cases as suggested by Ophüls(14). Anitschkow(14) pointed out that syphilitic infection of the aortic wall predisposes to the development of atheromatous lesions. The role of rheumatic fever in the pathogenesis of arteriosclerosis has also been considered.

Aschoff(12) himself argues forcefully for the imbibition theory with primary loosening of the ground substance due to wear and tear and followed shortly by an imbibition of the inner layer of the aorta with lipoid substances, especially cholesterol ester. In the infant and at puberty, the lesion is essentially an atheromatous one, since connective tissue proliferation does not occur and there is no tendency towards sclerosis. Indeed, complete regression of these lesions is possible. In old age, although the mechanism is still similar, the aortic wall is "worn" - the elastic tissue is inferior. There is marked proliferation of connective tissue which can undergo swelling and degeneration, giving rise to superimposed layers of hyaline degeneration. Other areas will be

composed chiefly of swollen and loosened ground substance infiltrated with fat from the plasma.

Reviewing the problem again, Aschoff[15] states his views more concisely. He regards arteriosclerosis as a trophic vascular disturbance closely connected with the wear and tear of advancing age, and brought on by a variety of factors of a physical and chemical nature. Some factors he considers are: excessive mechanical strain, alteration in the composition of blood plasma depending on nutritional factors, internal metabolism, endocrines, vitamines. Other factors of external source are temperature, humidity, light, etc.,

A combination of Aschoff's "wear and tear" theory and one now known as Hueper's theory is found in a publication by Hueck as early as 1920[16]. He considers arteriosclerosis as a consequence of a number of factors acting through a "Princip-einer fortschreitender Ernährungstörung der Gefässwand -" (a progressive disturbance of nutrition of the vascular wall). To him, arteriosclerosis is a disease of "wear and tear" depending on a constellation of processes ("Konstellation der Bedingungen") of which no single etiological factor can be discovered in the manner, for example, that the Tubercle Bacilla has been determined the cause of tuberculous lesions. He regards arteriosclerosis in the same light as other diseases of the connective tissue, such as arteritis deformans.

Interference with nutrition of the vessel wall and its oxygenation is the basis of what is now know as Hueper's theory[17,18,19,20,21,22] on the pathogenesis of arteriosclerosis. It has been pointed out disturbances in

the nutrition of the vessel wall have been invoked by earlier investigators, but in Hueper's hands, the theory has expanded and altered in such a manner as to become a comprehensive mechanism by which the variations in the morphological aspect of the disease can be explained. The basis of his theory appears sound and is supported by considerable experimental data and well known observations(23). Careful consideration is given to existing theories. This, together with his experimental findings, has provided the means with which he has constructed a Table of the classification of the etiologic factors of spontaneous and experimental arteriosclerosis(24). This Table is reproduced in contracted form on page 13. It serves to illustrate the great variety of factors incriminated in the production of this disease. Most of these agents have been considered by other investigators, although the mechanism of their action has been explained differently.

A somewhat different view is presented by Lange(25). In an extensive review concerned chiefly with the vascularization and innervation of arteries and with experimental data on these factors, he attempts to prove that arterial changes are effected by various stimulations of the nerve supply. He believes arteriosclerosis is produced by nerve influence on adventitial blood supply causing slowing of tissue fluid circulation with stretching of tissues and widening of the vessel lumen. This is followed by fatty infiltration of the intima and media leading to atheroma.

The neurogenic origin of arteriosclerosis has also been championed by Ricker(30), the process depending on the neurovascular tone, that is, vasoconstrictor and vasodilator action. Vasoconstriction through stimulation

ETIOLOGIC FACTORS OF SPONTANEOUS AND EXPERIMENTAL ARTERIOSCLEROSIS

	Vasculotonic Agents		Intravascular Hydrostatic Pressure		Colloidal Plasmatic Disturbances		
	Hypotonic Agents	Hypertonic Agents	Increased Hydrostatic Pressure	Decreased Hydrostatic Pressure	Lipoidal Plasmatic Disturbances	Carbohydratic Plasmatic Disturbances	Proteinic
Mechanism	Stagnant anoxemia increased permeability of vasc. walls.	Constrictory ischemic anoxemia	Ischemic anoxemia.		Films and precipitates on intima causing an impairment of exchange of gases and nutritive substances.		
Endogenous	Histamine, Orthostatic vasomotor insufficiency, Hypothyroidism, Hypopituitarism, Hypoadrenalism	Adrenalin, Adrenal cortical, Posterior pituitary, Thyroid, Parathyroid hormones, Angiotonine, Tyrosine, Tyramine, Guanidine	Congenital cardiac & vascular abnormalities of pulmonary circulation. Coarctation of aorta, Plethora, Sites of arterial bifurcation	Disuse and senile involution (umbilical omphalomesenteric, hypogastric, uterine, ovarian arteries)	Hyperlipoidemia in diabetes. Hypothyroidism essential Xanthomatosis, Pregnancy, Gaucher's disease, psoriasis	Glycogenosis	Amyloidosis, Hyperglobulinemia.
Exogenous	Nitrates, Nitrites, Cyanides, carbonmonoxide, Barbiturates, Arsenic, Mercury, Manganese, Traumatic shock, Reduced atmospheric oxygen pressure.	Suprarenine, Ephedrine, Ergotine, Hydrastine, Digitalis, Glycosides, Vit.D, Calcium salts, Cold, Acidosis, Barium chloride, Solarization, Vibration, Trauma, Strain, etc.	Excessive labour, Arteriovenous aneurysm, pulmonary fibrosis in infection or pneumoconiosis, Abnormal static condition, excessive liquids, General circulatory failure	Proximal & distal parts of ligated arteries, Arteries in scars, Scleroderma, Distal part of arteries in arteriovenous aneurysm	Excessive lipoid intake, Lipoid nephrosis, Starvation, Carbon disulfide poison, Loss of blood, Saponin, Goitrogenic substances	Polyvinylosis Methyl, Cellulosis, Pectinosis, Arabinosis	Allergic hyperglobulinemia, Experimental proteinosis

Hematinic Anoxemic Agents and Disturbances in the Oxygen Carbondioxide Balance:- carbon monoxide, sulfonamides, nitrites and reduced atmospheric oxygen pressure, oxygen poisoning.

leads to increased nutritive flow of the blood with proliferation of the elements of the vessel wall, in particular in the media. A stronger stimulus leads to partial constrictor paresis with oedematous imbitition of the whole wall due to slowing of the blood stream in the adventitial zone. The intimal stomata are widened, allowing greater flow of nutrient blood from the lumen. The intima hypertrophies. A decidedly strong irritation produces media paralysis with subsequent necrosis and calcification. The basis of this reasoning rests on the assumption of modifications in the circulatory movements of tissue fluids in the various layers of the vessel wall by either arterial contraction or dilatation, that is, increased or diminished nutrition.

Winternitz[26] has also studied vascularization of the arteries. By injecting Higgins Engrossing Ink he demonstrated vasa vasorum of the adventitia penetrating through the media to the intima. In addition, he was able to show in the cow's aorta the existence of intimal stomata arising directly from the lumen of the parent vessel and penetrating the intima. The distribution and extent of vascularity is a function of both age and disease. The diseased vessel is markedly vascular at any age. He suggests capillary and endothelial growth is stimulated by materials of the blood. Through the action of various agents, numerous small haemorrhages are produced in the vessel wall and upon these subsequent lesions develop.

The pertinent factor in the majority of the above theories is the disturbance of the nutrition of the vascular wall. An entirely new trend appears in the writings of Leary[27,28,29]. The disturbance of cholesterol

metabolism causes abnormal deposits in adrenal, liver and other organs rich in reticulo-endothelial cells. These cells must proliferate and pick up the excess cholesterol in the above mentioned organs. They then crowd into the sinuses and lymphatics and eventually reach the blood stream. They pass through the capillaries of the lung by means of ameboid motion. Lipoid-laden cells are freed from the mother organs in waves. The localization of the lipoid cells in the arterial wall is due to chemotaxis, related to the cholesterol content of the cell, since macrophages carrying other forms of particulate matter do not invade the subendothelial layer of the intima. Macrophages in man and in the experimental animals in which a great variety of particulate matter have been engulfed, including silica and silicates, carbon, magnesium dioxide, mercuric sulfide, colloidal dyes, bacteria, blood pigments and other particulate substances, exhibit no tendency to invade the arterial walls. The initial lesion of atherosclerosis (both in man and in the rabbit) consists in the invasion of the subendothelial layer of the intima by cholesterol lipoid cells. At the onset, there is no swelling or disintegration of the subendothelial layer of the intima. There is no inflammatory reaction. Oedema, mucoid degeneration and lymphoid infiltration are all secondary phenomena.

These statements are in contradiction with the findings of numerous other investigators[12,13,16]. Most of the writers reviewed have noted some change, such as swelling and thickening of the intima, and the presence of free fat in the intima and the internal media, before the appearance of foam cells has been found on numerous occasions.

Gordon[31] asserts his allegiance to the Leary theory, but with some modification. To him the cause of lipoid cell penetration into the aortic intima lies in their lightness. Due to their lesser weight they are pushed into the peripheral zone of the circulating blood, thus closer to the vessel wall. Then, due to the considerably lower pressure in the intima, in comparison with that of the circulating blood, the lipophage is forced into the inner layer of the wall and imprisoned there by the limiting internal elastic. Mechanical factors, such as eddying of the blood stream at the mouth of branching vessels, would enhance lipophage penetration into the intima.

Duff[32] is of a different opinion on the pathogenesis of arteriosclerosis. In his view hyperlipemia and hypercholesterolemia are essential factors but will not of themselves produce the lesion. He believes that swelling of the subendothelial ground substance occurs prior to the deposit of lipoid material. In this swollen ground substance, both of intima and media, free lipoid deposits. This lipoid is brought in with nutritive fluid. Cellular response of two types, macrophage and fibroblast, become evident.

An abrupt swing of the pendulum back to the early theory of Rokitansky is found in publications by Duguid[33,34,35]. This writer finds the origin of atherosclerosis in the fibrinous layer depositing on the intimal surface of the vessel wall in thrombus formation. Softening and fatty degeneration of the thrombus centre occurs, while organization is proceeding inward from the intimal border, the endothelium very early covering the thrombus. His argument is scarcely convincing, since he admits himself that

"in aortic atherosclerosis the fatty changes are mostly seen in the deeper layers of the intima or in the subintimal zone, where any connection with mural deposits would be hard to trace"(34).

In addition to the theories on the etiology and pathogenesis of atherosclerosis here presented, it is considered necessary to review briefly some factors influencing the development of this disease.

Tortuosity of arteries and irregularities of the arterial wall have been studied by Springorum(36), Maljatzkaja(37) and Thiersch(38). Whereas Springorum could not claim arterial wall weakness and the degree of coiling of the splenic artery and its accompanying arteriosclerosis as a product of age alone, Maljatzkaja traced the arterial changes through as a slow process beginning towards the end of the second decade of life, but taking on a far more rapid development in advanced age (beyond the fifth decade). This is also a finding of Staemmler(39). Thiersch found in some 90 splenic arteries examined that with age the tortuosity of the vessel increases, predisposing it to atherosclerosis and medial degeneration. Elastic fibre degeneration, fibrosis, mucoid degeneration and thinning of muscle fibres are in favour of mechanical trauma as causal agent, and may be associated with lengthening of the artery. Diseases have been incriminated in aiding the progress of atherosclerosis. Hypertension is found associated with atherosclerosis and is considered by Lange(40) as acting through the unusual responsiveness of the hypertonic vessel to stimulation. The incidence of atherosclerosis in various pathological conditions is summarily presented by MacCallum(8). Among the diseases more prominently

associated with atherosclerosis are diabetes[65] and nephritis of vascular origin. The influence of diet has been studied[12,41]. It is believed that the rich American diet may be responsible for the higher percentage of atherosclerosis in comparison with that of the Chinese. Yet Eskimos, whose diet is high in fats, do not show increased lipemia[3]. Hollman[42] produced "typical arterial lesions" by feeding a standard high fat diet over a period of eight weeks or longer to dogs and then inducing renal insufficiency. Blood lipoids and their relation to the production of arteriosclerosis have been extensively studied but results are conflicting[47]. Mjassnikow, Alvarez and Neuschlosz found considerable increase in blood cholesterol in arteriosclerosis. Elevated blood lipoids were found in diabetes with arterial complication by L'Abbé and Heitz. Koulkov, Veiland, Hunt and Maxwell have reported contrary findings. From their study on "healthy" individuals with sudden death, Landé and Sperry[43] concluded that there is no correlation between the concentration of cholesterol in the blood serum and the lipid content of the aorta. Buck and Rossiter[44] give evidence that atheroma lipid is not of the same chemical constitution as the lipid of the blood stream. Payne and Duff[45,46] suggest, as based on experimental findings, that the interrelationship of the different lipid components of the blood stream is much more important than the absolute level of cholesterol itself. Pomeranze and Kunkel[48] found that, in a group of diabetic patients, 92% of those with atherosclerosis showed the presence of some serum lipid abnormality. In marked hyperlipaemia, the greatest elevation was in the neutral fat fraction. The cholesterol levels gave no indication of total lipid elevation.

Some consideration must be given to the influence of endocrine secretions on atherosclerosis. Thyroid hormone has been shown to decrease or prevent atherosclerosis in the experimental animal[3,10,61]. Conversely, if thyroid function is decreased by the administration of thiouracil, atherosclerosis develops in dogs fed cholesterol, whereas without previous thyroid dysfunction, no lesions develop[49]. Clinical findings reveal that some relation exists between the adrenals and human arteriosclerosis[47]. Josué[50] as early as 1891 produced medial necrosis and calcification with thickening of the intima in the rabbit by injection of adrenalin. Lange[25] also produced arterial lesions with adrenalin injections, obtaining variations in degree and localization with different amounts injected. Adrenal injections accelerate the development of atherosclerotic changes in arteries[10]. Nor can the possibility of influence of sex hormones on the production of atherosclerosis be neglected. That hypertension frequently follows menopause is well known. Experimentally, Murata and Katsuoka[51] produced atherosclerosis with lanolin diet much more rapidly in castrated animals than in normal. The question whether the lipoid disturbances with increased "fatty streaks" of the aorta encountered at puberty and the higher incidence of atherosclerosis in old age are associated with endocrine function of the sex organs merits further investigation.

THE MORPHOLOGY OF ATHEROSCLEROSIS.

Prior to consideration of the morphological aspects of atherosclerosis, a brief review of angiogenesis(52) and the normal structure(53) of arteries is presented.

Intraembryonic vessels arise from clefts differentiating locally in the mesenchyme. The angioblast, originally in the form of solid chords or masses, hollows out and peripheral cells become flattened endothelium. Proliferative growth of the endothelium links the simple vascular spaces into continuous channels. The endothelium of early stages has capacities identical with the mesenchyme that gave it origin. The endothelium proliferates mitotically, and, as in the embryonic vessels of liver and spleen, it may take part in the production of hemocytoblasts(53), but these powers are soon lost. Around the endothelium the neighbouring mesenchyme later adds the accessory coats (1) the tunica intima (endothelial and fibrous), (2) the tunica media (muscular), and (3) the tunica externa or adventitia (fibrous). Capillary plexuses precede the formation of definite arterial and venous trunks in any region. The differentiation of channels into arteries and veins may be due to mechanical conditions of the blood flow.

In the adult[53] the endothelium of the blood vessels are structurally very similar to fibroblasts. The elongated oval nucleus is flattened and contains fine, dust-like chromatin particles like the fibroblast nucleus. It lacks the large nucleoli of the fibroblast and its membrane often shows longitudinal folds. This endothelial cell can turn into a fibroblast.

The morphology of arteries of different caliber differs. In large arteries the endothelium of the intima differs in that the cells are not elongated but have an oval or polygonal form. Directly beneath the endothelium is a thin layer composed of a few interlacing fibres and fibroblasts where a few wandering cells may be found. Then comes a layer of elastic fibres forming an elastic membrane. Between the elastic fibres are collagenous fibres, fibroblasts and small bundles of smooth muscle cells. Externally to this is found a fenestrated elastic membrane, which serves as a boundary between the intima and media. The tunica media consists mainly of elastic tissue with thin layers of connective tissue, collagenous fibres, fibroblasts and some smooth muscle cells in the interspaces. The thin adventitia fades gradually into the surrounding loose connective tissue.

As the caliber of the artery is reduced, the elastic tissue of the media is gradually replaced by muscle tissue. Here, the internal elastic membrane is well developed. In arteries of medium caliber, the media is constituted almost exclusively of smooth muscle cells arranged in concentric layers. The adventitia of loose connective tissue and elastic fibres, is sometimes thicker than the media.

In the arterioles, the intima is reduced to the endothelium over the internal elastic membrane. The tunica media consists of smooth muscle cells, orientated transversally. The adventitia is similar to that of the muscular arteries.

The question of blood supply for the arterial wall is of importance when considering atherosclerosis, but, although certain facts on the anatomical distribution of the vasa vasorum are fully established, other questions are still in a controversial state[55]. Maximow and Bloom[53] describe vasa vasorum arising from neighbouring vessels and a capillary network in the adventitia, as penetrating only to the external layer of the media. Lange, Benda and Kaufmann, Plotnikow[25], Horn and Finkelstein[54], Ramsey[56] have also followed vasa vasorum into the media, but the question of intimal nutrition is by no means settled. While some investigators believe that the intima derives its nutrient fluid directly from the blood through imbibition [12,57], others[26,58] have demonstrated vasa vasorum arising from minute openings in the intimal surface.

Because of his conviction that the pathogenesis of atherosclerosis is found in nervous action upon the walls of vessels, Lange[25] studied the nerve supply to blood vessels in some detail. He found that arteries are supplied by myelinated and non-myelinated nerves. There is a delicate lacework of nerve fibres in the adventitia from which fibres supply the muscle layer. Most investigators have been unable to demonstrate nerve fibres in the intima.

Another factor must be considered before delving into the morphology of atherosclerosis. Changes in the arteries occur, without necessarily leading to atherosclerosis, from birth throughout life into old age[62]. In a study of the vascularization of the aorta in health and disease, Schlichter[64] found that the aortas of infants showed the most marked vascularity which decreases with the increase of the size of the aorta. While the arteries acquire their three main layers in the fourth month of embryonic life[53], they reach their mature form only in adult life. Elastic tissue of the media increases after birth. Between the endothelium and the internal elastica an elastic muscular layer appears to be completely differentiated by the age of twenty-five. After birth, arteries of muscular type develop a connective tissue layer between the endothelium and the internal elastica as well as thickening of the wall as a whole. Not only does the aortic wall change as life progresses, but also sectional variations in intimal structure have been observed[62]. These variations, in the form of local thickening, may be due to over-developed musculo-elastic layer of the intima or to unequal development of the hyperplastic (fibro-elastic) layer of the intima (in the infant to 12 years).

Orsos[63] studied the intima of the ascending aorta in 200 specimens and found a system of musculo-elastic bundles in longitudinal arrangement, which he believes predispose this section to atherosclerotic lesions. Wear of arteries as the individual gains in years is expressed in an irregular thickening of the intima. From youth there is progressive

splitting of the internal elastic membrane[59], with new fibrous formation in the interstices. Dietrich[60] describes transverse ridges in abdominal arteries, due to muscle bundles in the media, as appearing in adult life and becoming more marked with age.

Although the pathological picture of atherosclerosis as an established lesion has been fully described[12,59] and others, there is no agreement as to what constitutes the earliest lesion. Fatty streaks of the infant and adolescent are regarded by the old school (Virchow, Sanders) as independent of the atherosclerotic lesion as found in the more advanced years of life. Aschoff[12] believed they could regress completely, since no fibrous proliferation occurred. Zinserling[59] holds that fatty streaks are irreversible and progress towards atherosclerosis. He studied their localization and found it similar to atherosclerotic plaques.

If the view is taken that fatty streaks are an early stage of atherosclerosis, their description must be given. Lange[25] describes them adequately in his report of 1924. They appear as round or oval, slightly elevated spots, yellowish in colour. They may be found along the posterior wall of the aorta between the mouths of the intercostal arteries, more frequently at the base of the aorta, in the sinuses of Valsalva or just above them. The very earliest lesion observed, according to Aschoff, is the appearance of fat in the form of fine droplets of cholesterol esters in the elastic fibres which have undergone loosening and swelling, between the musculo-elastic and fibro-elastic layers of the intima. There appears to be no change in the rest of the intima in

this early stage. The fatty deposit in the elastic fibres increases with the age of the lesion and a diffuse, finely granular, fatty infiltration of the fibro-elastic layer is seen with fat appearing in the cells as well. Some of these cells are connective tissue cells, others macrophages. The occasional lymphocyte may be found. The interstitial fat, however, remains the outstanding characteristic. This is not fully accepted by Ophuls(59). No further change appears microscopically in fatty streaks, except that the fatty deposit becomes abundant enough to cause the intimal surface to bulge into the lumen of the vessel. In the lesion progressing towards a true atherosclerotic plaque, the changes are somewhat more severe and connective tissue proliferation occurs over the plaque. The covering is thus thickened often consisting of dense hyaline fibrous tissue(3). Grossly, they now appear as grayish yellow spots, a few mm. in diameter, exhibiting a tendency to fuse and form plaques. The surface becomes white by the development of fibrous tissue, while the fatty parts break down and form a muschy material from which the condition has received its name. The surface may become permanently sealed to form a calcareous plaque or the degenerative process in the depths may extend to the surface and burst through the endothelial lining, establishing a ragged ulceration, at the bottom of which remains some remnant of the necrotic material. These ulcers may be partly or completely covered with thrombi. Such a picture is seen chiefly in the abdominal aorta, the thoracic lesions being less severe. It should be noted that differently from the fatty streaks, the atheromatous lesions tend to locate around the mouths of intercostal arteries. This suggests

that the lesions may not be identical in nature.

Microscopically the lesions in the earlier stages, reveals fibrous thickening with many connective tissue cells containing fine fat droplets. Many round cells "foam cells" are also found. Sometimes the lesion may be more marked in the superficial layers of the intima. Again, there may be a greater fibrous proliferation and relatively little fatty deposit and vice versa. Despite the obvious cellular proliferation, no mention is made of mitotic figures in the literature reviewed on human atherosclerosis. In experimental atherosclerosis McMillan and Duff[66] report seeing about one in every 4 cm. length of section. Altschul[67,68] favours amitotic divisions, not only in the experimental lesion but also in human atherosclerosis. He has attempted to show that the cellular proliferation of the intima is due to endothelial "dedifferentiation" migrating into the subendothelial layer to become foam cells. Some cells would also come from the media and undergo the same process of dedifferentiation.

Calcium deposits may infiltrate the degenerated collagen, usually in the deeper layers. Hyaline degeneration has also been demonstrated as occurring along with the intimal changes in some cases. The vasa vasorum of the adventitia may show a slight round cell infiltration. In more advanced stage the media also becomes the seat of fatty infiltration, found in the muscle cells. Degeneration and necrosis may extend far down into the media leaving only a narrow zone of muscle and elastic tissue adjacent to the adventitia.

The necrotic material contains many cholesterol crystals and fine fat granules as well as some minute granules of calcarious material.

When the superficial fibrous tissue remains intact and calcifies permanently sealing the atheromatous lesion, the lining connective tissue adjoining may develop bony spicules with fatty or cellular marrow.

Atheromatous lesions such as described above but not so severe may be found in pulmonary arteries following pulmonary hypertension.

Atherosclerosis in the coronary arteries is described by Horn and Finkelstein(54). In addition to the morphology of the lesion, as described in the aorta, that is fatty deposit and fibrosis, they describe vascularization of the lesion. These vessels give rise to haemorrhages in the other sclerotic plaques inducing acute degenerative and reactive responses leading to obstruction of the lumen. The fibrinous masses within the plaques are considered the sequelae of intramural haemorrhage originating from the mural capillaries within the plaque. Medial atrophy was a frequent finding and perivascular infiltration of lymphocytes is recorded. Calcification and bone formation characterize ultimate healing of the lesion.

Description of medial necrosis and arteriosclerosis are omitted here, since the experiment reported has no bearing on these lesions.

EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS

It is a well known fact that a variety of arterial lesions have been produced in a variety of animals by a variety of methods. To mention but a few, arterial lesions have been produced in the rhesus monkey by pyridoxine deficiency(69), in guinea pigs by feeding egg yolk and milk, in goats, rats and mice by cholesterol feeding(10), in hens and pigeons by feeding egg yolk(70), in dogs with thiouracil and cholesterol(19,71). Nerve stimulation, nerve section, mechanical trauma, injections of adrenalin, dyes, earths, toxins and bacteria have been used. The arterial lesions produced by these methods are but superficially comparable to atherosclerosis in the human. The experimental lesions produced by feeding cholesterol to rabbits, on the other hand, while not completely superimposable upon human lesions in all respects, can be compared in many aspects. It is, therefore, with the latter that we are chiefly concerned.

Since Ignotowski first noted fatty plaques on the intimal surface of the aorta of the rabbit fed a high protein diet, numerous dietary measures have been taken to produce arterial lesions in the rabbit. In addition, cholesterol has been administered intravenously(74), intraperitoneally(72), by gastric intubation, by rubbing it directly on the skin(67) in

an endeavour to produce atherosclerotic lesions. Again, cholesterol has been used with a variety of chemical(45), hormonal(12,25) substances. But the fact remained that cholesterol alone, administered simply with the diet or by gastric intubation would produce the same results. Agreement on the gross and microscopic picture of the lesions produced by the administration of cholesterol to rabbits is only partial. The gross descriptions of experimental atherosclerosis appears similar(10,72,73). But opinion diverges where the early lesions, as described microscopically, are presented. This problem will be dealt with more fully in the review of the histology of the lesion.

In the aorta the lesions appear first as minute yellowish-white opaque flecks, their borders poorly defined, about the mouths of vessels arising from the aortic arch or just above the aortic valve ring. These little thickenings grow into nodular projections with a glistening yellow or yellow-white surface. More of these appear along the posterior wall of the vessel about the mouths of the intercostal arteries, but they are found on the sides and anterior aspect of the aortic wall as well. These lesions may be found in the abdominal aorta, though to a lesser degree. Adjacent nodules coalesce forming large irregular plaques, or, as on the posterior wall of the thoracic aorta, streaks. The thickening may be sufficient to cause narrowing of the aortic lumen, later followed by dilatation. When the lesions of the aorta are well established, fatty flecks may be found in such arteries as the pulmonary, the carotid, the subclavian, the iliac and femoral, the coronary, the splenic and mesenteric,

the last two to a lesser degree. The arteries of the liver and kidney are even less affected, while the cerebral and retinal are found unchanged.

The microscopic alterations become perceptible some twenty to thirty days after cholesterol feeding is begun. Opinion differs as to where these early diffuse finely granular masses of lipoid occur. Anitschkow[10] and Duff[72] observed the earliest deposits of anisotropic fat in the swollen, thickened layer of ground substance between the endothelium and the internal elastic membrane. Bailey[75], on the other hand, believes that "the early lesions produced by cholesterol feeding consist of a cellular thickening of the intima, most of the cells present showing small droplets of doubly refractile fat". Leary[27,76] in his early publications was of the opinion that the fat appeared extracellular in the initial lesion. A completely opposite view is expressed in his later publications[29,77]. Here, Leary finds the initial lesion in the appearance of foam cells under the endothelium. At a somewhat later stage, the thickened intima contains a considerable accumulation of cells, among which the foam cells, loaded with fat, are the most prominent. These cells have a small centrally placed, dark staining nuclei. The protoplasm is generally abundant, pale staining, showing a delicate reticular structure forming a honey-combed network, which gives the cytoplasm a foamy appearance. Some of the large cells contain two or three nuclei. The origin of the foam cell has been widely discussed. Altschul[67,68] believes it can originate from the endothelium through a process of "dedifferentiation". Others possibly are invaders (muscle cells and fibroblasts) from the medial layer. Leary[26,27,76,77] believes that foam cells penetrate through the endothelial lining

of the lumen. They are cells originating in the reticulo-endothelial system of the adrenal, liver and spleen, which become loaded with fat and then take to the blood stream in waves. Duff[72] considers the foam cells akin to the large mononuclear phagocytes not only in appearance but also in their phagocytic properties. They are active in the phagocytosis not only of lipoids but are able to take up colloidal dyes. Among the foam cells small spindle shaped or stellate cells are found. These have been identified as fibroblasts. Their number is generally limited in the early lesion but becomes greater as the lesions advance. The occasional lymphocyte may also be encountered. The role of the endothelium is not established. Most authors have found that the intimal cells covering the lesion may appear swollen and contain fatty granules. But the question whether these are true endothelial cells remains unanswered. Frozen sections stained for fat show large amounts of fat in the atheromatous lesion. Innumerable fine granules are packed in the cytoplasm of the foam cell. Fat is also found in the stellate cells and in the ground substance. It is probable that the deposited lipoids consist chiefly of cholesterol and its esters[29]. As the lesion develops, the foam cells in the deepest part of the plaque become necrotic, leaving fatty material free in the granular debris. At the same time there is fibroblastic proliferation and connective and elastic fibres increase. Foam cells remain scattered singly or in groups throughout the lesion. Those about the free fat tend to become necrotic in their turn. This area of cellular debris and free fat tends to become calcified. Infiltration of fat into the media may be seen very early in experimental atherosclerosis. Indeed, Duff[72] has observed medial infiltration

in some instances prior to any intimal alteration.

The above description is that of the atheromatous lesion in the rabbit during the period of cholesterol administration. Cessation of cholesterol feeding will, after a month or longer, bring on a transition toward a more fibrous type, closely resembling the later stages of human atheroma(78).

Anatomic Comparison of Experimental Cholesterol Atherosclerosis and Human Atherosclerosis(72).

The gross appearance and the distribution of individual atherosclerotis lesions in the experimental animal closely resemble certain lesions of human atherosclerosis. The correspondence between the localization of the experimental lesions about the mouths of branching arteries with the distribution of lipoid flecks in children has been noted(10). However, while human lesions predominate in the abdominal aorta, the lesions in the rabbit tend to become most advanced in the arch. The secondary arteries, pulmonary, renal, coronary, cerebral, are affected differently in experimental and in human atherosclerosis.

Early microscopic lesions cannot be compared, since in the human lesion it is still undecided as to what constitutes the earliest change. In more advanced experimental lesions there is the localized intimal thickening in which doubly refractile and other lipoids are present, the foam cells are abundant and there is proliferation (to a lesser degree) of fibrous connective tissue cells. This is repetetive of the human lesion. But the intercellular fat of the experimental lesion does not tend to concentrate

along elastic fibrils of the intima as it does frequently in human arteries. The more fibrous lesion of the rabbit, when cholesterol feeding has been discontinued for a long time, very closely resembles human atherosclerosis. The lesions of the media present a different picture. In the rabbit lipid deposition in the media follows destruction and disappearance of muscle and elastic tissue. This may sometimes occur before intimal change is evident. Not so in the human - here medial deposits of fat is found beneath well developed intimal plaques. Also the lipoids appear between the muscle and elastic fibres.

The fact that, in experimental atherosclerosis, fats accumulate in other organs such as adrenal, liver, spleen, bone marrow, kidney, skin etc., must be considered. The lipoids appear before any changes are found in the arteries. This finding has not been duplicated in human atherosclerosis.

In conclusion, cholesterol atherosclerosis in the rabbit may be considered as resembling, more than any other experimentally produced arterial lesion, but is not identical with human atherosclerosis. In the cholesterol fed rabbit, the aortic lesion is part of a picture where saturation of the entire body with lipoids is a prominent feature. Human atherosclerosis, on the other hand, exhibits lipoids limited to the arterial wall lesion.

THE NATURE OF THE ARTERIAL ENDOTHELIUM.

ITS BEHAVIOUR TOWARDS MACROMOLECULAR AND COLLOIDAL SUBSTANCES.

VASCULAR ENDOTHELIUM AND RETICULO-ENDOTHELIUM.

It has been previously stated in this review that the endothelium of the blood vessels arises from primitive mesenchyme of the embryo(52). It was also remarked that Maximow and Bloom(53) and Arey(52) considered the endothelium capable of mitotic proliferation, and that it took part in the production of hemocytoblasts in the manner of the undifferentiated mesenchymal cell. There is no doubt that this power is lost by the end of foetal life. Studies on adult endothelium have shown that it possesses the ability to revert to its embryological function and properties. Malyschew(79) demonstrated its versatility by means of a doubly ligated artery. In the ligated area, the tissue cells are freed and, in the lumen they multiply (by mitosis). They may become fibroblasts, histiocytes, macrophages and cells identical to the hemocytoblast. Altschul(67) believes that the endothelium undergoes a "dedifferentiation" and penetrates into the subendothelial layer as a mesenchymal cell which may have one of a variety of final evolutions. It may remain as a mesenchymal cell, it may degenerate and become part of the atheromatous mass, it may turn into a fibroblast or become a lining cell of new vessels. Lastly, it may become a foam cell. This last possibility has

been considered by others, but is not accepted as fact[72].

Lewis and Lewis[80] have studied the behaviour of endothelium in tissue cultures. They found that while some endothelial cells retain their specific character, others become fibroblasts. In cultures of tissues containing both endothelium and mesenchyme, it is difficult or impossible to distinguish between these two types of cells after they enter the migratory zone.

McJunkin[81] produced granulomas by injecting rats with casein and then, three weeks after, injected India Ink intravenously. He observed no free particles in the lumen of vessels but found the substance in intramuscular mononuclear phagocytes. He also found carbon present in the vessel walls of granulation tissue. He concludes that the blood vascular endothelium must be considered as a possible source of mononuclear phagocytes.

Kux[82] injected India Ink, trypan blue and carmin intravenously in frogs and observed the fate of these substances in the vessels of the tongue. The free particles seen almost immediately after the injection in the lumen of vessels tend to cling to the endothelial lining. This, according to some authors, is due to a gelatinous secretion of the endothelial cells. Polynuclears, both loaded with India Ink and free of the substance, were observed passing through the endothelium. As the free particles disappear in the blood, their appearance is observed in the endothelial cell of the capillary. In larger vessels very little India Ink is taken up. Later the particulate substance also appears in the adventitial cells. When using carmin and trypan blue, he was unable to demonstrate these substances in any

endothelium of larger blood vessels or capillaries. A migration of the capillary endothelial cell into the lumen of the vessel or into the adventitia or changing into other cell forms was never observed. Although he admits the ability of capillary endothelium to phagocytose large particulate matter such as India Ink, he does not believe it capable of storing fine colloidal suspensions like carmin or trypan blue.

Okuneff[83] studied the penetration into the aortic intima of colloidal dyes injected intravenously into dogs, cats, rabbits, guinea pigs, white rats and white mice. He found significant differences in the deposits of the dye in the aorta of rats, rabbits, mice and guinea pigs on the one hand, and cats and dogs on the other. In the former the intima stained diffusely, while there appeared a distinct predilection for the dye for certain areas of the aortic wall in cats and dogs. These areas correspond to those of lipoid infiltration in atherosclerosis. His conclusions of intimal staining appear to be based mainly on macroscopic findings and he is not clear as to whether the dye is found in endothelial cells.

Anitschkow[84], and others quoted by him, believed they could demonstrate "areas of increased permeability" of the arterial wall to colloidal dyes. The localization of the dye spots are analagous to those of lipoid deposits in children and in early rabbit lesions. Glasunoff[84] showed that local injury increased local arterial permeability. The penetration of the dye comes from the lumen through the endothelium. This was also found by Lange[25]. Duff[85] performed similar experiments with trypan blue injected intravenously in rabbits. He found that the variations

in the degree of staining were due to variations in adventitial thickness, and not, as had been proposed by the authors mentioned above, due to variations in intimal permeability.

The penetration of macromolecular substances into the aortic intima, to be stored there, is viewed in a different light by Hueper[86]. He considered substances, both of endogenous and exogenous origin, with a molecular weight above 1000 calculated by the method of ultracentrifugation (Svedberg). Such substances as egg albumin, serum albumin, hemoglobin, casein, gelatin, virus, cellulose, starch, triaminophosphatides, polyvinyl alcohol, when introduced into the circulation tend to remain there for a long time because they do not pass readily through capillary walls. Also, because of their comparatively marked chemical inertness they resist degradation and are phagocytosed by various types of cells, the phagocytic cells of the liver, spleen and lymph nodes, and the endothelial cells of large and small blood vessels. These substances can give rise to atheromatous and arteriosclerotic lesions, because they interfere with oxygenation and nutrition of the vessel wall by formation of films.

Variations in the size of the lipoid molecules circulating in the blood were studied by Gage and Fish[87]. These men observed that, while there is only a slight increase in chemical concentration of lipids in the blood after a fatty meal, the number of visible lipid particles greatly increases, indicating that the average particle size of the absorbed lipid is greater after a fatty meal than in the fasting state. Moreton[88] after studying lipoid particle size in lipemia of nutrition believed that the

increased particle size of the lipids in sustained or alimentary hyperlipemia is a stimulus to the phagocytosis in the intima by macrophages and the formation of typical foam cells.

The significance of macromolecular lipids in the circulating blood gained in stature through the careful and interesting work of Gofman[89]. He divides lipoproteins into four groups: (1) Sf 70 and over (Svedberg flotation unit) the largest in size, (2) Sf 30 - 70, (3) Sf 10 - 20, (4) Sf 3 - 6. The two largest groups are increased after fat meals. They were not shown to be related to atherosclerosis. The group Sf 10 - 20 on the other hand, seems definitely related in concentration to atherosclerosis. The molecular weight of these latter particles is about 3 million, formed of 70% protein and 30% cholesterol. In rabbits the two groups Sf 3 - 6 and Sf 10 - 20 only are found. In experimental cholesterol atherosclerosis there is an initial increase in the Sf 3 - 6 types and finally the Sf 10 - 20 group shows a higher level. It is only with the increase of the latter that atherosclerosis develops and the extent of the disease is related to the degree of increase in the serum levels of this Sf 10 - 20 group.

In an investigation on humans, normal and patients with hypertension, infarction etc., Gofman showed that there is an increase in the Sf 10 - 20 molecular size in the patients with probable atherosclerosis. Zinn and Griffith[90], on the other hand, found that in the fasting state the chylomicrons or large fat particles were definitely higher in the sera of atherosclerotic and diabetic patients as opposed to normal controls.

It remains to be determined whether the molecular size of particles in the blood stream has any bearing on the phagocytosis of these substances by macrophages and other phagocytic cells. Their relation, if any, to endothelial permeability is as yet unknown. The possibility that endothelial permeability to, or phagocytosis of, macromolecular lipoid molecules as an early or initial factor in the development of atherosclerotic lesions may be considered following the finding of Duff and McMillan(91). These authors report that colloidal thorium dioxide, when injected intravenously into atherosclerotic rabbits, tends to deposit in foam cells and in some endothelial cells overlying the atherosclerotic plaque. Normal endothelium, between the plaques and in a group of normal control animals, does not take up this substance. The occurrence of Thorotrast only in those endothelial cells overlying the lesion indicates that these cells are different from their neighbours with respect to the accumulation of particulate matter.

Turning to a somewhat different aspect of the vascular endothelium, consideration is given to its relation with the reticulo-endothelial system as a whole. Foot(92) points out the similarities between vascular endothelium and reticulo-endothelium. "They have a like embryological origin, a similar mechanical function, the common characteristic of forming fibrils demonstrable by the same methods and that of phagocytosis". He believes that the two, vascular endothelium and reticulo-endothelium, may be separated on morphological grounds but should not be considered as being possessed of an irrevocable specificity. The possibility of vascular endothelium transforming into other cell types has already been discussed.

In the adult the origin of the free phagocyte is almost impossible to prove. But in pathological conditions it is probably that the vascular endothelium gives rise to the greater bulk of these cells. The relation of these two endothelial structures is of importance, since considerable evidence supports the opinion that the reticulo-endothelial system has a function in the utilization of the lipids in the blood(93). Harbitz(93) states that the reticulo-endothelial system in the metabolism of cholesterol has such importance that it has been regarded as an intermediary apparatus. It also participates in the removal of emulsified lipids from the blood stream.

A number of vital questions remain unanswered in the literature on atherosclerosis, as reviewed here. What is the relation between the molecular size of the lipoproteins circulating in the blood and the deposition of lipoid material in the vascular intima? Does the endothelium play a part? The decided difference between endothelium overlying atherosclerotic plaques and normal endothelium in its behaviour to macromolecular substances in the circulating blood raises the questions - When does this change occur? Has it any significance in the initial penetration of lipoid material into the intima? What is the part played by the reticulo-endothelial system in the production of atherosclerosis?

These are the questions which prompted the experiment reported in the following part of this thesis.

Summary.

A brief review of the literature on human atherosclerosis has been presented in an attempt to illustrate both the stepping stones to the insight into the problem possessed today and the numerous obstacles in the path towards a comprehension of the disease so that rational treatment may be sought. Following an attempt at definition, a brief review of the history was presented. The etiology, pathogenesis and factors influencing the development of the lesion were discussed to some length. The lesion was then pictured in its various morphological aspects preceded by a quick glance at angiogenesis and the normal structure of arteries.

Experimental atherosclerosis was then reviewed, sketching briefly the variety of lesions produced in various animals, but primary stress was layed on experimental cholesterol atherosclerosis as produced in the rabbit by cholesterol feeding. The morphology of the lesions produced was portrayed. Then a comparison with human atherosclerosis was made.

Because of the importance of the vascular endothelium, its behaviour towards macromolecular colloidal substances and its relation to the reticulo-endothelial system, a more detailed study of these factors concluded the review of the literature.

PART II.

REPORT OF THE EXPERIMENT

Materials and Methods:

Forty eight New Zealand White Rabbits of both sexes were used in the experiment. For the duration of the experimental period they were placed in individual cages. Prior to the feeding of cholesterol, they were weighed and bled for the determination of the blood cholesterol (free and total), cholesterol ester, phospholipids, fatty acids and neutral fats. Throughout the experiment the animals were given water ad libitum. They were fed standard Purina chow with 1 gm. cholesterol dissolved in ether thoroughly mixed with 100 gm. of food and evaporated on a shallow pan. The rabbit chow was changed to Miracle Rabbit Pellets during the eleventh week of the feeding because it was believed less powdery than the Purina. Also, cholesterol was mixed with food in 7% Mazola oil from the tenth week of the experiment. This change was effected because of the danger involved in evaporating large quantities of ether and its high cost. It had been previously determined that mixing cholesterol with oiled food was well tolerated by the animals and produced comparative lesions. The cholesterol was mixed with the oiled food in a cement mixer in the following proportions:-

Cholesterol ..... 1 gm.
Mazola oil .....6.37 gm. (7 cc.)
Miracle Rabbit Pellets q.s. 100 gm.

The animals were fed 100 gm. per day of the above mixture for five days every week. The residue was weighed and recorded daily. For the remaining two days of the week they received a total of 200 gm. of normal food.

Two animals (M.48 and M.55) died prior to any experimental procedures and will not be considered in the experiment. Another animal (0.28) suffered marked autolysis and his material was not suitable for examination. The animals were divided into groups of four and five each. Each group represented a fixed and predetermined period of cholesterol feeding. The entire series of animals ran thus:-

Group I: Animals 0.57, M.23, M.24, L.23, L.24, were normal animals receiving no cholesterol with their food.

Group II: Animals 0.18 and 0.21 to 0.24 inclusive were fed cholesterol for one week.

Group III: Animals 0.25 to 0.29 inclusive were fed cholesterol for two weeks.

Group IV: Animals M.41 to M.45 inclusive were fed cholesterol for three weeks.

Group V: Animals Q.6 to Q.9 inclusive were fed cholesterol for three weeks.

Group VI: Animals M.46 to M.50 inclusive were fed cholesterol for four weeks.

Group VII: Animals M.51 to M.54 inclusive were fed cholesterol for five weeks.

Group VIII: Animals M.56 to M.61 inclusive were fed cholesterol for six weeks.

Group IX: Animals M.62 to M.65 inclusive were fed cholesterol for seven weeks.

Group X: Animals M.66 to M.70 inclusive were fed cholesterol for eight weeks.

A detailed protocol of every animal will be found in Appendix A.

On the morning of the last day of cholesterol feeding the animals were again weighed and bled for blood chemistry (complete tables of the chemical findings are recorded in Appendix B). Each animal of the group concerned then received warmed Thorotrast, 3 cc. per kilo of body weight, in the external marginal vein of the ear at the rate of approximately 2 cc. per minute. During the twenty four hours following the injection 2 cc. of blood was drawn at varying intervals. The blood was allowed to clot and was fixed in saline formol 10% in preparation for paraffin section. The animals in Group I were treated somewhat differently. Animal 0.57 received one injection of Thorotrast and was killed twenty four hours after. Animals M.23, M.24 and L.24 received two injections of 7.5 cc. each at twenty four hour intervals and L.24 received three injections of 12 cc. each at twenty four hour intervals. (See Protocols, Appendix A). Blood specimens at varying intervals were taken after each injection. Some difficulty was encountered in the control of bleeding following the taking of blood specimens. Due to the Thorotrast, some of the animals had prolonged bleeding times and died of haemorrhage during the night. All animals were killed approximately twenty four hours after the injection of Thorotrast. The mode of death is given below:-

Haemorrhage 4

Overdose of thorotrast .. 1 (L.24)

Broken back 1

Sudden death on thorotrast injection. 2

Blow to nape of neck 1

Cause not determined 1

Nembutal 5 cc. I.V. 36

Just prior to autopsy a specimen of blood for section was taken from the jugular vein. Cholesterol deposit in eyes was noted. At autopsy a specimen of the principal organs and tissue was taken, including lung, liver and spleen. In addition, one animal (Q.9) had a complete autopsy done, that is, a specimen of every tissue was taken in order to study the behaviour of thorotrast in the entire organism. These were fixed in Zenker-formol. Special attention was paid to the aorta. It was carefully dissected in its entire length. A number of sections at approximately the same level in each animal were fixed in Zenker-formol (Appendix C). Other sections were fixed in formol 10% for frozen sections. The remaining sections were fixed in a variety of fixatives in an effort to find an ideal one for endothelium (Appendix C), but it must be admitted none proved superior or even equal to Zenker-formol as a fixative for the endothelium itself, although fixative No. 9 (alcohol, formol, trichloracetic acid) and Du Bosq - Brazil proved superior for the fixation of elastic tissue.

In Groups IX and X, where well developed atherosclerotic lesions were expected, the aorta was cut in approximately 1" lengths and the proximal end was marked with India Ink. This was done in order to study the deposit

of Thorotrast in the lesion in relation to the current of the blood. Again, in Group II, III and V, a portion of the aorta from the distal half of the arch to the upper half of the abdominal, was opened and rolled in order that the endothelium could be studied over a considerable length. These groups were considered of great importance, since they immediately preceded, or demonstrated the very earliest changes in aortic intima.

A block of all the tissues taken was embedded in paraffin in the routine way. Specimens of aorta, liver and spleen were preserved in formol 10% for frozen sections. Gelatin embedding was tried for fat and haemotoxylin and eosin stains on alternate sections in animals of Groups II, III, VI, VII, VIII, IX and X. It was hoped that by staining a section with H. & E. followed immediately by a Sudan stain for fat it could be determined whether Thorotrast deposited prior to the appearance of fat. The attempt proved unsuccessful, since the Thorotrast was not visible either in the H. & E. stain or the fat stain. Other methods for staining fat in such a way that Thorotrast could be detected at the same time were equally fruitless. Sudan Green, Sudan Blue and Osmic acid were tried. Because of difficulty in the photography of the earliest visible deposits of fat in the lighter stains, Sudan black was tried.

An embedding medium of British Drug Houses called "Ester Wax" was also used for the tissues of M.50. It was hoped that by using it the fat content of tissues would be preserved and could then be stained in the routine way. But the clearing agent (Cellusolve) necessary, dissolved the fat almost as readily as those used in paraffin embedding. An attempt to

saturate the Cellusolve with cholesterol prior to immersing the tissues in it did not decrease solubility of the tissue fats in it.

Routine H. & E. stains were done on all sections, including the blood specimens embedded in paraffin. For further morphological detail, Masson's trichrome was done on the aortae of animals M.41 to M.70. The Van Gieson Verhoeff stain for the study of elastic tissue was done on most aortae. An attempt was made to demonstrate the Golgi apparatus of the endothelium by means of Aoyama's silver technique as well as the Osmic acid impregnation. The results have not been satisfactory, although it is felt that further attempts should be made. Laidlaw's reticulin stain was done on animals of Groups II, III and V. A Giemsa-Jenner stain was done on the blood specimen of 0.18 and M.65 to compare the visibility of Thorotrast in polynuclears to that in the ordinary H. & E. stain.

In an effort to demonstrate Thorotrast in the atherosclerotic lesions of the aorta, a portion of the aorta of animal M.63 was stripped of its adventitia (because Thorotrast deposits in the periadventitial fat cells) and an X-ray was taken. This was not successful because of the excessive thinness of the rabbits aorta so that even the softest rays pass through.

Microscopic Examination:

All sections were at first studied with the apochromatic objective (X44). However, the oil immersion proved essential to discovering the minute particles of Thorotrast in the very early lesions of the aorta. Thereafter, Thorotrast accumulation in tissues as a whole and cells in

particular, was studied under oil immersion. Entire lengths of aortae were thus examined and all sections of aortae of every animal were thoroughly studied. The specimens of blood were examined in a regular pattern, so as not to miss any portion of the section.

Observations:

Blood specimens taken at various intervals following the injections of Thorotrast were examined under oil immersion to discover particles of the substance free or in any of the various cells circulating in the blood. One hundred and sixteen such sections were examined. Thorotrast was demonstrated as free particles among the elements of the blood, in neutrophils and/or in what will here be called "macrophages". Of the 116 sections examined:

Thorotrast was not found in 2

Thorotrast was found free in 9

Thorotrast was found free and in neutrophils in 17

Thorotrast was found free, in neutrophils and in macrophages in 61

Thorotrast was found in neutrophils and macrophages in 1

Nine sections were not suitable for examination (See graph on page 49).

Some of these cells are undoubtedly the ordinary monocyte of the circulating blood (Fig.1). Others are similar to the histiocytic or reticulo-endothelial cells of the spleen, lymph nodes and other organs. These cells have a large, rounded or somewhat elongated nucleus with a well marked nuclear

THOROTRAST IN BLOOD

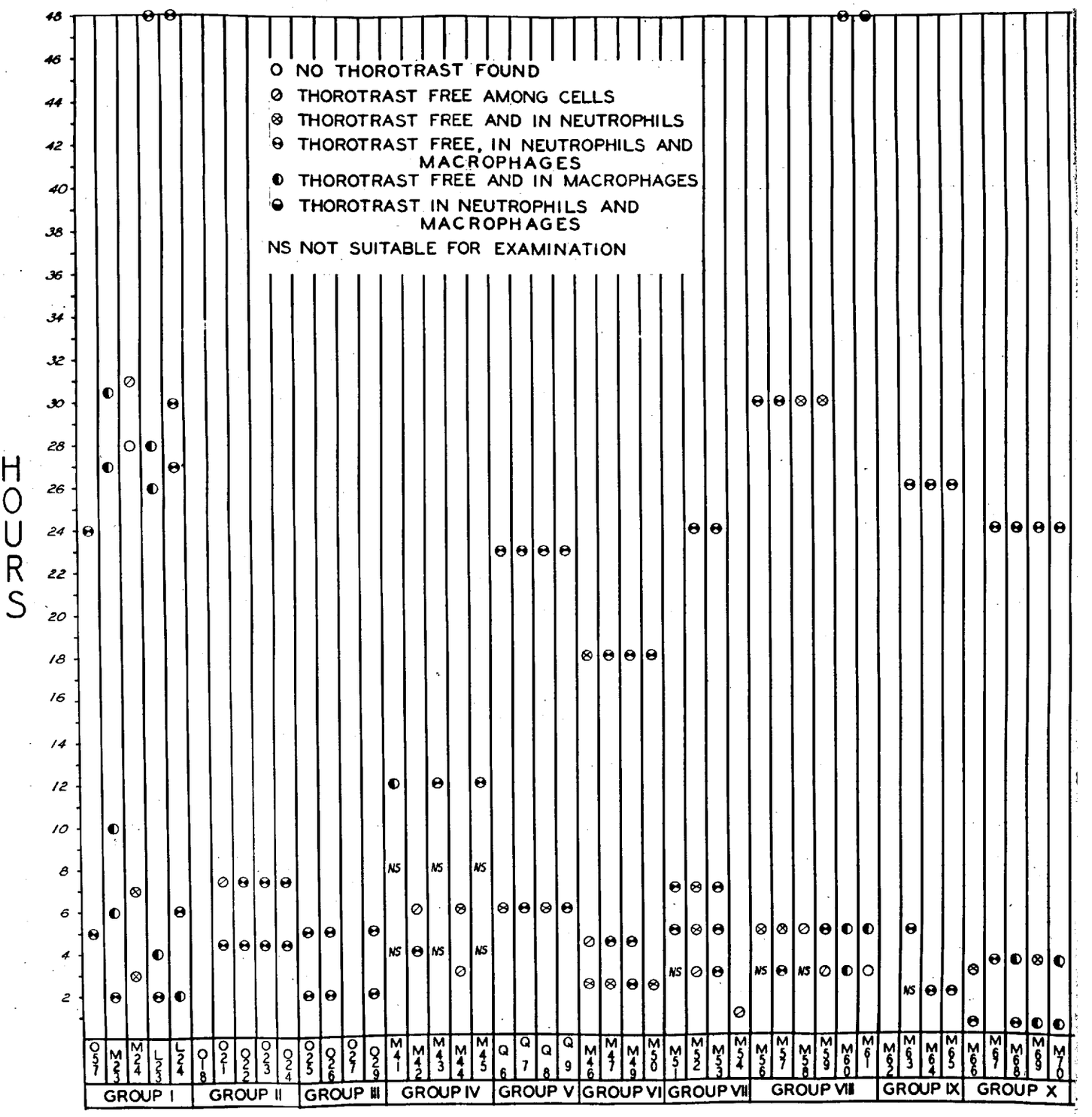

Fig. 1

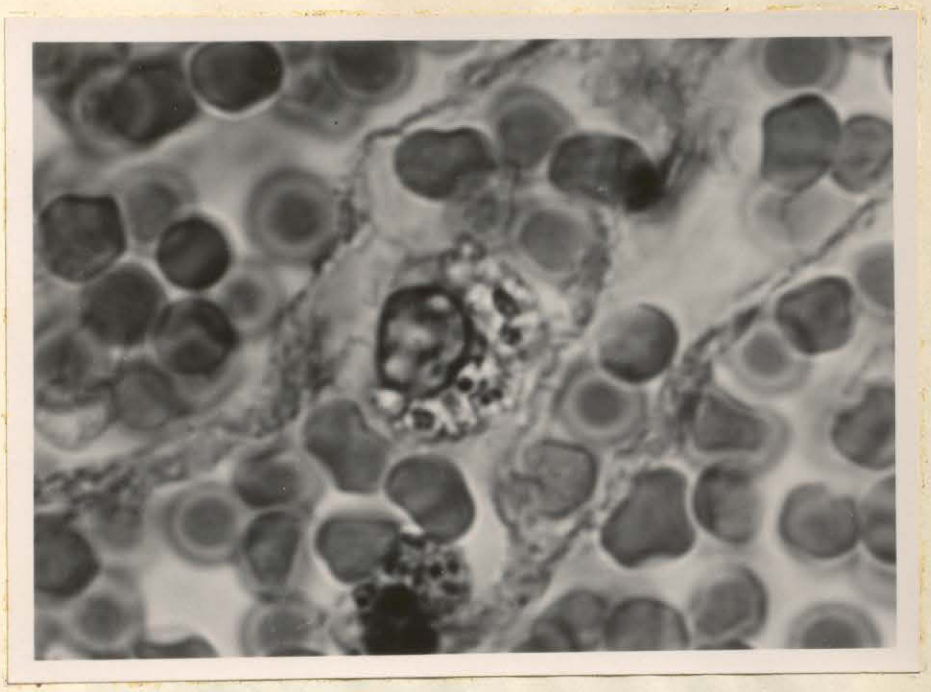

Fig. 1. Paraffin section of blood specimen taken 18 hours after Thorotrast injection. Monocyte with numerous large particles of Thorotrast in its cytoplasm.
Animal No. M.50 B. X 2600. Haematoxylin and Eosin stain.

membrane. The chromatin is finely scattered with one or two larger, dark staining granules. The protoplasm is quite abundant and has an irregular outline. The cells containing Thorotrast appeared greatly distended, the protoplasm being broken up by the coarse granules. It was extremely difficult to study the cytoplasm of these cells for their possible content of fat since the Thorotrast frequently masked the entire cytoplasm (Fig. 2 & 3). In the few cells of this aspect not containing Thorotrast in such abundance, the cytoplasm appeared homogeneous, taking a slightly basophilic stain with H. & E. The typical honeycomb aspect of the foam cell was never observed.

Fig. 2

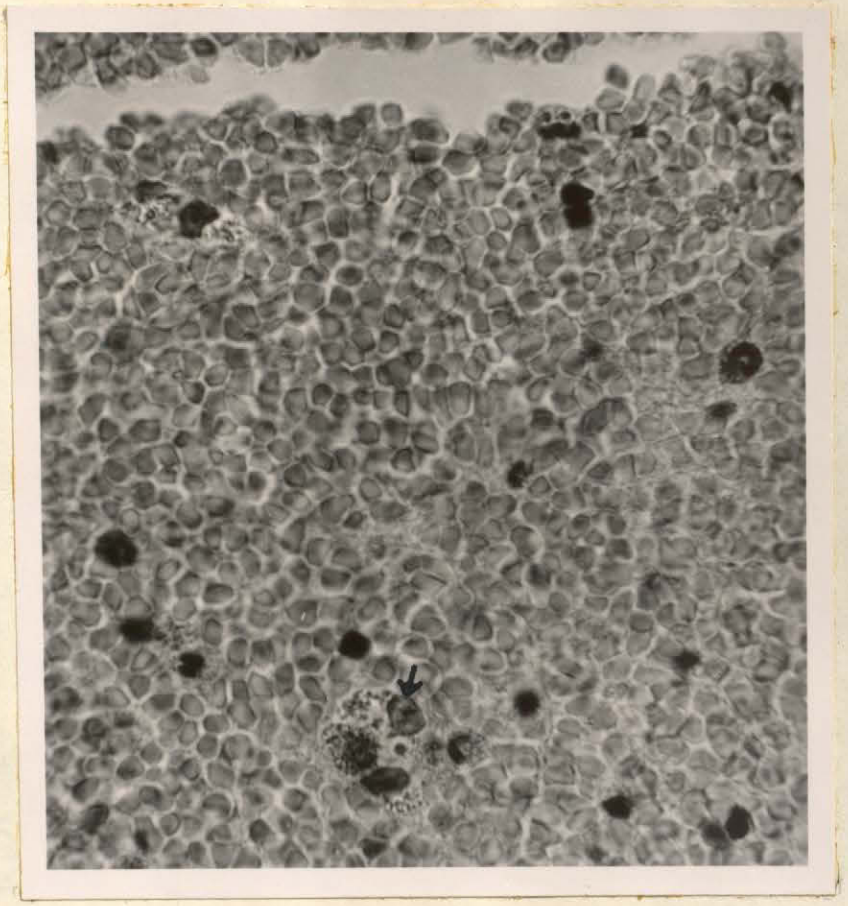

Fig. 2 Paraffin section of blood specimen taken 26 hours after Thorotrast injection. Arrow points to macrophage of the aspect described above. Below and to the left can be seen a neutrophil without Thorotrast. A second macrophage appears directly below. This also contains Thorotrast granules in its cytoplasm. It should be noted that the normal granules of the neutrophil may be distinguished by the absence of the "halo" always present around the Thorotrast granule in micro-photographs.

Animal M.65 B. X 920. Haematoxylin and Eosin stain.

Fig. 3

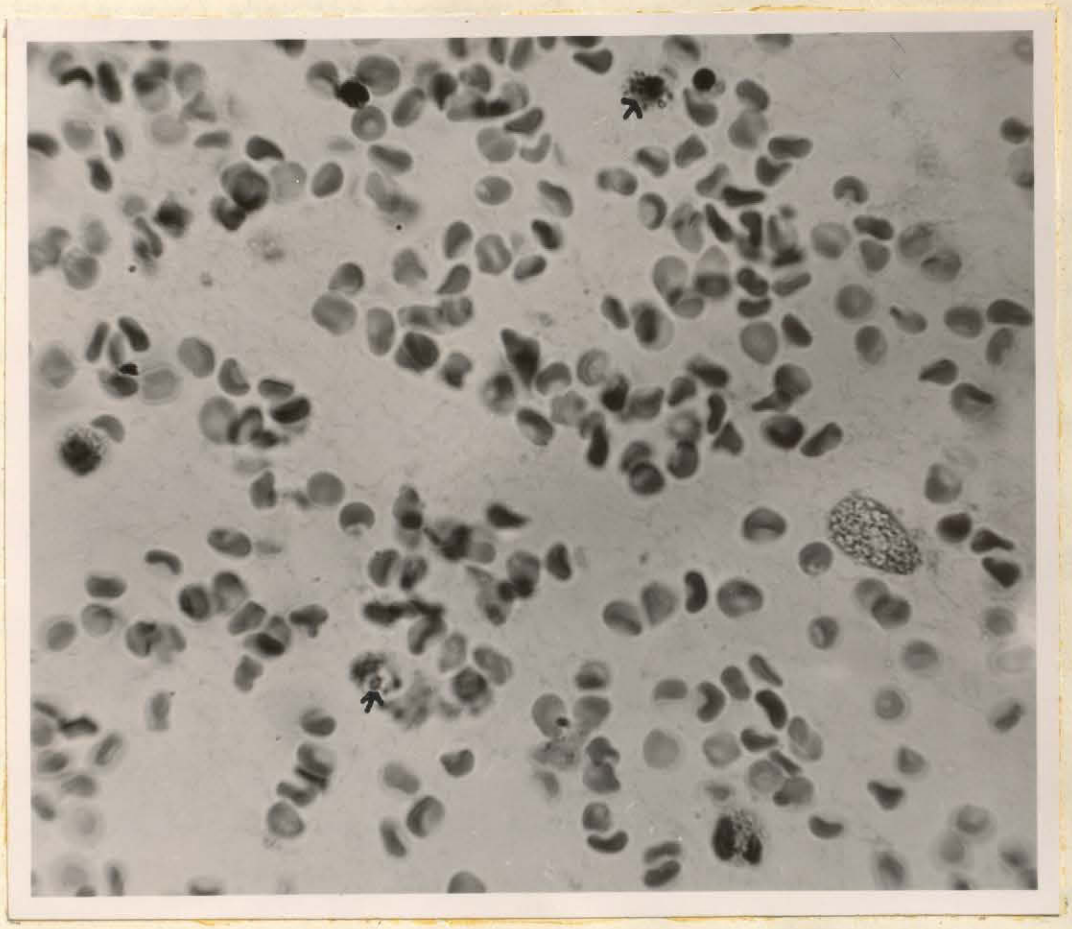

Fig. 3. Paraffin section of blood specimen taken 26 hours after Thorotrast injection showing a large macrophage loaded with Thorotrast. The nucleus is not visible in this plane. An arrow points to neutrophils, each containing a granule of Thorotrast in their cytoplasm.
Animal M.65 B. X 1200. Haematoxylin and Eosin stain.

The relation of the presence of macrophages with Thorotrast to the time-interval when the specimen of blood was taken is difficult to assess because although they appear in sections taken two to twelve hours following Thorotrast injection as well as in those taken twenty four hours or more afterwards, there was a decided increase in the number of these macrophages in the later specimens. In the early sections (i.e. those taken from twenty minutes to one half hour to twelve hours after Thorotrast injection) the macrophages were few indeed, generally from one or two to 10 to 12 in the entire section. The specimens taken twenty to forty eight hours after Thorotrast injection showed numerous macrophages, sometimes from five to ten in a microscopic field (oil immersion).

Microscopic sections of aortae of 46 animals were suitable for observations.

In Group I five normal animals that had received 1, 2 and 3 injections of Thorotrast, showed no accumulation of the substance in the normal aortic endothelium. Where the latter showed natural arteriosclerosis, as in one section of L.24, Thorotrast deposits were seen in fine granules in the intact and normal appearing endothelial cells. The intima of this section of aorta was markedly thickened with considerable fibroblastic proliferation. Other sections of the aorta of this animal showed a normal intima with no deposits in the endothelial cells.

The findings of the 41 animals cholesterol fed over varying periods of time are presented in table form on page 54.

Microscopic Findings in Aortic Sections of Cholesterol-fed Animals

Lesion	No. of Animals	Thorotrast in endothelium
No lesion	15	3
Swelling of intima (fat stain negative)	1	1
Swelling of intima (no fat stains done)	4	4
Swelling of intima (no foam cells) Fat stain +	9	5
Fully developed atherosclerotic lesion with foam cells	8	8
Spontaneous arteriosclerosis	4	3

Two aortae (M.54 and Q.9) showed a granulomatous lesion with marked thickening of the intima through fibroblastic proliferation. The lesion in Q.9 occurred in the sinus of Valsalva. Many of the fibroblasts contained fat granules in their cytoplasm, but no definite foam cells could be recognized. There was abundant accumulation of thorotrast in the fibroblasts containing lipoid material as well as in the endothelium overlying the lesion (Fig.4) .

As seen in the above table three animals who demonstrated no intimal change whatsoever did, however, contain tiny particles of thorotrast in some endothelial cells. Of these animals, 0.21 and 0.22 had been on cholesterol feeding for one week only. The third animal (0.25) to contain thorotrast in the aortic endothelium without microscopically visible change in the intima or media had been on cholesterol feeding for two weeks. Attempts to photo-

graph the sections illustrating the above finding were unsuccessful.

The remaining twelve of the animals who showed no microscopic change in the aortic intima or media did not have any thorotrast in the endothelial cells or in the subendothelium.

Fig. 4.

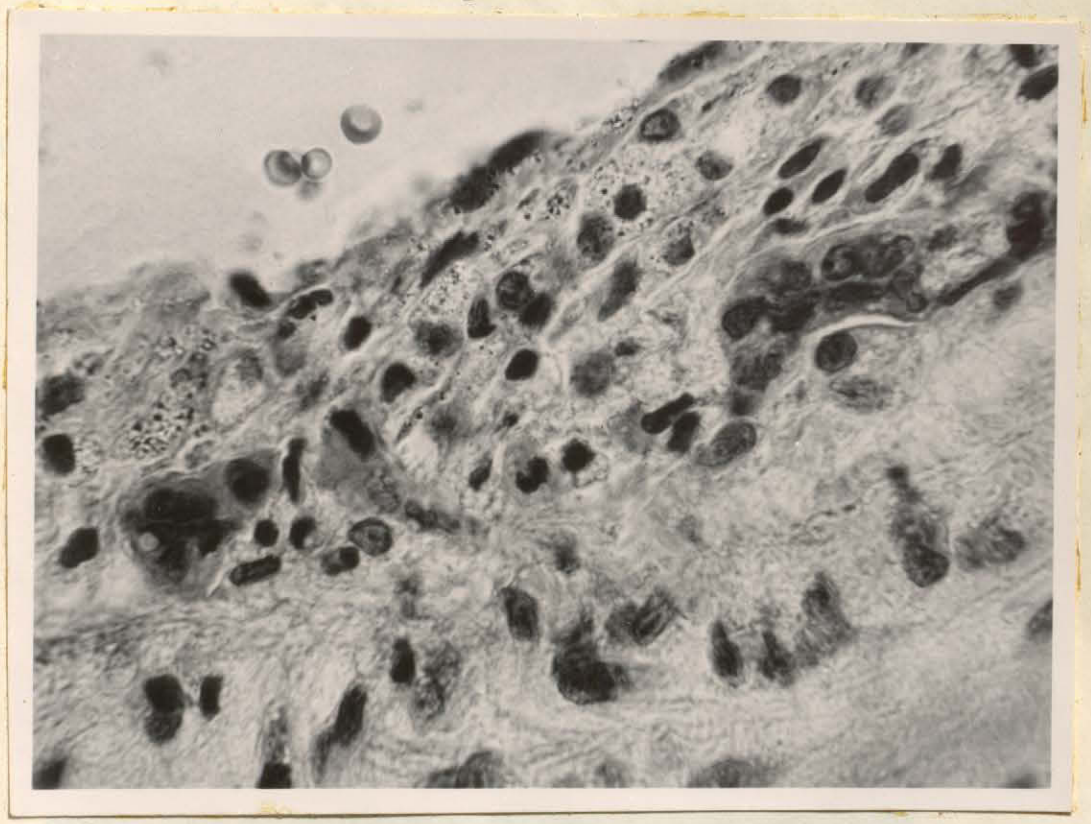

Fig. 4. Aorta: Section taken at the sinus of Valsalva. Atheromatous lesion with giant cell formation showing abundant thorotrast in endothelium, fibroblasts and lipoid cells (not typical foam cells).
Animal Q.9 - cholesterol fed 3 weeks. Haematoxylin & Eosin. X 1120.

Only one animal (M.51) with swelling of the ground substance failed to show Sudan positive material in the intima. Thorotrast was found in the endothelium. A frequent finding was the presence of stainable fat in the endothelium. It was noted chiefly in groups on cholesterol feeding for

from one to three weeks (See Figs.5,6 & 7). Although repeated attempts were made with various stains and in serial sections in which a haematoxylin eosin stain alternated with a fat stain in frozen sections, thorotrast could not be demonstrated conclusively (it was seen as probably present in many cases) in these sections. The paraffin sections of lesions very similar to these did show the accumulation of thorotrast in the endothelium. The particles of thorotrast appearing in the early lesions were minute and less in number compared with those appearing in the foam cells of fully developed atherosclerotic plaques. In the endothelium the particles were always of smaller

Fig. 5.

Fig. 5. Aorta - showing endothelial cells staining for fat with osmic acid stain.
Animal Q.8 - cholesterol fed 3 weeks. Osmic acid stain. X 1200.

Fig. 6.

Fig. 6. Aorta showing endothelial cells staining for fat with osmic acid. Animal Q.7 - cholesterol fed for 3 weeks. Osmic acid stain. X 475.

Fig. 7.

Fig. 7. Aorta showing endothelial cells staining for fat with Sudan Red. Animal Q.7 - cholesterol fed for 3 weeks. Sudan Red Stain X 500.

size than those encountered in the foam cells of the same lesion (Fig.8).

The eight animals presenting typical early atherosclerotic lesions had thorotrast accumulated in endothelial cells overlying the plaque, in foam cells of the superficial layers and in the occasional fibroblast. In the majority of cases there appeared to be no predelection for any area of the

Fig. 8.

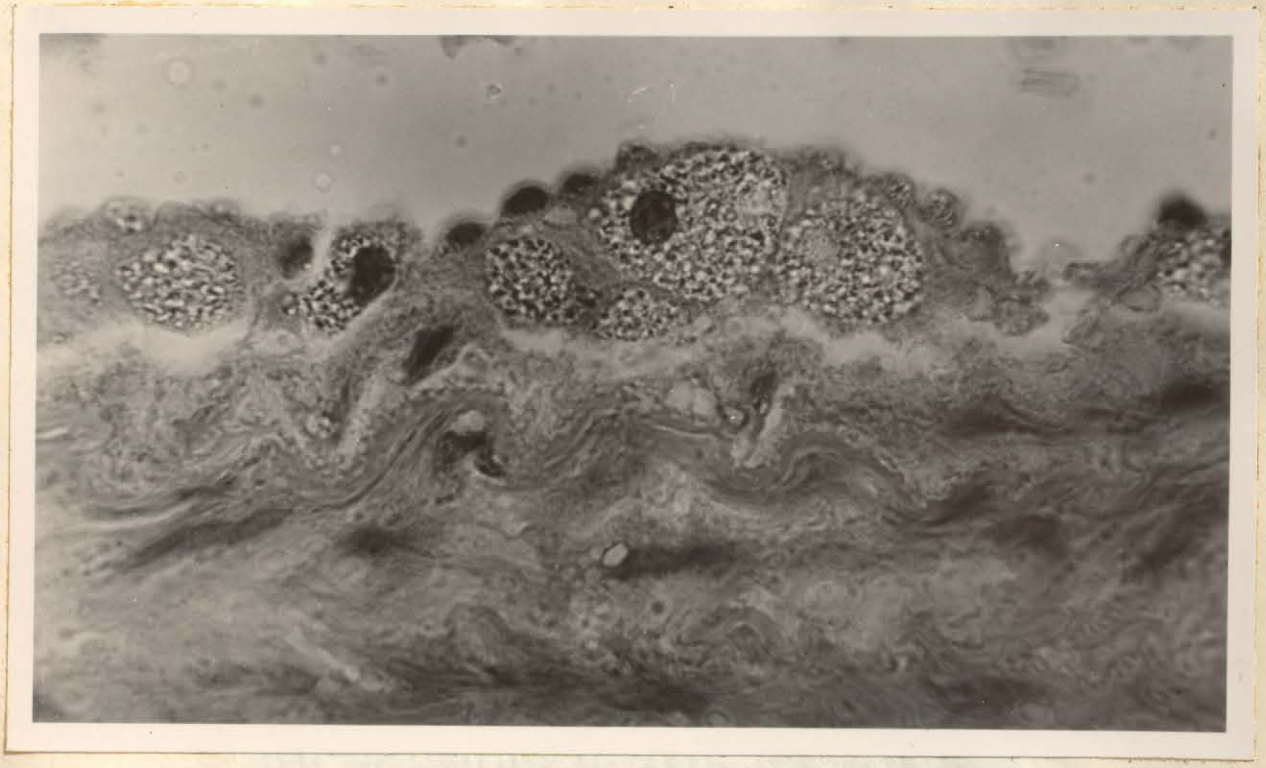

Fig. 8. Aorta: High power to show thorotrast in abundance in foam cells and in endothelial cells. Note the difference between the size of particles in endothelium and those in foam cells.
Animal M.63 - cholesterol fed 7 weeks. Haematoxylin & Eosin stain X 1200.

lesion save,as just mentioned, the predominance of thorotrast accumulation in the superficial layers (as in Fig. 4). All cells are not equally rich in this substance. While one cell has the entire cytoplasm peppered with thorotrast granules, its neighbouring cells may be quite free of the substance, or contain but one or two granules. The same holds true for the endothelial accumulation. Cells with thorotrast are found intermittently, averaging about 2 - 4 per oil immersion field over the atherosclerotic lesion.

The above picture holds true for the majority of lesions. Some exceptions must be pointed out. The very occasional lesion shows thorotrast deposits in the endothelium overlying an atherosclerotic plaque and in fibroblasts, while none at all can be seen in the foam cells (Fig. 9). Another lesion (Fig.10) illustrates an abundant deposit in almost all foam cells and in deeplying fibroblasts.

Fig. 9.

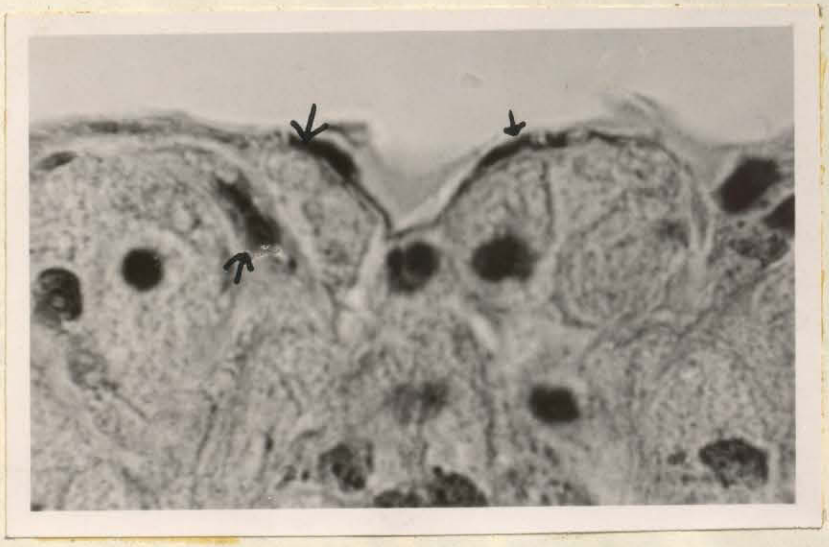

Fig. 9. Aorta showing fine granules of thorotrast in two normal appearing endothelial cells and in one fibroblast (arrows) while neighbouring foam cells are free of the substance.
Animal M.53. Cholesterol fed 5 weeks. Haematoxylin & Eosin stain X 1200.

The aortae of Groups IX and X were sectioned longitudinally, a section being approximately 2 cm. long and the proximal end marked with India Ink on the adventitial aspect in an effort to demonstrate any relation of thorotrast deposit to the direction of the blood current. In the sections thus examined, there appeared to be no relation to the hemodynamic factors

Fig. 10

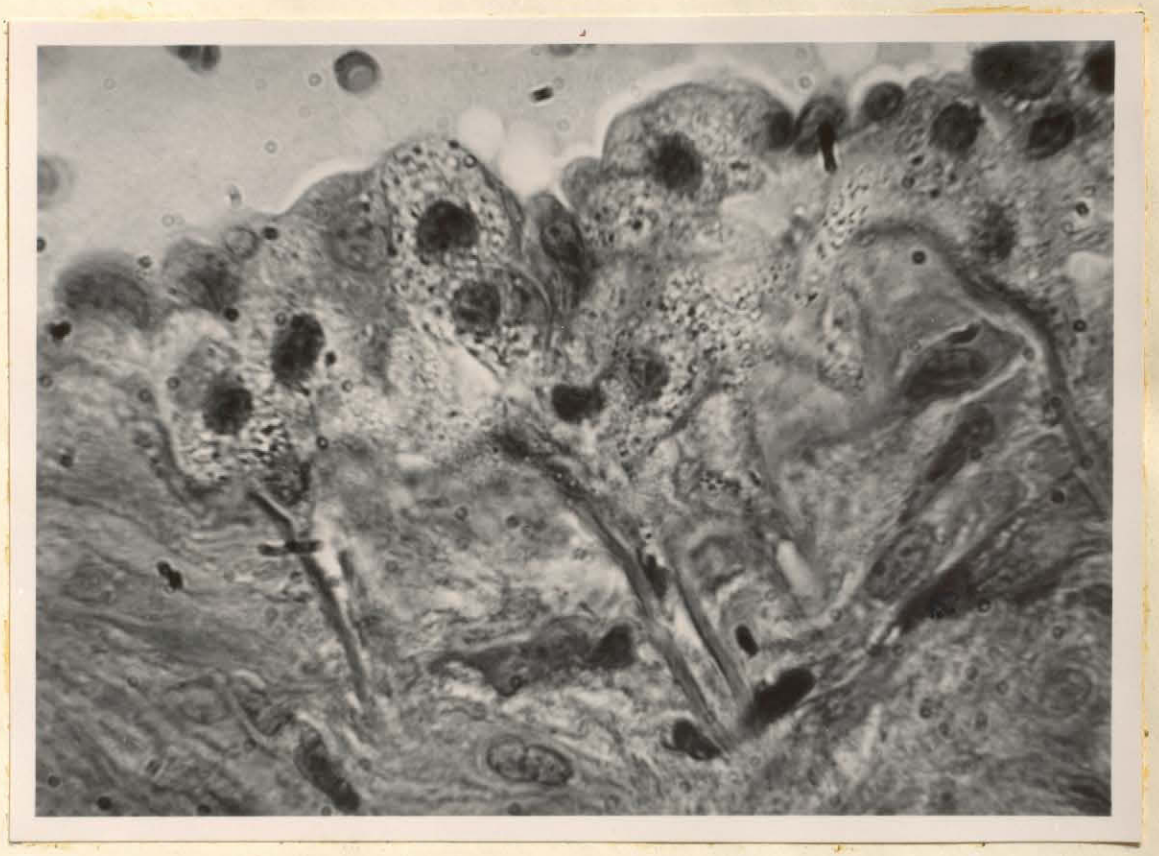

Fig. 10. Coronary showing atheromatous plaque with abundant accumulation of thorotrast in foam cells throughout the lesion. Arrow indicates a fibroblast of deep layer also containing thorotrast. Animal Q.9 - cholesterol fed 3 weeks. Haematoxylin & Eosin stain X 1150.

as thorotrast appeared in foam cells scattered throughout the lesion. This was also true of the distribution in endothelial cells.

The accumulation of thorotrast in endothelium and foam cells assumed a definite localization in some lesions of four animals (M.56, 57, 63 and Q.9). Here, thorotrast was found primarily at the periphery of the lesion (Figs.11,12). This could not be related to the blood flow, since it was found at both extremities of the lesion.

Fig. 11.

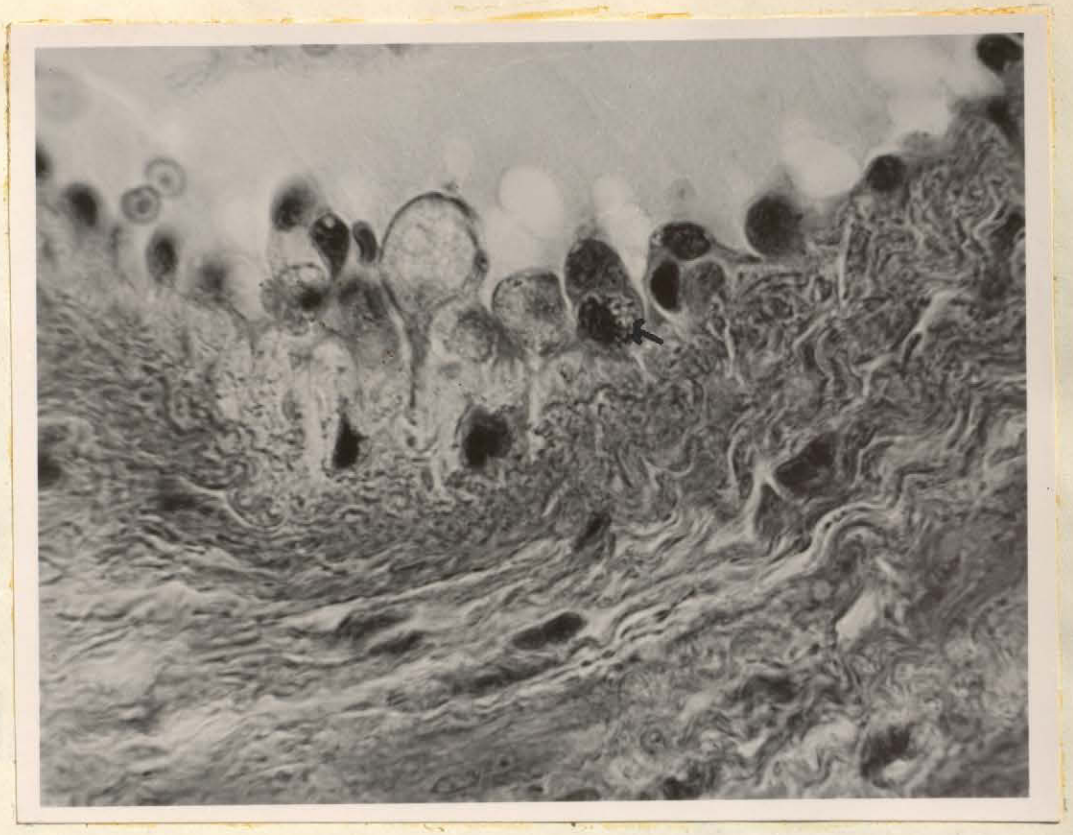

Fig. 11. Coronary showing thorotrast deposited at periphery of lesions. Arrow indicates cell (containing thorotrast) which closely resembles the macrophages found in the circulating blood. Animal Q.9 - cholesterol fed for 3 weeks. Haematoxylin & Eosin. X 1150.

In favour of an influence exerted on the localization of atherosclerotic lesions is the lesion seen in Fig. 13. This is an inciidental finding in Q.9 and illustrates a very definite predilection for the mouth of a branching vessel.

Fig. 12.

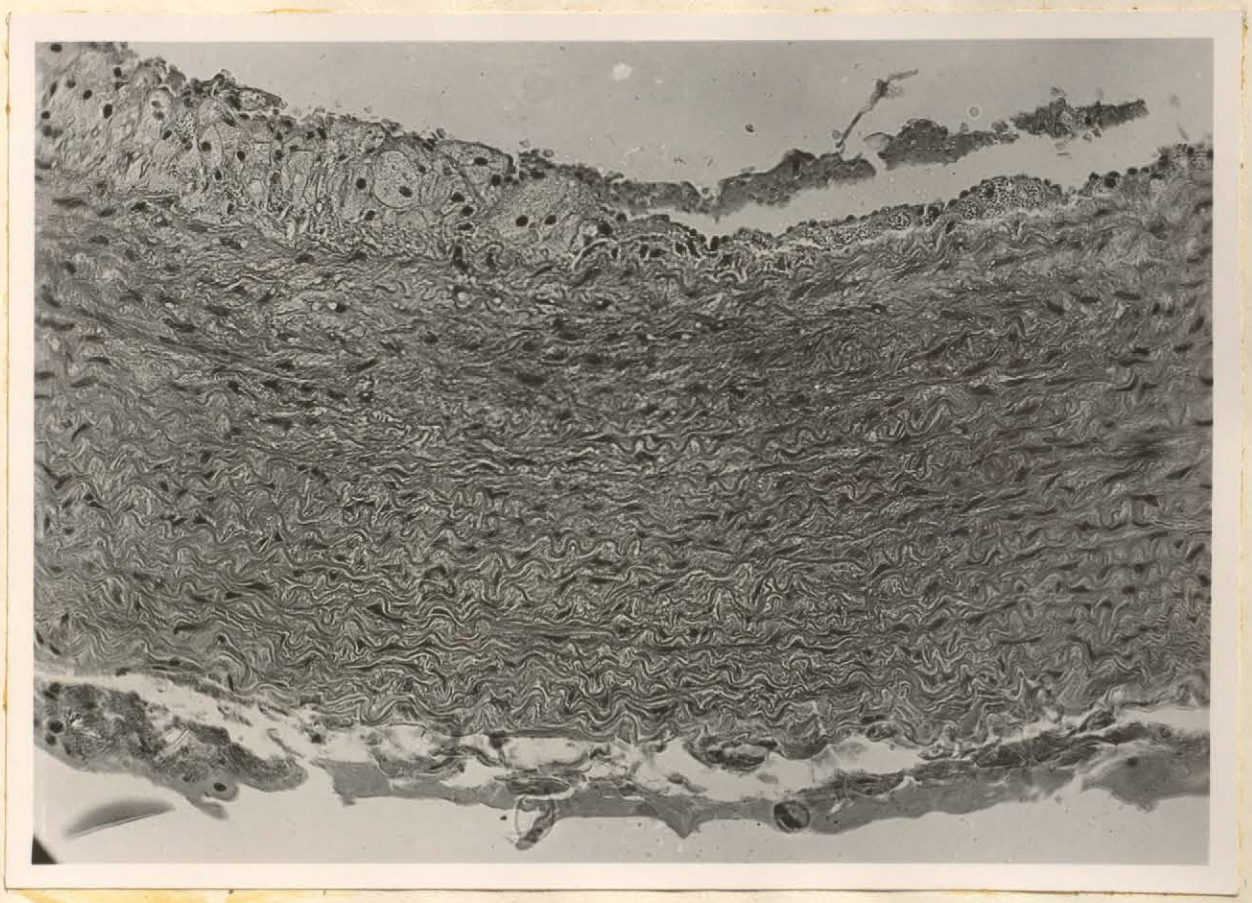

Fig. 12. Aorta showing a decided predilection of thorotrast for peripheral areas of the lesion. A very few cells in the central portion of the plaque also contain thorotrast.
Animal M.63 - cholesterol fed for 7 weeks. Haematoxylin & Eosin stain.
X 245.

Fig. 13.

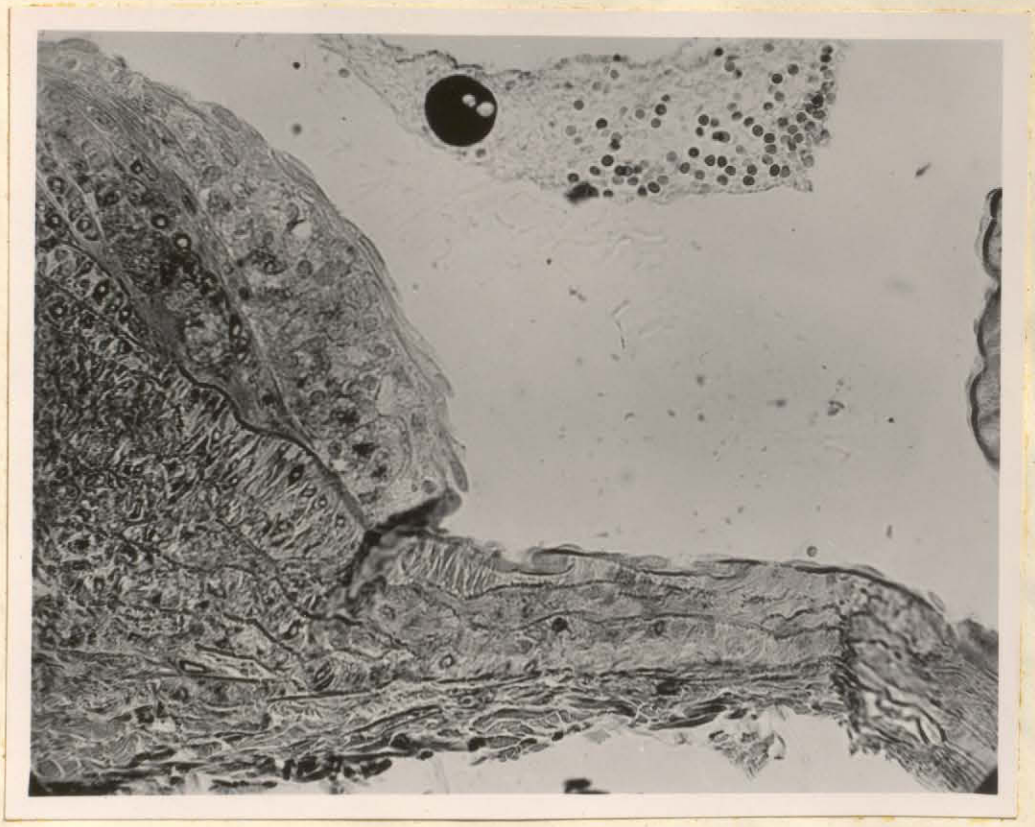

Fig. 13. Aorta showing an isolated atheromatous plaque at the mouth of a branching vessel.
Animal Q.7 - cholesterol fed 3 weeks. Osmic acid stain. X 400.

The study of the organs other than aorta revealed the predilection of thorotrast for the reticulo-endothelial system. It was found, without exception, that this substance tended to accumulate most abundantly in the reticulo-endothelial cells of the spleen, frequently causing marked necrosis. The lymphoid tissue and their germinal centres showed little tendency to accumulate thorotrast, remaining free of the substance when virtually all

the reticulo-endothelial cells were loaded with it (Fig.14 illustrates the massive accumulation of thorotrast as generally found in this organ. Where the reticulo-endothelial cells were already burdened with fat, they still took up innumerable particles of thorotrast (Fig.15).

Thorotrast accumulated chiefly in the Kupffer cells of the liver. The cells with lipoid material again picked up this substance avidly (Fig.16). The endothelium of the glomerular tufts in the kidney was the site of thorotrast deposit in the kidney. The capillaries of the glomerular tuft were frequently obstructed with thorotrast (Fig.17). The tubules remained almost completely free of the substance.

Abundant deposits of thorotrast were expected in the adrenals. This was not the case. Thorotrast was found most frequently only in the reticulo-endothelial cells, the parenchymal cells remaining relatively free (Fig.18). The pituitary presented a similar picture (Fig.19).

The cytoplasm of the periadventitial fat cells of the aorta was frequently the site of thorotrast accumulation, though it was never encountered in the cellular structures of the adventitia itself (Fig.20). A striking picture is presented in the bone marrow. Here the lacy network of the reticulo-endothelial system is completely engorged with thorotrast, while the hematopoietic cells suspended in it are relatively free (Fig.21).

All vessels of the various organs of the body were thoroughly examined for any evidence of thorotrast accumulation in the endothelial lining, with or without atherosclerotic lesions. The findings were generally negative with only two exceptions. The first was in the coronaries of Q.9

Fig. 14. Spleen showing the massive accumulation of thorotrast in the reticulo-endothelial cells, while the lymphocytes remain free.

Animal Q.57 - normal rabbit- 1 injection of thorotrast. Haematoxylin & Eosin. X 1200.

Fig. 15. Spleen showing fat laden reticulo-endothelial cells. These cells also demonstrated thorotrast on microscopic examination.

Animal Q.9 - cholesterol fed 3 weeks. Osmic acid stain. X 425.

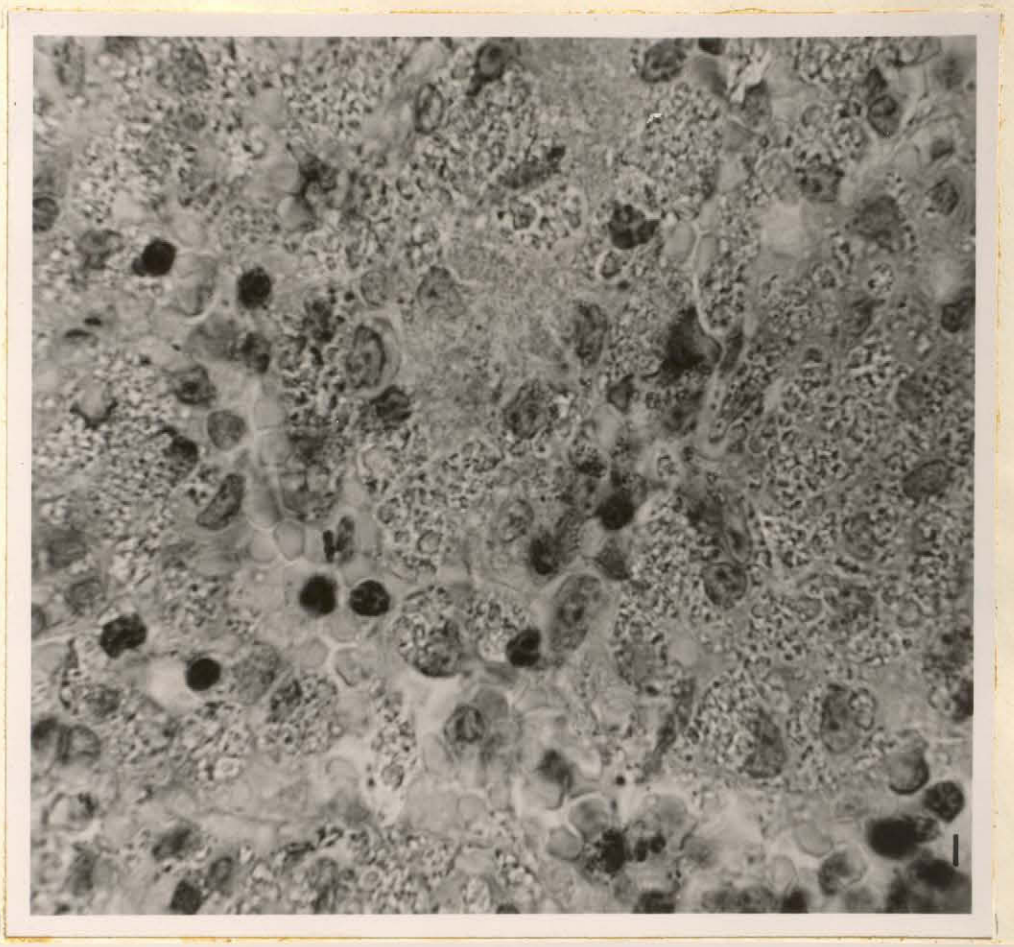

FIG. 14

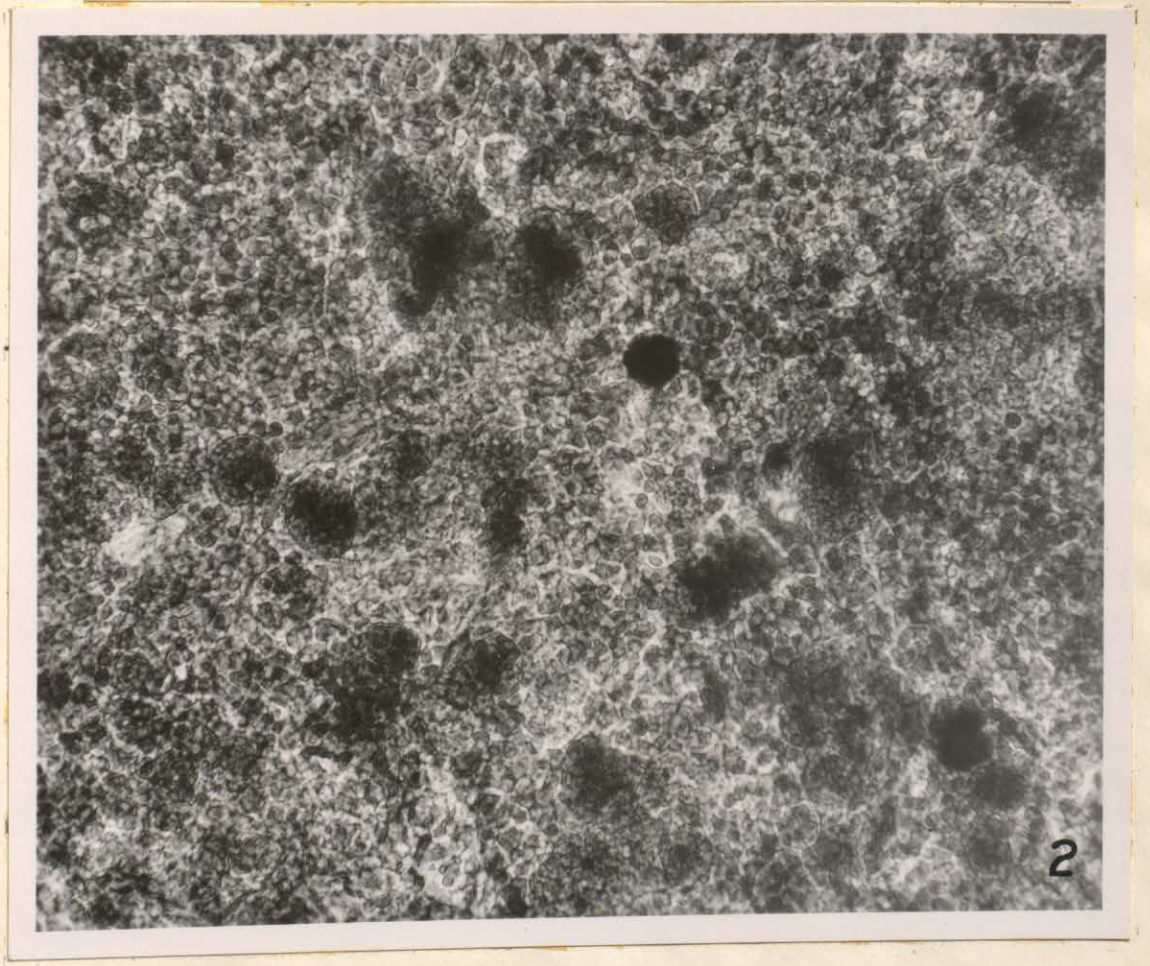

FIG. 15

Fig. 16 Liver showing Kupffer cells staining for fat and containing numerous particles of thorotrast. The parenchymal cells are relatively free of the substance.
Animal Q.7 - cholesterol fed 3 weeks. Sudan Red stain X 475.

Fig. 17 Kidney showing part of a glomerular tuft with thorotrast in the endothelial cells and obstructing capillary.
Animal L.24 - normal rabbit - 3 injections of thorotrast.
Haematoxylin & Eosin stain. X 1200.

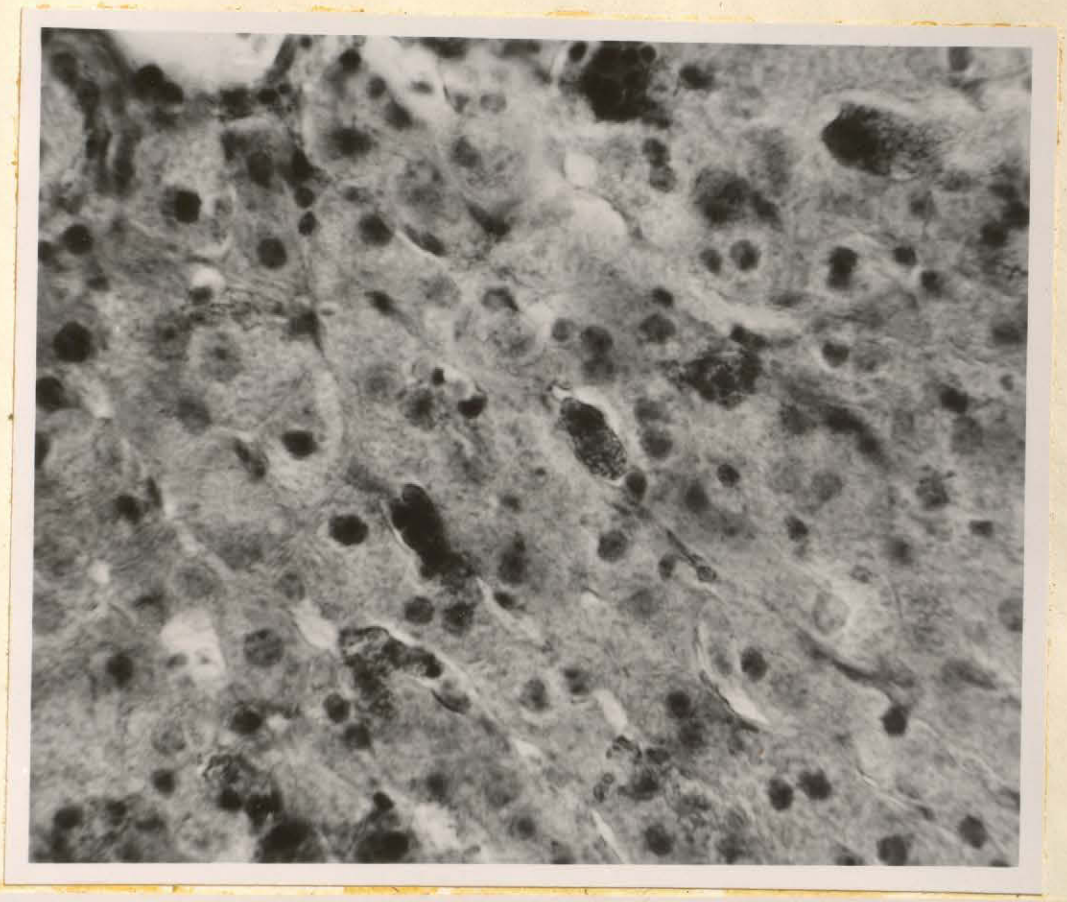

FIG. 16

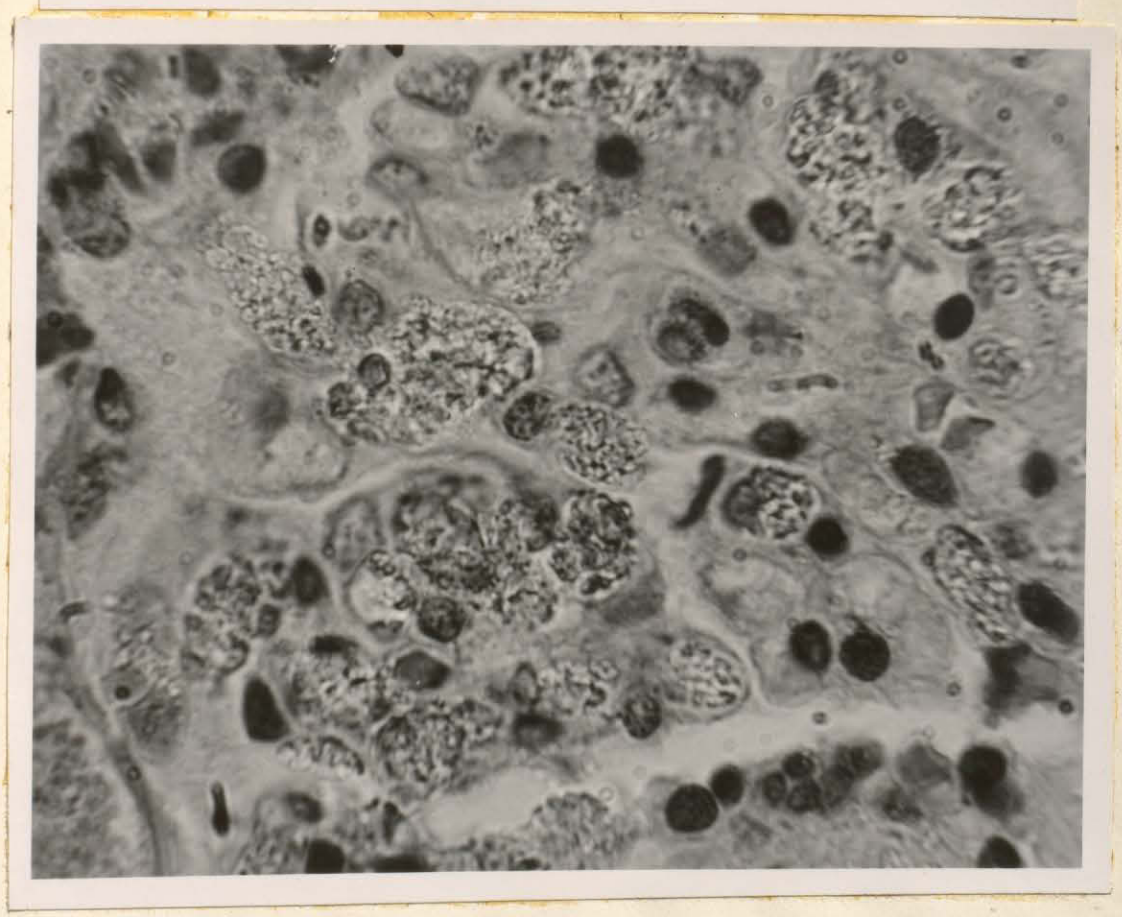

FIG. 17

Fig. 18. Adrenal showing predilection of thorotrast for the reticulo-endothelial cells in the adrenal.

Animal M.56 - cholesterol fed 6 weeks. Haematoxylin & Eosin stain. X 1050.

Fig. 19. Pituitary: The thorotrast is almost exclusively accumulated in the reticulo-endothelial cells.

Animal Q.8 - cholesterol fed for 3 weeks. Haematoxylin & Eosin stain. X 1200.

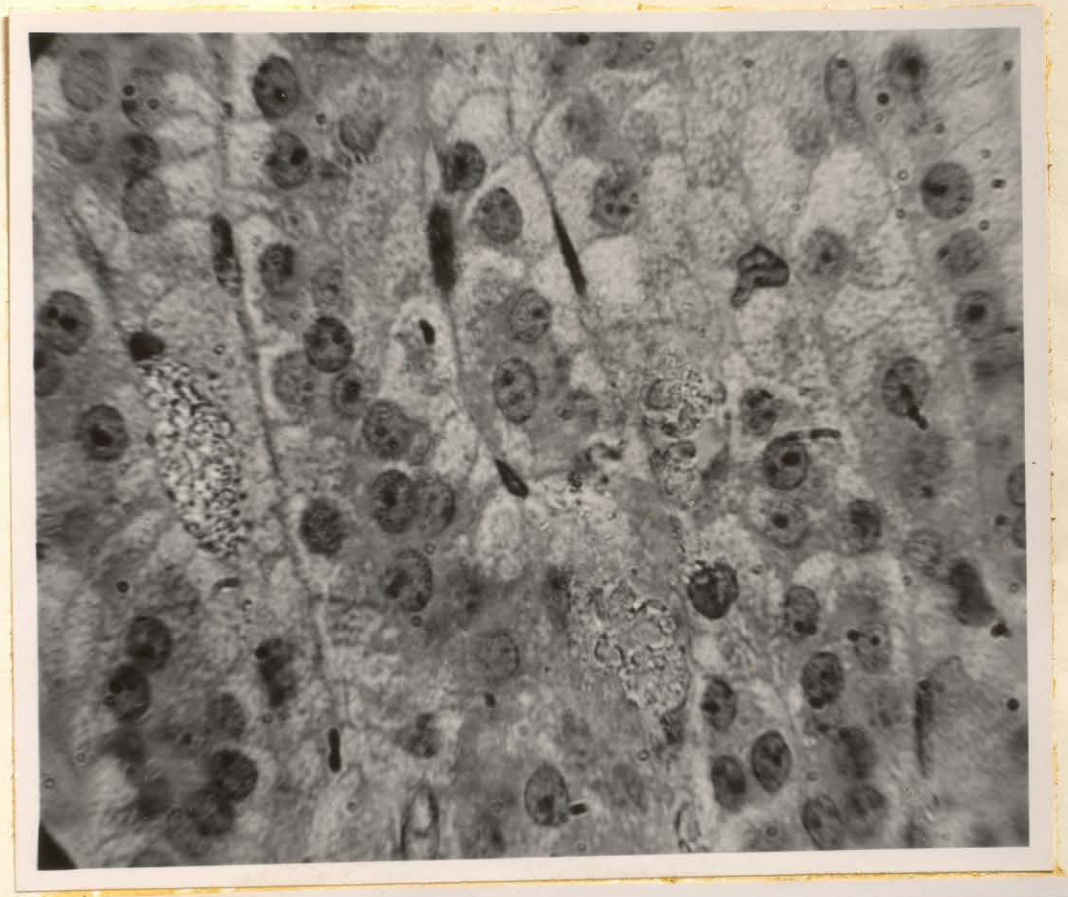

FIG. 18

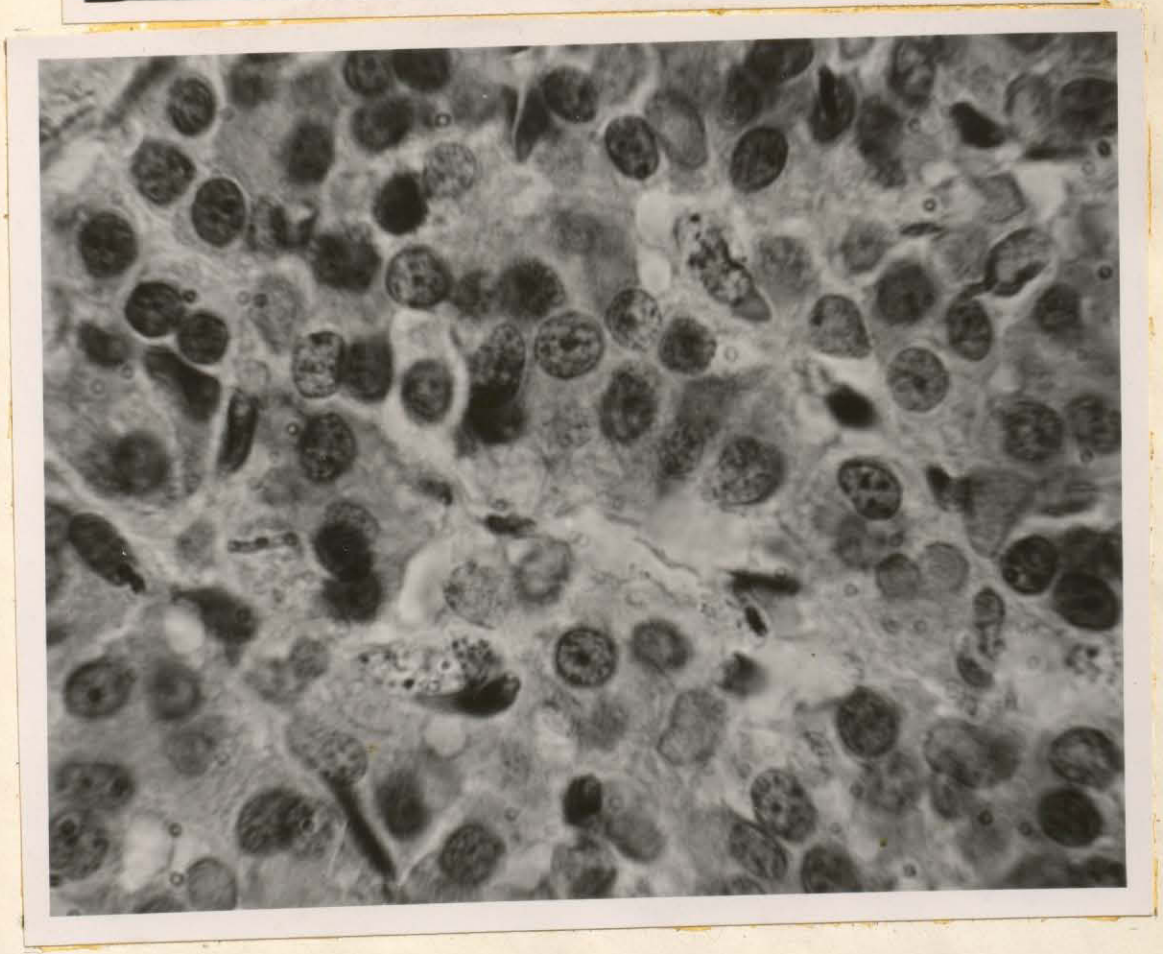

FIG. 19

Fig. 20 Periadventitial fat of aorta showing thorotrast accumulation in the fat cells.
Animal L.24 - normal rabbit - 3 injections of thorotrast.
Haematoxylin & Eosin stain. X 1150.

Fig. 21 Bone marrow showing thorotrast in abundance in the reticulo-endothelial net-work, while hematopoietic cells remain free.
Animal Q.6 - cholesterol fed for 3 weeks. Haematoxylin & Eosin stain. X 1150.

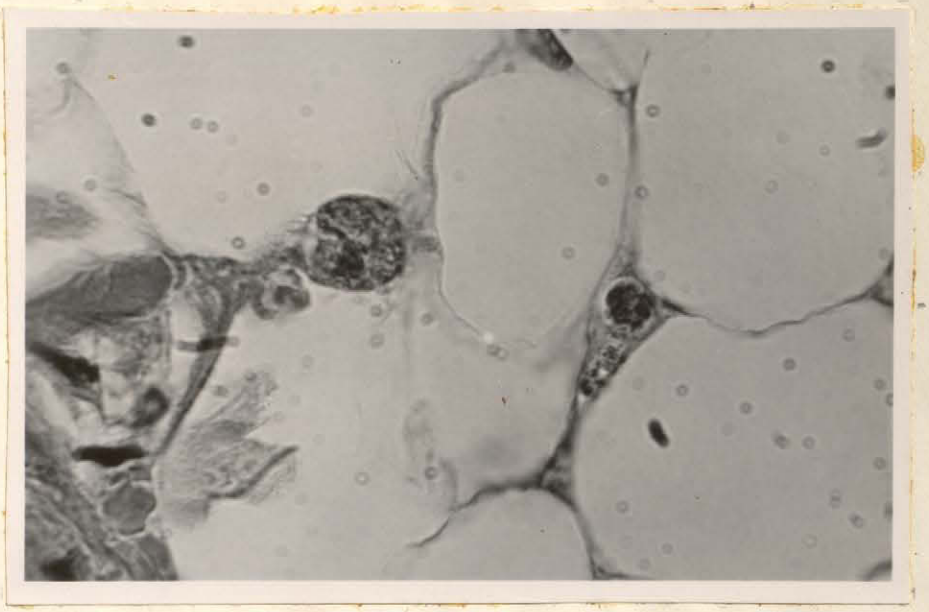

FIG. 20

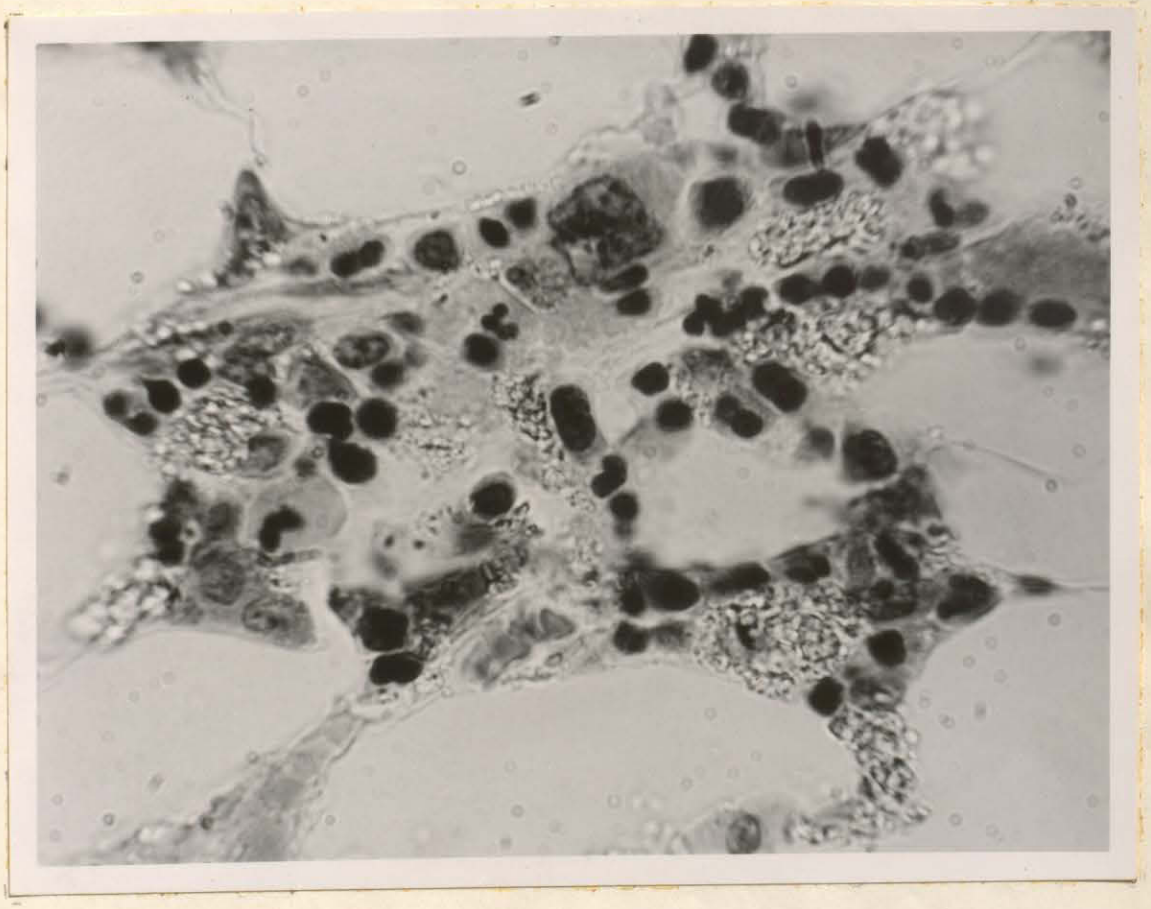

FIG. 21

and which have been previously reported. The second finding was the accumulation of thorotrast in several endothelial cells of what appeared as a normal pulmonary artery (L.24).

A constant finding was the presence of monocytes and macrophages loaded with thorotrast in the pulmonary arteries and veins (Fig.22). Whenever there was blood fixed in the aortic wall or in the heart cavities, the same cells could be found.

The remaining organs of the body remained relatively free of thorotrast. Many lymph nodes were examined and it was noted that thorotrast deposited in the reticulo-endothelial cells in this tissue only rarely and in very small amounts.

Fig. 22.

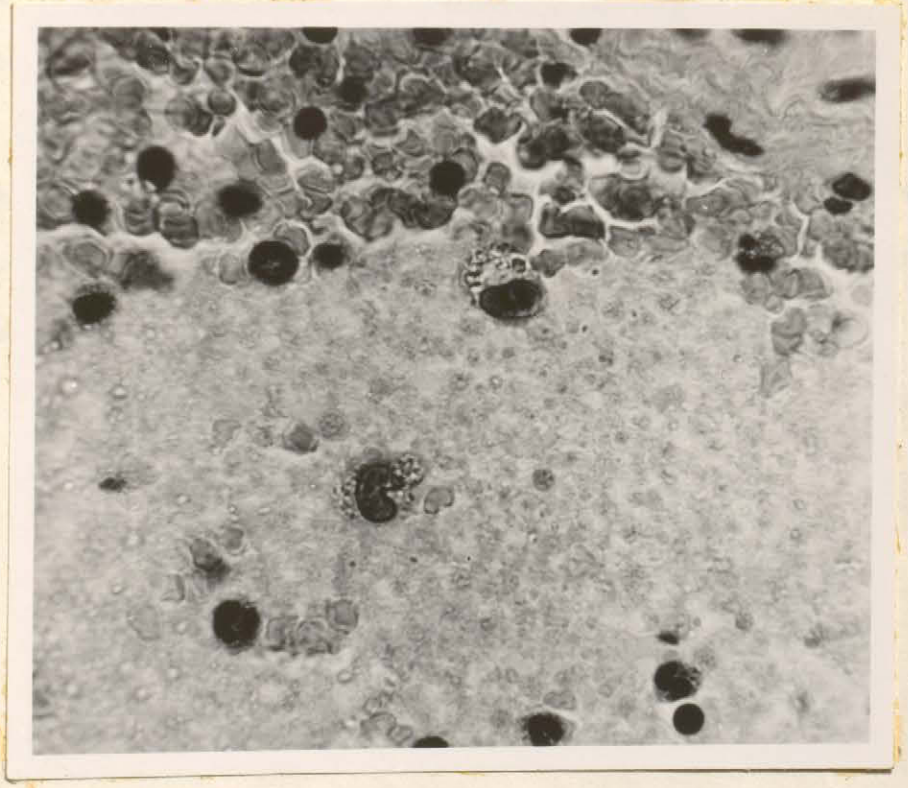

Fig. 22. Artery in lung showing two monocytes with large particles of thorotrast in their cytoplasm.
Animal M.64 - cholesterol fed for 7 weeks. Haematoxylin & Eosin X 1050.

General Remarks:

While thorotrast is recorded as found in the aortic endothelium in three animals with apparently normal intimas, after only a week or two of cholesterol feeding, it should be noted that the particles of thorotrast were very minute and fewer in number than in the endothelial cells overlying developed lesions. The number of the cells containing the thorotrast were also fewer.

It is felt that the group of normal animals was not sufficient in number to act as adequate controls. However, this omission was partially compensated by the fact that findings in altered areas of the treated animals could be compared with areas where the intima remained normal.

The fixatives and staining methods used, while proving of definite value in most instances, need further study and manipulation to adapt them to the morphology of the delicate endothelium.

Throughout the microscopic studies of the aortae, the possibility of encountering mitotic figures in the atherosclerotic lesions was borne in mind. Mitotic activity was demonstrated in one slide (M.70) where two foam cells were undergoing mitotic division.

DISCUSSION

There is possibly a very close relation between the findings as reported in this experiment and those found by Gofman[89]. As has been previously noted, the very first particles to appear in the endothelial lining are generally very minute. It may be questioned then, whether the normal endothelium would pick up macromolecular particles, in size inferior to visibility in the microscope by our present-day methods, but could perhaps be demonstrated by the electron microscope. It may be postulated that due to the increased number of the Sf 10 - 20 cholesterol molecules in the circulating blood, the endothelium accumulates the larger molecules in its cytoplasm, eventually becoming visible as lipoid granules. Certainly it can be stated that in this experiment the endothelium, first demonstrating an accumulation of tiny particles, few in number, gradually increases its ability to pick up this substance in greater quantities and in larger particles.

Viewing the problem from a somewhat different angle, it is clearly illustrated in this experiment that the reticulo-endothelial system is the great accumulator of macromolecular substances, in this case Thorotrast. Considering the embryological properties of the endothelium and its linkage with tissues of mesenchymal origin, it can be

conceived that a "metaplasia" occurs in answer to the altered surroundings with the addition of macromolecular substances in the plasma. The feeding of cholesterol produces a change in the surrounding medium of the endothelium in such a way that the latter, previously impregnable to thorotrast injection in double and triple quantities, now begins to accumulate this substance in its cytoplasm. An argument against the above lies in that the same change in endothelial properties occurs in spontaneous arteriosclerosis. Although cholesterol cannot be incriminated in this latter case, it is possible that some change in the proteinic factor in the blood has occurred causing the same disturbance in the colloidal state of the plasma.

The fact that thorotrast particles in the foam cells are larger than those in the overlying endothelium need not invalidate the hypothesis presented, since foam cells may actively aggregate the particles in their voluminous protoplasm. Although these cells are heavily loaded with lipoid material, they still are capable of mitotic division(66) (this experiment).

The findings of the experiment reported here are in contradiction to Leary's views, as presented in his later writings(29). According to him the earliest evidence of lesions in atherosclerosis is the appearance of foam cells under the aortic endothelium. The earliest changes observed here, with the rare exception, consisted of intimal swelling, intercellular fat, and occasionally fat in the endothelial cells. Foam cells generally appear later in the development of the lesion. Leary believes foam cells are of the reticulo-endothelial system from the adrenal, spleen and liver, which become loaded with fat and take to the blood stream. Again, the findings of this experiment do not substantiate him. Macrophages loaded with

thorotrast, probably originating in the lung, were regularly seen in blood sections, in blood of lumen of vessels in various organs. However, this is not an unusual finding, since it is known that the injection of thorotrast acts as a great stimulator towards the reticulo-endothelial system because of the great tendency to accumulate in this tissue(105). It must be added that foam cells, recognizable as such, were never observed in the many sections carefully studied. Foam cells were never observed penetrating through the endothelium into the intima. The occasional foam cell appeared between two endothelial cells, but here the picture was more of the foam cell bursting from the subendothelial layers rather than penetrating the intima already crowded with foam cells.

The findings reported here invalidate the theory of Gordon(31) that foam cells are pushed out of the axial blood stream because of their lightness. The high specific gravity would cause the substance or cells carrying the substance to be carried away in the blood stream.

Thorotrast demonstrated as accumulating only in altered endothelium after a minimal period of cholesterol feeding is contrary to the findings of Okuneff(83), Glasunoff(84) and Anitschkow, but is in agreement with those of Duff(85). No particulate matter sufficiently large to be observed under oil immersion was found in the endothelium of normal aortae.

The significance of the predilection of thorotrast for endothelial and foam cells at the periphery of the lesions is not clear.

A possibility to be considered lies in the hemodynamic factor. Because of the protrusion of the tiny lesion above the endothelium of the aortic intima there is interference with the peripheral blood current.

The proximal surface of the plaque is exposed to the stronger pressure of the blood stream deflecting from it to continue around the plaque. The undersurface of the plaque, on the other hand, is exposed to eddying of the blood stream after it rounds the most protruding area of the plaque.

SUMMARY AND CONCLUSIONS

An experiment to study the deposition of colloidal thorium dioxide (Thorotrast) in the lesions of experimental cholesterol atherosclerosis in the rabbit has been presented. Particular stress was layed on the endothelial accumulation of the substance in very early lesions and the period just prior to the appearance of any change in the intima or media. A group of normal animals were given thorotrast in one, two and three injections at twenty-four hour intervals to determine if a "threshold" when normal endothelium would accumulate the substance could be arrived at. Studies were made of more advanced lesions to determine the predilection of the accumulation of thorotrast in the atherosclerotic plaques with relation to the direction of the blood current.

Blood sections taken at various intervals following thorotrast injection and imbedded in paraffin were studied.

The deposition of thorotrast in other organs and in particular the reticulo-endothelial system was briefly studied. The findings may be summarized as follows -

1. Normal animals exhibiting no microscopic changes of the aortic wall do not show any accumulation of thorotrast in the endothelium of the aortic intima.

2. Animals fed cholesterol for one or two weeks showed the accumulation of very minute particles of thorotrast in endothelial cells of the aortic intima.

3. With the very earliest changes, that is swelling of the ground substance of the intima, thorotrast was also demonstrable in the endothelial cells with or without demonstrable fatty infiltration. Methods used to determine the relationship of thorotrast deposition to the very early fatty infiltration were not entirely satisfactory. It is suggested that further studies of this problem are necessary.

4. The accumulation of thorotrast in the endothelial cells in the pre-atherosclerotic stages is restricted to very minute, and a minimal number of particles. As the lesion progresses the number and size of the thorotrast particles in individual cells increase.

5. Foam cells mainly in the superficial two or three layers directly under the endothelium accumulated thorotrast in large particles, breaking up the cytoplasm.

6. In many lesions, the thorotrast-laden foam cells were dispersed among others entirely free of the substance. In four cases there was a decided predilection of thorotrast accumulation at the periphery of the plaque, showing its presence in both the endothelium and the few foam cells directly under it.

7. Thorotrast is accumulated in intima undergoing changes accompanying spontaneous arteriosclerosis in the rabbit, but not in the same abundance as in foam cells of cholesterol produced lesions.

8. Two granulomatous lesions of the intima with giant cell formation showed thorotrast in intima and in fibroblast cells throughout the lesion.

9. Thorotrast demonstrated a predilection for the reticulo-endothelium system. Organs supplied with this tissue were the site of heavy deposits, while others remained relatively free.

10. Thorotrast was demonstrated in macrophages (resembling cells of the reticulo-endothelial system) in the circulating blood. These were found at various time intervals, but appeared in greater numbers from 18 to 24 hours after the injection of the substance. Monocytes of the circulating blood also contained thorotrast.

11. Blood in arteries and veins of the lungs constantly showed the presence of macrophages loaded with thorotrast. Where blood was left in the aortic lumen these cells were also found.

12. The macrophages with thorotrast in the circulating blood do not resemble the foam cells. When loaded with thorotrast it was impossible to study their cytoplasm but the occasional cell without particles of the substance showed a fat free cytoplasm. (No fat stains were done to substantiate this finding).

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APPENDIX A

PROTOCOLS

Animal No. O-57 M. Age: 2 mths. 1 wk.

Date	Day	Time	
23/11/50			Weighed 2.040 kgms.
			Bled for chemistry.
			No cholesterol given.
			Course: not remarkable.
			Thorotrast.
		10:15 a.m.	Given 7.5 ccs. i.v.
		3:15 p.m.	Specimen for paraffin section (blood clot).
24/11/50		10:15 a.m.	" " " " " "
24/11/50			Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.
Aorta: Negative.
Liver: Coccidiosis.
Spleen: Enlarged spleen.

Microscopic.

Fixatives: Formol 10%
Saline Formol
Zenker Formol

Imbedding: Paraffin
Frozen section.

Stains: H. & E.
Van Gieson Verhoeff
Sudan Red
Laidlaw.

Findings.

Lung: Considerable amount of thorotrast in alveolar cells. Numerous poly-nuclears in alveolar wall.

Spleen: Abundant deposit of thorotrast in reticulo-endothelial cells.

Adrenals: Few reticulo-endothelial cells with thorotrast. No thorotrast in parenchymal cells.

Aorta: No thorotrast in intima. Macrophages in blood with thorotrast. Thorotrast in periadventitial fat. No mitoses, no spontaneous atherosclerosis seen.

Heart: Coronaries negative save for one endothelial cell close to a swelling containing thorotrast. Few connective tissue cells also contain thorotrast between muscle fibres. No thorotrast in epicardium or endocardium.

Animal No. M-23 M. Age: 4 mths.

Date	Day	Time	
21/8/50			Weighed 2.592 kgms.
			Bled for chemistry.
			No cholesterol given.
			Course: not remarkable.
			Thorotrast.
21/8/50		12:00 a.m.	Given 7.5 ccs. i.v.
21/8/50		3:00 p.m.	Specimen for paraffin section (blood clot).
		7:00 p.m.	" " " " " "
		11:00 p.m.	" " " " " "
22/8/50		12:00 a.m.	Given 7.5 ccs. i.v.
		3:00 p.m.	Specimen for paraffin section (blood clot).
		6:30 p.m.	" " " " " "
23/8/50			Killed: hit on the back of the head.

Microscopic.

Fixatives:

Zenker Formol
Formol 10% (for blood)
Fixative 11

Imbedding:

Paraffin.

Stains:

H. & E.
Trichrome.

Findings.

Spleen: Considerable amount of thorotrast in the reticulo-endothelial cells. Very necrotic.

Lung: Considerable deposit of thorotrast in alveolar cells. Thickening of alveolar walls. White blood cell infiltration. Numerous macrophages loaded with thorotrast in vessels.

Aorta: No thorotrast in intima. Thorotrast in periadvential fat cells.

Animal No. M-24 M. Age: 4 mths.

Date	Day	Time	
21/8/50			Weighed 2.570 kgms.
			Bled for chemistry.
			No cholesterol given.
			Course: not remarkable.
			Thorotrast.
22/8/50		12:00 a.m.	Given 7.5 ccs. i.v.
		3:00 p.m.	Specimen for paraffin section (blood clot).
		7:00 p.m.	" " " " " "
23/8/50		12:00 a.m.	Given 7.5 ccs. i.v.
		3:00 p.m.	Specimen for paraffin section (blood clot).
		6:30 p.m.	" " " " " "
24/8/50			Died: Haemorrhage.

Microscopic.

Fixatives:

Formol 10%
Saline Formol
Zenker Formol
Bouin
Du Bosq Brésil.

Imbedding:

Paraffin.

Staining:

H. & E.
Trichrome
Van Gieson Verhoeff.

Findings.

Lung: Slight amount of thorotrast in alveolar cells. Numerous macrophages with thorotrast in the lumen of blood vessels. No thorotrast in endothelium of vessels.

Spleen: Moderate amount of thorotrast in reticulo-endothelial cells. Necrotic.

Adrenals: Slight deposit of thorotrast in reticuloendothelial cells. Very little thorotrast seen in connective tissue cells and in ground substance.

Aorta: Numerous macrophages in lumen of aorta.

Animal No. L-23 M. Age: 4 mths.

Date	Day	Time	
21/8/50			Weighed 4.13 kgms.
			Bled for chemistry.
			No cholesterol given.
			Course: not remarkable.
			Thorotrast.
22/8/50		12:00 a.m.	Given 12 ccs. i.v.
		4:30 p.m.	Specimen for paraffin section (blood clot).
		7:30 p.m.	" " " " " "
23/8/50		12:00 a.m.	Given 12 ccs. i.v.
		4:30 p.m.	Specimen for paraffin section (blood clot).
		6:00 p.m.	" " " " " "
24/8/50			Killed: found dead. No haemorrhage.

Gross Findings.

Moderate haematoma of soft tissues of neck. Slight peticteal haemorrhage around, but not into, right adrenal.

Adrenals: Very necrotic.

Microscopic.

Fixatives:

Zenker Formol
Formol 10% (for blood clots).
Bouin
Du Bosq Brésil

Imbedding:

Paraffin

Stains: H. & E.
Trichrome
Van Gieson Verhoeff

Findings:

Lung: Moderate amount of thorotrast in alveolar cells. Oedema. Very numerous macrophages.containing thorotrast in vessels (arteries and veins). No thorotrast seen in endothelium of vessels.

Spleen: Considerable amount of thorotrast in reticulo-endothelial cells. Very necrotic.

Aorta: Numerous macrophages with thorotrast in blood of lumen. Also some white blood cells contain thorotrast. No thorotrast seen in endothelium. No mitoses.

Animal No. L-24 M. Age: 4 mths.

Date	Day	Time	
21/8/50			Weighed 3.740 kgms.
			Bled for chemistry.
			No cholesterol given.
			Course: not remarkable.
			Thorotrast.
22/8/50		12:00 a.m.	Given 12 ccs. i.v.
		3:00 p.m.	Specimen for paraffin section (blood clot).
		6:00 p.m.	" " " " " "
23/8/50		12:00 a.m.	Given 12 ccs. i.v.
		4:30 p.m.	Specimen for paraffin section (blood clot).
		7:00 p.m.	" " " " " "
24/8/50		12:00 a.m.	Given 12 ccs. i.v.
		4:30 p.m.	Specimen for paraffin section (blood clot).
		7:00 p.m.	" " " " " "
25/8/50			Killed: Found dead.

Microscopic.

Fixatives:

Zenker Formol
Formol 10% (for blood clots)
Du Bosq Brésil
Bouin.

Imbedding: Paraffin.

Stains:

H. & E.
Trichrome.

Findings.

Lungs: Considerable amount of thorotrast in alveolar cells. Macrophages in the blood of arteries and veins. Few particles found in endothelium. Some long cells. loaded with thorotrast found in the blood of vessels.

Spleen: Abundant deposit of thorotrast in reticulo-endothelial cells. Very necrotic.

Liver: Considerable amount of thorotrast in Kupffer cells. Numerous Kupffer cells with thorotrast. Necrosis.

Adrenals: Abundant thorotrast in reticulo-endothelial cells, but only few granules in parenchymal cells..(artefact).

Kidney: Abundant thorotrast in glomerular endothelium with obstructing glomerular tufts.

Lymph Node: Thorotrast in reticulo-endothelial cells.

Aorta: Normal aorta does not show thorotrast in endothelium. One cell had a few granules on or in it. One section, however, with natural atherosclerosis, fibrosis of intima, and calcifications of media, showed thorotrast in endothelium beautifully.

Animal No. O-18 M. Age: 2 mths. 1 wk.

Date	Day	Time	
16/10/50			Weighed 1.857 kgms.
			Bled for chemistry.
16/10/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	7		Weighed 1.931 kgms.
			Bled for chemistry.
			Thorotrast.
		11 a.m.	Given 8 ccs. i.v.
			No specimen for paraffin section.
24/10/50	8		Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.
Liver: Coccidiosis.

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10%
Formol 10%
Bouin

Imbedding: Paraffin
Frozen sections.

Stains: H. & E.
Giemsa - Janner of blood clot.
Sharlack Red (fat stain) - not suitable
Van Gieson Verhoeff
Sudan Red
One section: haematoxylin xyline followed by one section Sudan in series.

Findings.

Lung:	Moderate deposit of thorotrast in alveolar cells.
	Macrophages in the vessel of lumen with thorotrast.
	Endothelium of vessels does not contain thorotrast.
	No atherosclerosis of pulmonary vessels.
Liver:	Slight amount of thorotrast in Kupffer cells.
	Thorotrast mainly in Kupffer cells.
Spleen:	Considerable amount of thorotrast in the reticulo-endothelial cells. Necrotic.
Pancreas:	Very little thorotrast in connective tissue cells.
Intestine:	No thorotrast seen.
Aorta:	No atherosclerosis; no foam cells; no swelling.
	No thorotrast in intima. Some thorotrast in connective tissue cells of peri-adventitial fat.

Animal No. O-21 M. Age: 2 mths. 1 wk.

Date	Day	Time	
16/10/50			Weighed 2.085 kgms.
			Bled for chemistry.
16/10/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	7		Weighed 2.238 kgms.
			Bled for chemistry.
			Thorotrast.
		11:00 a.m.	Given 8 ccs. i.v. in 3¼"
		3:30 p.m.	Specimen for paraffin section (blood clot).
		6:30 p.m.	" " " " " "
24/10/50	8		Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.
Aorta: Negative.

Microscopic:

Fixatives: Formol 10%
Saline Formol
Zenker Formol
Bouin

Imbedding: Paraffin
Frozen section

Staining: H. & E.
Sudan Red
Van Gieson Verhoeff.

Findings.

Lung: Slight deposit of thorotrast in alveolar cells. Thorotrast in macrophages in vessels. None in the bronchial epithelium.

Spleen: Moderate amount of thorotrast in reticulo-endothelial cells.

Liver: Moderate amount of thorotrast in Kupffer cells. Thorotrast found in macrophages in lumen of large vein; also in several indothelial cells. Some cells of parenchyma also contain thorotrast.

Aorta: (H.&E.) Thorotrast in endothelium at intervals. No swelling, no cellular proliferation, no foam cells. Internal elastica intact.

Sudan stain for fat:

There is a staining of endothelial cells, but it is not sufficiently clear to be conclusive.

Animal No. 0-22 M. Age: 2 mths. 1 wk.

Date	Day	Time	
16/10/50			Weighed 1.984 kgms.
			Bled for chemistry.
16/10/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	7		Weighed 2.116 kgms.
			Bled for chemistry.
			Thorotrast.
		11:00 a.m.	Given 8 ccs. i.v. in 3½ "
		3:30 p.m.	Specimen for paraffin section (blood clot).
		6:30 p.m.	" " " " " "
24/10/50	8		Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.
Aorta: Negative.

Microscopic.

Fixatives: Zenker Formol
Formol 10% (for blood)
Bouin

Imbedding: Paraffin
Frozen sections.

Stains: H. & E.
Sudan (for fat)
Van Gieson Verhoeff

Findings.

Lung: Moderate amount of thorotrast in alveolar cells.

Thorotrast in macrophages in arteries and veins.

No thorotrast seen in endothelium of vessels.

Liver: Moderate amount of thorotrast in Kupffer cells.

Aorta: (rolled - H. & E.)

Three or four endothelial cells with thorotrast.

Macrophages in lumen with thorotrast. One white blood cell has penetrated into the intima.

No atherosclerotic lesions.

Sudan stain: Not done. Accidentally immersed in Absolute Alcohol.

Animal No. O-23 M. Age: 2 mths. 1 wk.

Date	Day	Time	
16/10/50			Weighed 2.024 kgms.
			Bled for chemistry.
16/10/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	7		Weighed 2.271 kgms.
			Bled for chemistry.
			Thorotrast.
		11:00 a.m.	Given 8 ccs. i.v. in 3 "
		3:30 p.m.	Specimen for paraffin section (blood clot).
		6:30 p.m.	" " " " " "
24/10/50	8		Died during the night. Autolysis.

Gross Findings.

Eye: Negative.

Microscopic.

Fixatives: Zenker Formol
Formol 10%
Bouin

Imbedding: Paraffin
Frozen sections.

Stains:
H. & E.

Findings.

Lung:	Slight deposit of thorotrast in alveolar cells. Oedema. Thorotrast in macrophages in lumen of vessels, none in endothelium.
Liver:	Moderate deposit of thorotrast in Kupffer cells.
Aorta:	Only one endothelial cell seen with thorotrast. No atherosclerosis; thorotrast in periadventitial fat. No mitoses.

Animal No. 0-24 M. Age: 2 mths. 1 wk.

Date	Day	Time	
16/10/50			Weighed 2.185 kgms.
			Bled for chemistry.
16/10/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	7		Weighed 2.251 kgms.
			Bled for chemistry.
			Thorotrast.
		11:00 a.m.	Given 8 ccs. i.v. in 3¼".
		3:30 p.m.	Specimen for paraffin section (blood clot).
		6:30 p.m.	" " " " " "
24/10/50	8		Killed: given 5 ccs.Nembutal i.v.

Gross Findings.

Eye: Negative.
Aorta: Two very small patches of atherosclerosis in ascending portion.

Microscopic.

Fixatives:
Zenker Formol
Formol
Du Bosq Brésil (not useful)
Saline Formol 10%

Imbedding:
Paraffin
Frozen sections.

Stains: H. & E.
Sudan Red
Van Gieson Verhoeff

Findings.

Lung: Moderate deposit of thorotrast in alveolar cells. Thorotrast in vessels.

Liver: Slight deposit of thorotrast in Kupffer cells. Cloudy swelling.

Intestine: No thorotrast in wall. Numerous mitoses of epithelial lining.

Aorta: Spontaneous atherosclerosis with calcification of media and fibrosis of intima. Where fibrosis layer is thick and very compact, little or no thorotrast is found in endothelium, but where connective tissue proliferation is loosely knitted, thorotrast can be found in endothelium (6 or 7 cells). Also, a few cells containing only one or two granules. No mitoses. Some thorotrast in macrophages in lumen, some thorotrast in periadventitial fat.

Animal No. O-25 M. Age: 2 mths. 1 wk.

Date	Day	Time	
16/10/50			Weighed 1.640 kgms.
			Bled for chemistry.
16/10/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	14		Weighed 1.990 kgms.
			Bled for chemistry.
			Thorotrast.
		1 p.m.	Given 8 ccs. i.v.
		3 p.m.	Specimen for paraffin section (blood clot).
		6 p.m.	" " " " " "
	15	6 p.m.	" " " " " "
31/10/50	15		Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.
Aorta: Negative.

Microscopic.

Fixatives:
Formol 10%
Zenker Formol
Bouin
Saline Formol 10%

Imbedding:
Paraffin
Frozen sections

Stains: H. & E.
Sudan.
Van Gieson Verhoeff.

Findings.

Lung: Moderate amount of thorotrast in alveolar cells. Thorotrast in macrophages of vessels.

Spleen: Considerable deposit of thorotrast in reticulo-endothelial cells. Necrosis.

Liver: Moderate amount of thorotrast in Kupffer cells. Areas of necrosis.

Adrenals: Slight amount of thorotrast in reticulo-endothelial cells. Some thorotrast free in central veins.

Aorta: No atherosclerotic changes; one or two endothelial cells contain thorotrast; no mitoses. Some thorotrast in connective tissue cells of adventitia.

Sudan stain for fat: No fat in intima or endothelial cells. No thorotrast seen.

Animal No. 0-26 F. Age: 2 mths. 1 wk.

Date	Day	Time	
16/10/50			Weighed 1.995 kgms.
			Bled for chemistry.
16/10/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	14		Weighed 2.180 kgms.
			Bled for chemistry.
			Thorotrast.
		1 p.m.	Given 8 ccs. i.v.
		3 p.m.	Specimen for paraffin section (blood clot).
		6 p.m.	" " " " " "
	15	6 p.m.	" " " " " "
31/10/50	15		Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.
Aorta: Negative.

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10% (blood clots)
Bouin
Du Bosq Brésil

Imbedding: Paraffin
Frozen sections

Stains: H. & E.
Sudan (on frozen sections).

Findings.

Lungs: No swelling on alveolar wall. Moderate amount of thorotrast in alveolar cells. Thorotrast in macrophages and white blood cells in vessels.

Spleen: Abundant amount of thorotrast in reticulo-endothelial cells.

Liver: Moderate amount of thorotrast in Kupffer cells.

Adrenals: Thorotrast in reticulo-endothelial cells and free in sinuses.

Aorta: (H. & E.)

No changes. No thorotrast found in endothelium. No mitoses. Macrophages with thorotrast in the blood.

Sudan Red:

One swollen endothelial cell staining faintly for fat. No thorotrast seen: section thick.

Animal No. 0-27 F. Age: 2 mths. 1 wk.

Date	Day	Time	
16/10/50			Weighed 1.816 kgms.
			Bled for chemistry.
16/10/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	14		Weighed 2.09 kgms.
			Bled for chemistry.
			Thorotrast.
		1:00 p.m.	Given 8 ccs. i.v.
			No specimen for paraffin section (blood clot).
30/10/50	14	1:15 p.m.	Died of broken back.

Gross Findings.

Eye: Negative.
Aorta: Negative.

Microscopic:

Fixatives: Formol Saline 10%
Zenker Formol.

Imbedding: Paraffin
Gelatin
Frozen sections.

Stains: H. & E. (on all organs)
Sudan Red (frozen sections)) on Aorta.
Laidlow)

Findings.

Lung:	Slight deposit of thorotrast in alveolar cells. Some thorotrast in alveolar wall cells and in macrophages in vessels.
Liver:	No thorotrast seen. Pinpoint abscess.
Aorta:	No atherosclerosis, no thorotrast in endothelium.
Laidlaw:	The endothelium fails to demonstrate reticulum fibrils.
Fat:	Some swelling of the intimal ground substance staining faintly for fat.
Gelatin sections:	Stained for fat: negative.

Animal No. O-28 M. Age: 2 mths. 1 wk.

Date	Day	Time	
16/10/50			Weighed 1.832 kgms.
			Bled for chemistry.
16/10/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	14		Weighed 2.345 kgms.
			Bled for chemistry.
			Course: animal appeared ill before injection of thorotrast.
			Thorotrast.
		1 p.m.	Given 8 ccs. i.v.
30/10/50	14		Course: animal appeared ill - rapid respiration, ears and mouth cyanotic.
			Given 25,000 u. S.C. Penicillin.
			No specimen for paraffin section.
31/10/50	15		Found dead. Acute infection of the lungs.

Gross Findings.

Eye: Negative.
Aorta: Negative.
G.I. Tract: Bowel markedly dilated and had undergone complete autolysis.

Microscopic.

Paraffin sections: Inadvertently delayed.

Frozen sections for Sudan: Negative for aortic intima. No thorotrast seen.

Animal No. O-29 M. Age: 2 mths. 1 wk.

Date	Day	Time	
16/10/50			Weighed 1.976 kgms.
			Bled for chemistry.
16/10/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	14		Weighed 2.324 kgms.
			Bled for chemistry.
			Thorotrast.
		1 p.m.	Given 8 ccs. i.v.
		3 p.m.	Specimen for paraffin section (blood clot).
		6 p.m.	" " " " " "
31/10/50	15		Died in the morning. Hemorrhage.
			Autopsy performed immediately.

Gross Findings.

Eye: Negative.

Microscopic.

Fixatives: Formol
Zenker Formol
Saline Formol
Bouin

Imbedding: Paraffin
Frozen sections.

Stains: H. & E.
Sudan Red.

Findings.

Lung: Moderate deposit of thorotrast in alveolar cells. Thorotrast in macrophages in veins and arterial lumen.

Liver: Moderate amount of thorotrast in Kupffer cells. Thorotrast in Kupffer cells.

Spleen: Considerable amount of thorotrast in reticulo-endothelial cells.

Aorta: There is a very moderate "loosening" of intima. Endothelial cells contain thorotrast in groups of three and four cells at intervals. Numerous macrophages loaded with thorotrast in blood of lumen of aorta.

Sudan Red: no fat demonstrated in endothelium.

Animal No. M-41 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.907 kilos.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	20		Weighed 2.370 kgms.
			Bled for chemistry.
			Thorotrast.
		11 a.m.	Given 7.5 ccs. i.v. (marginal vein of ear).
		3 p.m.	Specimen for paraffin section (blood clot).
		7 p.m.	" " " " " "
		11 p.m.	" " " " " "
22/8/50	21		Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: No cholesterol deposits seen macroscopically.

Microscopic.

Fixatives:
Zenker Formol 9:1
Saline Formol 10%
Du Bosq Brésil

Imbedding:
Paraffin

Staining:
H. & E.
Trichrome (Masson)

Findings.

Lung: Considerable amount of thorotrast in alveolar cells. Vessels contain macrophages loaded with thorotrast.

Spleen: Considerable amount of thorotrast in reticulo-endothelial cells. Very necrotic.

Liver: Moderate amount of thorotrast in Kupffer cells.

Adrenals: Moderate amount of thorotrast in reticulo-endothelial cells. Some thorotrast free in sinuses.

Aorta: Several endothelial cells contain thorotrast granules. There is loosening and thickening of intima with proliferation of connective tissue cells. No foam cells are seen. A few connective tissue cells also contain fine particles of thorotrast.

Another section shows spontaneous arteriosclerosis with calcification of the media and fibrotic thickening of the intima. Here, where the intimal cells are loosened, thorotrast can be found in the connective tissue cells and in endothelium at intervals.

Animal No. M-42 F. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.578 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	20		Weighed 2.26 kgms.
			Bled for chemistry.
			Thorotrast.
	21	11 a.m.	Given 7.5 ccs. i.v.
		3 p.m.	Specimen for paraffin section (blood clot).
		6 p.m.	" " " " " "
23/8/50	22		Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.

Microscopic.

Fixatives:
Saline Formol 10%
Zenker Formol
Du Bosq Brésil
Fixative II - (has possibilities).

Imbedding:
Paraffin.

Stains:
H. & E.
Trichrome.

Findings:

Lung: Considerable amount of thorotrast in alveolar cells.

Thorotrast in macrophages in lumen of vessels: very abundant.

No thorotrast in endothelium of vessels.

Liver: Slight amount of thorotrast in Kupffer cells.

Spleen: Abundant deposit of thorotrast in reticulo-endothelial cells.

Aorta: One section shows swelling of intima with connective tissue proliferation, some endothelial cells contain thorotrast as well as some of the cells in the subendothelial layer. There is also an area of spontaneous arteriosclerosis.

Animal No. M-43 F. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.558 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	20		Weighed 2.285 kgms.
			Bled for chemistry.
			Thorotrast.
		11 a.m.	Given 7.5 ccs. i.v.
		3 p.m.	Specimen for paraffin section (blood clot)
		7 p.m.	" " " " " "
		11 p.m.	" " " " " "
22/8/50	21		Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative

Microscopic.

Fixatives:
Saline Formol 10%
Zenker Formol
Du Bosq Brésil.

Imbedding:
Paraffin.

Stains:
H. & E.
Trichrome
Van Gieson Verhoeff.

Findings.

Lung: Moderate amount of thorotrast in alveolar cells. Arteries and veins contain macrophages with thorotrast in lumen.

Spleen: Considerable amount of thorotrast in the reticulo-endothelial cells.

Aorta: There is swelling of the ground substance and loosening of tissues in the intima and internal media with some connective tissue proliferation in the intima. A very few endothelial cells contain tiny particles of thorotrast. There is also some in a few connective tissue cells.

Animal No. M-44 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.543 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	20		Weighed 2.230 kgms.
			Bled for chemistry.
			Thorotrast.
	21	12 a.m.	Given 7.5 ccs. i.v.
		3 p.m.	Specimen for paraffin section (blood clot).
		6 p.m.	" " " " " "
23/8/50	22		Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.

Microscopic.

Fixatives:
Saline Formol 10%
Zenker Formol
Fixative 11

Imbedding:
Paraffin
Frozen section
Gelatin.

Staining:

H. & E.
Trichrome
Van Gieson Verhoeff
Soudan Red and H. & E. in serial sections
Soudan Blue
Soudan Green
Laidlaw for Reticulin.

Findings.

Lung: Moderate amount of thorotrast in alveolar cells.

Macrophages with thorotrast in blood vessels.

Spleen: Considerable amount of thorotrast in reticulo-endothelial cells.

Liver: Considerable amount of thorotrast in Kupffer cells.

Thorotrast abundant in Kupffer cells and in peri-lobular spaces.

Aorta: Swelling with foam cells.

Thorotrast in some endothelial cells and in foam cells.

The Soudan Red and H. & E. serial stains do not show thorotrast in endothelium (sections too thick).

Laidlaw - Endothelium does not appear to have reticular fibrils surrounding it.

Animal No. M-45 F. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.827 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable
	20		Weighed 2.370 kgms.
			Bled for chemistry.
			Thorotrast.
		11 a.m.	Given 7.5 ccs. i.v.
		3 p.m.	Specimen for paraffin section (blood clot).
		7 p.m.	" " " " " "
		11 p.m.	" " " " " "
22/8/50	21		Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.

Microscopic.

Fixatives:
Saline Formol 10%
Zenker Formol
Fixative 8
" 15

Imbedding:
Paraffin.

Staining:
H. & E.
Trichrome.

Findings.

Lung: Slight deposit of thorotrast in alveolar cells. Thorotrast in macrophages in blood vessel lumen.

Liver: Moderate deposit of thorotrast in Kupffer cells and in some sinuses.

Spleen: Moderate deposit of thorotrast in reticulo-endothelial cells.

Adrenals: Cells of the reticulo-endothelial system contain thorotrast. Some cells of the clear zone also contain thorotrast.

Ovary: Very few cells in the follicles contain thorotrast. None in the stroma.

Aorta: Some swelling of aortic intima with few granules of thorotrast in the endothelium and in ground substance.

Animal No. Q-6 M. Age: 2 mths.

Date	Day	Time	
9/2/51			Weighed 3.108 kgms.
			Bled for chemistry.
12/2/51	0		Cholesterol feeding begun
			Course: not remarkable.
	21		Weighed 3.35 kgms.
			Bled for chemistry.
			Thorotrast.
		10:30 a.m.	Given 9 ccs. i.v.
		4:30 p.m.	Specimen for paraffin section (blood clot)
	22	9:30 a.m.	" " " " " "
6/3/51			Killed: given 5 ccs. Nembutal. i.v.

Gross Findings.

Eye: Negative.

Aorta: Negative. One small elevated spot in ascending branch of arch.

Microscopic.

Fixatives:

Zenker Formol
Saline Formol 10%
Formol 10% followed by Osmic Acid for fat - 24 hours
Formol 10% " " " " " Golgi - 6 days
Aoyama fixative.

Imbedding:

Paraffin
Frozen section.

Stains: H. P. S.
Van Gieson Verhoeff
Aoyama
Osmic acid for fat
Osmic acid for Golgi
Frozen section for H. & E. and Soudan IV in serial sections.
Reticulin stain-Laidlaw.

Findings:

Lung: There is a polynuclear infiltration of the alveolar wall. Some congestion. Slight deposit of thorotrast in alveolar wall cells. One thick walled vessel (artery) has five or six endothelial cells of the lining loaded with thorotrast. There are also macrophages with the substance in the lumen of the vessels.

Spleen: Abundant deposit of thorotrast in reticulo-endothelial system.

Liver: Slight deposit of thorotrast limited to Kupffer cells.

Adrenals: A few cells of the reticulo-endothelial network contain thorotrast.

Bone: Abundant thorotrast in reticulo-endothelial cells and fat cells. The hematopoetic cells remain free.

Aorta: H. & E. stain. Endothelium appears as a very flat single layer with dark staining nucleus applied immediately unto unbroken elastic. There are a number of these endothelial cells containing unmistakable thorotrast granules (very small in size). There is no swelling, no connective tissue proliferation. No foam cells are seen.

Aoyama: The Golgi apparatus of the cells of the media are stained but nothing appears in endothelial cells.

Osmic acid for fat: Where endothelial cell stains for fat, thorotrast can also be found occasionally.

Osmic acid for Golgi: Not suitable; too much precipitate.

Van Gieson Verhoeff: Internal elastic quite intact.

Frozen section-:

One frozen section was stained for fat and the one immediately following was stained with H. & E. in an attempt to find thorotrast in the cells staining for fat. The material was not adequate for thorough study. The sections appeared too thick although thorotrast could be suspected in the H. & E. stain.

No fat was visible in the endothelial cells.

Animal No. Q-7 M. Age: 2 mths.

Date	Day	Time	
9/2/51			Weighed 3.195 kgms.
			Bled for chemistry.
12/2/51	0		Cholesterol feeding begun
			Course: not remarkable
	21		Weighed 3.318 kgms.
			Bled for chemistry.
			Thorotrast.
	21	10:30 a.m.	Given 8.5 ccs. i.v.
		4:30 p.m.	Specimen for paraffin section (blood clot).
	22	9:30 a.m.	" " " " " "
6/3/51			Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.

Aorta: Negative.

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10%
Formol 10% followed by Osmic acid for fat
" " " " " " " Golgi.

Imbedding medium:
Paraffin blocks and frozen sections.

Stains: H. P. S.
Van Gieson Verhoeff
Aoyama
Osmic acid for fat
Osmic acid for Golgi
Frozen sections for H. & E. and Soudan IV in serial sections
Laidlaw for reticulin.

Findings.

Lung: Slight deposits of thorotrast in alveolar wall cells. Polynuclear infiltration. Thorotrast is seen in macrophages in lumen of arteries and veins.

Liver: Slight deposit of thorotrast limited to Kupffer cells. These also stain positive for fat with Soudan IV.

Spleen: Abundant deposits of thorotrast in reticulo-endothelial cells. These cells are loaded with fat as shown by Osmic acid stain.

Aorta: Swelling of intima and internal media. Swelling and bulging of endothelial cells. Some with thorotrast. These cells and the swollen intercellular ground substance stain + for fat with Sudan. Some foam cells also found containing granules of thorotrast.

Van Gieson Verhoeff stain shows elastica intact.

Aoyama: Some endothelial cells show granulations around nuclei but cannot affirm Golgi apparatus.

Sudan IV stains positive for fat in: swollen ground substances, endothelial cells and foam cells.

H. & E. on frozen section shows thorotrast in foam cells and endothelium.

Animal No. Q-8 M. Age: 2 mths.

Date	Day	Time	
9/2/51			Weighed 2.636 kgms.
			Bled for chemistry.
12/2/51	0		Cholesterol feeding begun.
	1		Course: refused food, loose stool, looked poor.
	21		Weighed 2.469 kgms.
			Bled for chemistry.
			Thorotrast.
		10:30 a.m.	Given 8.5 ccs. i.v.
		4:30 p.m.	Specimen for paraffin section (blood clot)
	22	9:30 a.m.	" " " " " "
7/3/51	23		Killed: given 5 ccs. nembutal i.v.

Gross Findings.

Eye: Negative.

Aorta: Negative.

Microscopic.

Fixatives:

Zenker Formol
Saline Formol 10%
Formol 10% followed by osmic acid for fat stain
" " " " " " 2% for Golgi.

Imbedding medium:

Paraffin blocks and frozen sections.

Stains: H. P. S.
Van Gieson Verhoeff
Aoyama
Osmic acid for fat
Osmic acid for Golgi apparatus
Frozen sections for H. & E. and Sudan IV in serial sections
Laidlaw- Reticulin stain.

Findings.

Lung: Moderate deposit of thorotrast in alveolar walls. Some oedema of walls with polynuclear infiltration. Macrophages with thorotrast in veins and arteries.

Liver: Considerable thorotrast in Kupffer cells.

Spleen: Abundant thorotrast in reticulo-endothelial cells. Hemorrhagic infiltration, oedema and necrosis.

Kidney: Thorotrast in endothelial cells of glomeruli and obstructing capillaries of the tufts. None found in tubules. Thorotrast is found in macrophages, in veins and arteries of Hilus. No thorotrast is found in endothelium of vessel lining.

Adrenals: Moderate thorotrast deposit in reticulo-endothelial cells. None seen in parenchymal cells.

Tongue: No atherosclerosis of vessels. No thorotrast. Some fatty infiltration of muscle. Only one connective tissue cell containing thorotrast is seen.

Heart: Coronary vessels are negative for atherosclerosis and no thorotrast is seen in endothelium. There is no trace of the substance in the endocardial lining although a clot in left ventricle contains many macrophages and some white blood cells with thorotrast.

Pituitary:
Thorotrast found in macrophages in vessels. Also in reticulo-endothelial cells between the parenchymal tissue. No fatty changes observed.

Brain: Is entirely negative for thorotrast deposit.

Lymph nodes:
Lymph tissue itself remains entirely free of thorotrast. There is surprisingly little in sinuses. Vessels and fat in the vicinity contain thorotrast.

Testicle: Thorotrast in macrophages in vessels; none in parenchyma.

Pancreas: No thorotrast is found in islet cells, acini or ducts. The rare connective tissue cell contains thorotrast. There is a large artery below pancreas; contains no thorotrast in its endothelium.

Digestive Tract:
Stomach- No thorotrast in mucosal cells. A few connective tissue cells in submucosa contain thorotrast. The same is true for the intestinal tract.

Skin: No thorotrast is found in any of the skin layers.

Note: The blood vessels of all organs were carefully examined for thorotrast in the endothelium but none was found.

Aorta: No changes observed in intima or media. No thorotrast found in endothelium. One area has spontaneous arterio-sclerosis but no thorotrast could be detected in the cells of this area. A section in the osmic acid for fat shows fat in the endothelial cells with thorotrast present in these. The frozen sections stained with Sudan however were negative.

Animal No. Q-9 F. Age: 2 mths.

Date	Day	Time	
9/2/51			Weighed 2.630 kgms.
			Bled for chemistry.
12/2/51	0		Cholesterol feeding begun.
			Course: not remarkable.
	21		Weighed 2.749 kgms.
			Bled for chemistry.
			Thorotrast.
		10:30 a.m.	Given 8.5 ccs. i.v.
		4:30 p.m.	Specimen for paraffin section (blood clot).
	22	9:30 a.m.	" " " " " "
7/3/51	23		Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.

Aorta A few fatty streaks on arch.

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10%
Formol 10% followed by Osmic acid 2% for fat - 24 hours
" " " " " " " " Golgi apparatus - 6 days

Aoyamas fixative.

Imbedding medium:
Paraffin blocks and frozen sections.

Staining: H. P. S.
Van Gieson Verhoeff
Aoyama
Osmic acid for fat
" " " Golgi apparatus
Frozen section for H. & E. followed by section for Sudan.
Laidlaw for reticulin.

Findings.

Lung: Slight deposit of thorotrast in alveolar wall cells. Thorotrast in macrophages in vessel lumen but none in endothelial lining.

Liver: Considerable thorotrast in Kupffer cells. The reticulo-endothelial cells stain for fat and are loaded with thorotrast.

Spleen: Abundant thorotrast in reticulo-endothelial cells which also stain positive for fat.

Thymus: Very little thorotrast found in reticulo-endothelial cells. None in lymphoid tissue.

Adrenals: Thorotrast in reticulo-endothelial cells; none seen in parenchymal cells. The vessel lumen contain macrophages with thorotrast.

Coronaries: Two coronary arteries are seen with atheroma. In one plaque, followed through a series of four sections, the thorotrast deposits chiefly in the peripheral foam cells and the endothelial cells overlying these while the central foam cells remain free of the substance. The second plaque has thorotrast in the foam cells scattered throughout the superficial half of the plaque.

Aorta: There is an arteriosclerotic plaque consisting mainly of connective tissue proliferation and giant cell formation although some foam cells can be seen. Thorotrast found in endothelium, fibroblastic cell and foam cell. At a branching off of an artery there is an elevated atheromatous plaque with thorotrast in endothelium and staining moderately for fat (Osmic acid).

Animal No. M-46 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.688 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	27		Weighed 2.321 kgms.
			Bled for chemistry.
			Thorotrast.
		2:30 p.m.	Given 7.5 ccs. i.v.
		5:00 p.m.	Specimen for paraffin section (blood clot)
		7:00 p.m.	" " " " " "
	28	10:00 a.m.	" " " " " "
29/8/50	28		Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.

Microscopic.

Fixatives:
Zenker Formol.

Imbedding:
Paraffin
Frozen Section.

Staining:
H. & E.
Trichrome
Van Gieson Verhoeff
Soudan Red and H. & E. in serial sections.

Findings.

Lung: Moderate deposit of thorotrast in alveolar cells. Thorotrast in monocytes in blood vessels.

Spleen: Considerable amount of thorotrast in reticulo-endothelial cells.

Liver: Slight deposit of thorotrast in Kupffer cells. Some autolysis.

Thymus: Very little thorotrast found: this in the connective tissue cells.

Adrenals: Slight deposit of thorotrast in the sinuses and in the reticulo-endothelial cells.

Kidney: Some thorotrast in the endothelial cells and in the capillary spaces of the glomerular tufts.

Aorta: The intima is thickened throughout. There is deposition of fat (Soudan Red^{+}) in intimal and medial intercellular spaces. Connective tissue cell proliferation in the intima. Some thorotrast in endothelial cells in areas (3-6 in a group) and some fine granules in connective tissue cells as well.

Animal No. M-47 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.531 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	27		Weighed 2.17 kgms.
			Bled for chemistry.
			Thorotrast.
		2:30 p.m.	Given 7.5 ccs. i.v.
		5:00 p.m.	Specimen for paraffin section (blood clot).
		7:00 p.m.	" " " " " "
	28	10:00a.m.	" " " " " "
29/8/50	28		Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10%
Fixative 9

Imbedding: Paraffin
Gelatin
Frozen sections.

Stains: Hematoxylin and Eosin
Scarlet Red)
Hematoxylin and Eosin) in series.
for gelatin frozen sections)

Findings.

Lung: Considerable thorotrast in alveolar walls. Thorotrast also seen in macrophages in blood vessels.

Spleen: Considerable cellular necrosis. Moderate deposit of thorotrast in the reticulo-endothelial cells.

Liver: Slight deposit of thorotrast in Kupffer cells. There are tiny intralobular collections of polynuclears and some infiltration of the portal triads. Fatty degenerations.

Aorta: No intimal change could be detected. No thorotrast found in endothelium. The Sudan stain is negative. H. & E. stain on frozen section followed by section with scarlet red stain was not suitable.

Animal No. M-49 F. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.572 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: refused food for four days.
	27		Weighed 2.00 kgms.
			Bled for chemistry.
			Thorotrast.
		2:30 p.m.	Given 7.5 ccs. i.v.
		5:00 p.m.	Specimen for paraffin section (blood clot).
		7:00 p.m.	" " " " " "
	28	10:00a.m.	" " " " " "
29/8/50	28		Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.

Microscopic.

Fixatives: Saline Formol 10%
Fixative 9.

Imbedding: Paraffin
Gelatin
Frozen sections

Stains: Hematoxylin and Eosin
Van Gieson Verhoeff
Scarlet Red)
Hematoxylin and Eosin) in series.
for gelatin frozen sections)

Findings.

Lung: Slight thorotrast deposit in alveolar walls as well as in macrophages in blood vessels.

Spleen: Considerable deposit of thorotrast in reticulo-endothelial cells. Necrotic.

Liver: Pseudo-tuberculosis. Thorotrast in considerable quantity in Kupffer cells, also some fine granular deposit in the parenchymal cells.

Aorta: Some swelling of the endothelial lining cells and intima. Some endothelial cells contain fine granules of thorotrast. One section of aorta contains blood in the lumen. Here numerous macrophages loaded with thorotrast can be found. The Sudan stains for fat show intercellular fat, some foam cells, but no thorotrast is seen. Section is too thick.

Animal No. M-50 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 2.052 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	27		Weighed 2.530 kgms.
			Bled for chemistry.
			Thorotrast.
		2:30 p.m.	Given 7.5 ccs. i.v.
		5:00 p.m.	Specimen for paraffin section (blood clot).
		7:00 p.m.	" " " " " "
	28	10:00a.m.	" " " " " "
29/8/50	28		Died of hemorrhage.

Gross Findings.

Eye: Negative.
Liver: Minute area of yellowish cream with soft cheesy centre; 10 or 12 in number on cut surface (oocysts.)

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10%
Fixative 9.

Imbedding: Paraffin
Ester wax
Frozen section

Stains: Hematoxylin and Eosin
Trichrome
Van Gieson Verhoeff
Scarlet Red)
Hematoxylin and Eosin) in series.
for frozen sections)

Findings.

Lung: Moderate deposit of thorotrast in alveolar wall. Thorotrast seen in macrophages in veins.

Spleen: Necrotic. Moderate deposit of thorotrast in the reticulo-endothelial cells and in sinuses.

Liver: Not examined.

Adrenals: No thorotrast seen.

Aorta: Some swelling and connective tissue proliferation with intercellular fat staining with Sudan Red. Only thorotrast as very rare granule seen in endothelium or intima.

Animal No. M-51 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.718 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	35		Weighed 2.365 kgms.
			Bled for chemistry.
			Thorotrast.
		11 a.m.	Given 7.5 ccs. i.v.
		2 p.m.	Specimen for paraffin section (blood clot).
		4 p.m.	" " " " " "
		6 p.m.	" " " " " "
	36	12 a.m.	" " " " " "
6/9/50			Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.
Liver: White irregular area along lower margin of gall bladder lobe.

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10%

Imbedding: Paraffin
Frozen sections

Stains: Hematoxylin and Eosin
Trichrome
Van Gieson Verhoeff
Scarlet Red

Findings.

Lung: Oedema. Moderate deposit of thorotrast in alveolar wall. Thorotrast found in macrophages in lumen of veins, but none found in arteries.

Spleen: Considerable thorotrast found in reticulo-endothelial cells. Necrotic.

Aorta: Some swelling of intima not staining for fat with Sudan Red. The H. & E. stain shows thorotrast free in fine granules in the intima and some in endothelial cells. There is connective tissue proliferation and some cells appear to be invading the intima from the media.

Animal No. M-52 F. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.697 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	35		Weighed 2.365 kgms.
			Bled for chemistry.
			Thorotrast.
		12:00 a.m.	Given 7.5 ccs. i.v.
		2:00 p.m.	Specimen for paraffin section (blood clot).
		4:00 p.m.	" " " " " "
		6:00 p.m.	" " " " " "
	36	12:30 a.m.	" " " " " "
6/9/50			Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10%
Fixative 3

Imbedding: Paraffin
Frozen section.

Stains: Hematoxylin and Eosin
Trichrome
Van Gieson Verhoeff
Scarlet Red

Findings.

Lung: Oedema. Moderate deposit of thorotrast in alveolar wall cells. There is also some accumulation in the macrophages of blood vessels, both arteries and veins.

Spleen: Considerable thorotrast accumulates in the reticulo-endothelial cells.

Necrosis marked.

Lymph Nodes: A very few granules in some reticulo-endothelial cells.

Aorta: Slight swelling of internal layer. The endothelium, normal in appearance, contains thorotrast in individual cells here and there. The swollen intercellular substance stains positive for fat with Sudan Red.

Animal No. M-53 F. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.897 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	35		Weighed 2.4 kgms.
			Bled for chemistry.
			Thorotrast.
		12:00 a.m.	Given 7.5 ccs. i.v.
		2:00 p.m.	Specimen for paraffin section (blood clot).
		4:00 p.m.	" " " " " "
		6:00 p.m.	" " " " " "
	36	1:00 p.m.	" " " " " "
6/9/50	36		Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative.

Microscopic:

Fixatives: Zenker Formol.
Saline Formol 10%.

Imbedding: Paraffin.
Frozen Sections.

Stains: Hematoxylin and Eosin.
Trichrome.
Laidlow.
Scarlet Red.

Findings.

Lung:	Moderate deposit of thorotrast in alveolar walls. Numerous macrophages loaded with thorotrast in vessels.
Spleen:	Considerable thorotrast accumulation. Necrosis.
Ovaries:	No thorotrast seen.
Lymph Nodes:	No thorotrast seen in reticulo-endothelial cells or the lymphoid tissue.
Aorta:	Blood in the lumen of aorta contains numerous macrophages loaded with thorotrast. There is intimal swelling and some foam cells are seen. Other cells of connective tissue aspect are swollen and appear to be transforming into foam cells. Some of these as well as some endothelial cells contain thorotrast.

Animal No. M-54 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.849 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	35		Weighed 2.445 kgms.
			Bled for chemistry.
			Thorotrast.
		12:00 a.m.	Given 7 ccs. i.v.
			Died suddenly after injection of thorotrast.

Gross Findings.

Eye: Negative.

Microscopic:

Fixatives: Zenker Formol.
Saline Formol.10%
Fixative 6.
Fixative 12.

Imbedding: Paraffin.
Frozen Sections.

Stains: Hematoxylin and Eosin.
Trichrome.
Van Gieson Verhoeff.
Scarlet Red.

Findings.

Lung:	No thorotrast found in alveolar walls. A few granules seen in the lumen of veins.
Spleen:	No thorotrast found.
Aorta:	Spontaneous arteriosclerosis. No thorotrast found.

Animal No. M-56 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.811 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	41		Weighed 2.681 kgms.
			Bled for chemistry.
			Thorotrast.
		11:00 a.m.	Given 7.5 ccs. i.v.
		2:00 p.m.	Specimen for paraffin section (blood clot).
		5:00 p.m.	" " " " " "
	42	5:00 p.m.	" " " " " "
12/9/50			Killed: given 4 ccs. Nembutal i.v.

Gross Findings.

Eye: Some cholesterol deposit in cornea 1+
Aorta: Atherosclerosis visible in sections cut.

Microscopic:

Fixatives: Zenker Formol.
Saline Formol 10%.
Fixative 2.

Imbedding: Paraffin.
Frozen Sections.

Stains: Hematoxylin and Eosin.
Trichrome
Van Gieson Verhoeff.
Scarlet Red.

Findings.

Lung: Thorotrast abundant in alveolar walls. Macrophages with thorotrast in lumen of vessels.

Spleen: Abundant deposits of thorotrast. Very little necrosis.

Aorta: Here, the atheromatous lesion is developed fitting the classical description. The intima is thickened with fat free in the swollen ground substance and in foam cells. There are some endothelial lining cells loaded with thorotrast just a little beyond the edge of the lesion. There appears too a predilection for the foam cells in the periphery of the lesions. Some endothelial cells are swollen and appear to contain fat. The section stained for fat showed no atheromatous change and failed to stain with Sudan Red.

Animal No. M-57. F. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.598 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	41		Weighed 2.521 kgms.
			Bled for chemistry.
			Thorotrast.
		11:00 a.m.	Given 7.5 ccs. i.v.
		2:00 p.m.	Specimen for paraffin section (blood clot).
		5:00 p.m.	" " " " " "
	42	5:00 p.m.	" " " " " "
12/9/50			Killed: given 4 ccs. Nembutal i.v.

Gross Findings.

Eye: Cholesterol in cornea 1+

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10%
Fixative 2.

Imbedding: Paraffin
Gelatin
Frozen Sections.

Stains: Hematoxylin and Eosin
Scarlet Red
Hematoxylin and Eosin for Frozen Sections. } in alternating sections.

Findings.

Lung:	Moderate thorotrast in alveolar walls. Numerous macrophages with thorotrast in veins.
Spleen:	Abundant thorotrast in reticulo-endothelial cells and sinuses. Necrosis.
Liver:	Moderate amount of thorotrast in Kupffer cells.
Aorta:	Small atheromatous lesions with foam cells containing thorotrast, but not universally. There are also intact endothelial cells with thorotrast. The internal elastica is intact but thickened. The lesions stain positive with Sudan Red.

Animal No. M-58 M.. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.953 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	41		Weighed 2.683 kgms.
			Bled for chemistry.
			Thorotrast.
		11:00 a.m.	Given 7.5 ccs. i.v.
		2:00 p.m.	Specimen for paraffin section (blood clot).
		5:00 p.m.	" " " " " "
	42	6:00 p.m.	" " " " " "
12/9/50			Killed: given 4 ccs. Nembutal i.v.

Gross Findings.

Eye: Faint trace of cholesterol deposits.

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10%
Fixative 2.
Fixative 16.

Imbedding: Paraffin
Frozen Sections.

Stains: Hematoxylin and Eosin
Trichrome
Van Gieson Verhoeff.
Scarlet Red.

Findings.

Lung:	Moderate amount of thorotrast in alveolar wall cells. Some macrophages with thorotrast in vessel lumen.
Spleen:	Considerable accumulation of thorotrast. Some necrosis.
Aorta:	Fully developed atherosclerosis. Thorotrast in endothelium and foam cells scattered throughout the plaque. The internal elastic is fragmented and takes the elastic stain poorly. No relation to the blood flow can be determined.

Animal No. M-59 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.717 kgms.
			Bled for chemistry
1/8/50	0		Cholesterol feeding begun
			Course: not remarkable
	41		Weighed 2.6 kgms.
			Bled for chemistry
			Thorotrast:
		11:00 a.m.	given 7.5 ccs. i.v.
		2:00 p.m.	specimen for paraffin section (blood clot)
		5:00 p.m.	" " " " " "
	42	6:00 p.m.	" " " " " "
12/9/50			Killed: given 4 cc. Nembutal i.v.

Gross findings:

Eye: Negative to cholesterol deposits

Microscopic:

Fixatives: Zenker formol
Saline formol 10%
Fixative 2

Embedding: Paraffin
Frozen sections

Staining: Hematoxylin and Eosin
Trichrome
Van Gieson Verhoeff
Scarlet Red

Findings:

Lungs:	Thorotrast in alveolar wall is abundant; almost equal to that usually seen in the spleen. Numerous macrophages in the blood of vessel lumen.
Spleen:	Moderate deposit of thorotrast. Considerable necrosis.
Liver:	Thorotrast in Kupffer cells in moderate quantity. Slight polynuclear infiltration.
Aorta:	Some swelling of the intima with a few foam cells, some containing thorotrast. There is also some accumulation in the endothelial lining at irregular intervals.

Animal No. M-60 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.729 kgms.
			Bled for chemistry.
1/8/51	0		Cholesterol feeding begun.
			Course: not remarkable.
	41		Weighed 2.686 kgms.
			Bled for chemistry.
			Thorotrast.
		11:00 a.m.	Given 7.5 ccs. i.v.
		2:00 p.m.	Specimen for paraffin section (blood clot).
		5:00 p.m.	" " " " " "
	43	11:00 a.m.	" " " " " "
13/9/50			Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Cholesterol deposit 1+

Microscopic:

Fixatives: Zenker Formol.
Saline Formol 10%
Fixative 2
Fixative 16.

Imbedding: Paraffin
Frozen Sections.

Stains: Hematoxylin and Eosin.
Trichrome
Aoyama
Scarlet Red
Hematoxylin and Eosin for Frozen Sections
in series.

Findings.

Lung:	Moderate thorotrast in alveolar wall. Macrophages containing thorotrast in arteries and veins.
Spleen:	Considerable thorotrast deposited. Moderate necrosis.
Aorta:	There is no evidence of change in the intima of the sections examined. There is no stainable fat in the intima. No thorotrast was found. Some peri-adventitial fat cells contain thorotrast.

Animal No. M-61 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.781 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding began.
			Course: not remarkable.
	41		Weighed 2.766 kgms.
			Bled for chemistry.
			Thorotrast.
		11:00 a.m.	Given 7.5 ccs. i.v.
		2:00 p.m.	Specimen for paraffin section (blood clot).
		5:00 p.m.	" " " " " "
	43	12:00 a.m.	" " " " " "
13/9/50			Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Negative to cholesterol deposits.

Microscopic:

Fixatives: Zenker Formol.
Saline Formol 10%
Fixative 2.

Imbedding: Paraffin.

Stains: Hematoxylin and Eosin.
Trichrome.

Findings.

Lung:	Considerable thorotrast in alveolar wall. Also some in macrophages in arteries and veins.
Spleen:	Considerable thorotrast accumulated in reticulo-endothelial cells and sinuses. Considerable necrosis.
Aorta:	Intima unchanged. No thorotrast in endothelium although numerous macrophages in the thorotrast are seen in the lumen.

Animal No. M-62 F. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.611 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	48		Weighed 2.686 kgms.
			Bled for chemistry.
			Thorotrast.
		12:30 a.m.	Given 8 ccs. i.v.
		2:30 p.m.	Specimen for paraffin section (blood clot).
		5:30 p.m.	" " " " " "
19/9/50	49		Found dead.

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10%

Imbedding: Paraffin
Frozen sections
Gelatin

Stains: Hematoxylin and Eosin
Trichrome
Van Gieson Verhoeff
Scarlet Red
Hematoxylin and Eosin for gelatin and frozen sections
) in series

Findings.

Lung: Oedema. Considerable thorotrast in alveolar wall cells. Macrophages with thorotrast in the lumen of both arteries and veins.

Aorta: No intimal changes in H. & E. stain. No thorotrast found in endothelium. In one frozen section stained with Sudan Red, there is some moderate swelling of the intimal ground substance, but this does not take the fat stain. No thorotrast could be seen. Section too thick.

Animal No. M-63 Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.924 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
	47		Course: loose stool.
	48		Weighed 2.383 kgms.
			Bled for chemistry.
			Thorotrast.
		12:30 p.m.	Given 7.5 ccs. i.v.
		2:30 p.m.	Specimen for paraffin section (blood clot).
		5:30 p.m.	" " " " " "
	49	2:00 p.m.	" " " " " "
19/9/50			Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Cholesterol deposits ++

Aorta: Atherosclerosis ++

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10%

Imbedding:
Paraffin
Frozen sections

Stains: Hematoxylin and Eosin
Trichrome
Laidlaw
Scarlet Red
Sudan Red
Sudan Green
Sudan Blue
Sudan Green with H. & E.

Findings.

Eye: Thorotrast seen in any tissues of this organ.

Lung: Moderate deposit of thorotrast in alveolar wall. Thorotrast seen in mononuclear cells and macrophages in lumen of blood vessels.

Spleen: Abundant thorotrast seen in reticulo-endothelial cells. Not much necrosis.

Aorta: The H. & E. shoed atheromatous lesions of intima with foam cells several layers thick. Thorotrast tends to deposit in cells of the superficial layers to a greater degree, but some cells in the deepest layers also show an accumulation of thorotrast. One section shows a smaller lesion with thorotrast accumulating in the foam cells and endothelial cells at the periphery of the lesion. There is distinctly less deposit of the substance in the endothelium and the foam cells more centrally placed. Sudan Green stains fat well, but does not help in looking for thorotrast.

Sudan Blue - the same is true as for Sudan Green.

Serial sections of H. & E. alternating in the fat stain are not useful. Some endothelial cells in situ, stained deeply for fat.

Animal No. M-64 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.915 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	48		Weighed 2.735 kgms.
			Bled for chemistry.
			Thorotrast.
		12:30 p.m.	Given 8 ccs. i.v.
		2:30 p.m.	Specimen for paraffin section (blood clot).
		5:30 p.m.	" " " " " "
	49	2:00 p.m.	" " " " " "
19/9/50			Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Cholesterol deposit in faint traces.
Aorta: Atherosclerosis +

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10%

Imbedding: Paraffin
Frozen sections.

Stains: Hematoxylin and Eosin
Trichrome
Laidlaw
Scarlet Red

Findings.

Lung: Abundant thorotrast in alveolar wall. Macrophages with thorotrast in lumen of both arteries and veins.

Spleen: Moderate deposits of thorotrast. Considerable necrosis.

Aorta: Spontaneous arteriosclerosis. Some swelling of intima with considerable fibroblastic proliferation causing marked thickening of the intima. The endothelial lining cells are intermittently loaded with thorotrast. There is associated atherosclerosis in the form of foam cells in the intima. These foam cells also contain thorotrast. No thorotrast is seen in endothelium overlying the normal areas.

Animal No. M-65 F. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.749 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	48		Weighed 2.511 kgms. Bled for chemistry.
			Thorotrast.
		12:30 p.m.	Given 8 ccs. i.v.
		2:30 p.m.	Specimen for paraffin section (blood clot).
		5:30 p.m.	" " " " " "
	49	2:00 p.m.	" " " " " "
19/9/50			Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: No cholesterol deposits in eyes.
Aorta: No atherosclerosis.

Microscopic.

Fixatives:
Zenker Formol
Saline Formol 10%

Imbedding:
Paraffin
Gelatin
Frozen sections.

Stains: Hematoxylin and Eosin
Trichrome
Laidlaw
Scarlet Red)
Hematoxylin and Eosin for) in series
gelatin and frozen sections)
Scarlet Red stain and H. & E.
stain on alternate sections in series.

Findings.

Lung: Considerable thorotrast in alveolar wall. Numerous macrophages loaded with thorotrast in veins, much less in arteries. No thorotrast in endothelium lining vessels or bronchial epithelium.

Liver: Thorotrast in Kupffer cells. Considerable diffuse polynuclear infiltration.

Spleen: Considerable thorotrast accumulated. Very necrotic.

Aorta: No intimal changes seen. No thorotrast found in any endothelial cells.

Animal No. M-66 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.842 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	56		Weighed 2.845 kgms.
			Bled for chemistry.
			Thorotrast.
		2:00 p.m.	Given 10 ccs. i.v.
		5:30 p.m.	Specimen for paraffin section (blood clot).
27/9/50	57		Killed: given 5 ccs. of Nembutal i.v.

Gross Findings.

Eye: Cholesterol deposit +
Aorta: No gross atherosclerosis.

Microscopic.

Fixatives: Zenker Formol
Saline Formol
Fixative 16

Imbedding: Paraffin
Frozen sections

Stains: Hematoxylin and Eosin
Trichrome
Van Gieson Verhoeff
Scarlet Red

Findings.

Lung: Considerable thorotrast in alveolar walls. None observed in bronchial epithelium.

Spleen: Abundant thorotrast deposit. Marked by polynuclear infiltration.

Aorta: There is no intimal change. The fat stains are negative. No thorotrast is found in endothelial lining except for a single flat cell which does contain accumulated particles of thorotrast.

Animal No. M-67 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.894 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	56		Weighed 3.073 kgms.
			Bled for chemistry.
			Thorotrast.
		2:00 p.m.	Given 10 ccs. i.v.
		2:30 p.m.	Specimen for paraffin section (blood clot).
		5:30 p.m.	" " " " " "
	57	2:30 p.m.	" " " " " "
27/9/50			Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Aorta: No macroscopic lesions present.

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10%
Fixative 16.

Imbedding: Paraffin
Frozen sections

Stains: Hematoxylin and Eosin
Trichrome
Scarlet Red

Findings.

Lung: Considerable thorotrast in alveolar walls. There are a few tiny particles of thorotrast in the bronchial epitheliums. The macrophages in the arteries and veins contain thorotrast.

Spleen: Abundant thorotrast deposits in necrotic masses infiltrated with numerous red blood cells.

Liver: Thorotrast in Kupffer cells. Considerable diffuse polynuclear infiltration.

Aorta: No intimal changes. No thorotrast seen in endothelial cells. There are a few cells of the periadventitial fat loaded with thorotrast.

Animal No. M-68 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.775 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	56		Weighed 2.985 kgms.
			Bled for chemistry.
			Thorotrast.
		2:00 p.m.	Given 10 ccs. i.v.
		2:30 p.m.	Specimen for paraffin section (blood clot).
		5:30 p.m.	" " " " " "
	57	2:30 p.m.	" " " " " "
27/9/50			Killed: given 5 ccs. Nembutal i.v.

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10%
Fixative 16.

Imbedding: Paraffin
Gelatin
Frozen sections.

Stains: Hematoxylin and Eosin
Trichrome
Van Gieson Verhoeff
Scarlet Red
H. & E. for frozen sections and gelatin
) in series.

Findings.

Lung: Slight deposit of thorotrast in alveolar walls.

Spleen: Abundant deposit of thorotrast. The capsule remains free of any penetration of the substance.

Aorta: Atherosclerotic flecks show microscopically as foam cells one or two layers thick under the endothelium. Thorotrast can readily be seen in some foam cells and in what appear as normal endothelial cells. There appears to be no difference which could be attributed to the effect of the flow of blood.

Animal No. M-69 M. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.555 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	56		Weighed 2.540 kgms.
			Bled for chemistry.
			Thorotrast.
		2:00 p.m.	Given 5 ccs. i.v.
		2:30 p.m.	Specimen for paraffin section (blood clot).
		5:30 p.m.	" " " " " "
	57	2:30 p.m.	" " " " " "
27/9/50			Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: traces of cholesterol deposits.
Aorta: traces of atherosclerosis.

Microscopic.

Fixatives: Zenker Formol
Saline Formol 10%
Fixative 16

Imbedding: Paraffin.

Stains: Hematoxylin and Eosin
Trichrome
Van Gieson Verhoeff
Scarlet Red

Findings.

Lung: Moderate deposit of thorotrast in alveolar walls. Some macrophages and a few polynuclears containing thorotrast are seen in the lumen.of the blood vessels. No thorotrast can be seen in the epithelium of the bronchi .

Spleen: Abundant thorotrast accumulation.

Aorta: (Poorly fixed) No atheromatous plaques. No thorotrast in endothelial lining or in entire intima. There are a considerable number of cells in the periadventitial fat containing thorotrast. The fat stains are negative.

Animal No. M-70 F. Age: 2 mths. 1 wk.

Date	Day	Time	
27/7/50			Weighed 1.66 kgms.
			Bled for chemistry.
1/8/50	0		Cholesterol feeding begun.
			Course: not remarkable.
	56		Weighed 2.92 kgms.
			Bled for chemistry.
			Thorotrast.
		2:00 p.m.	Given 10 ccs. i.v.
		2.30 p.m.	Specimen for paraffin section (blood clot).
		5.30 p.m.	" " " " " "
	57	2.00 p.m.	" " " " " "
27/9/50			Killed: given 5 ccs. Nembutal i.v.

Gross Findings.

Eye: Cholesterol deposit 1+

Aorta: Diffuse streaks, more marked on posterior surface.

Microscopic:

Fixatives: Zenker Formol.
Saline Formol 10%
Fixative 16.

Imbedding: Paraffin
Frozen sections.

Staining: Hematoxylin and Eosin.
Trichrome
Van Gieson Verhoeff.
Scarlet Red.

Findings.

Lung: Slight accumulation of thorotrast in alveolar wall cells. Thorotrast also found in macrophages in lumen of vessels but none found in endothelial lining of vessels or in bronchial epithelium.

Spleen: Considerable thorotrast deposit, very necrotic.

Aorta: Atheromatous lesions present. The foam cells in some areas are up to 14 layers deep. They are covered by a layer of fibroblasts under the endothelium. Here the thorotrast is definitely localized in the superficial layers being found as frequently among fibroblasts as among foam cells. In a microscopic field (oil immersion) focused on the superficial layers, approximately 3-4 cells have accumulated thorotrast. The endothelial cells are swollen and contain thorotrast granules in their protoplasm. (3 out of every 4 cells over some areas of the plaque). One section has been marked with India ink - but no relation to the blood flow can be determined. These mitotic figures are seen in one section. This animal also received Trypan Blue and deposition of the dye runs paralled to that of thorotrast. The atheromatous areas stain well for fat with Sudan Red.

APPENDIX B

CHEMISTRY

MICROMETHODS USED IN THE DETERMINATION OF FREE AND TOTAL CHOLESTEROL PHOSPHOLIPIDS AND FATTY ACIDS ON BLOOD SERUM.

Free and Total Cholesterol:

Method by Schoenheimer and Sperry modified by Sperry.

References:

Schoenheimer, R., and Sperry, D.W.: A Micromethod for the Determination of Free and Combined Cholesterol. J. Biol. Chem., 106: 745, 1934.

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

Phospholipid:

Method by Youngburg and Youngburg, modified by Hawk, Oser and Summerson who employed the procedure of Fisk and Sunbarrow.

References:

Youngburg, G.E., and Youngburg, M.V.: Phosphorous Metabolism; system of blood phosphorus analysis. J. Lab. & Clin. Med., 16: 138, 1930.

Hawk, P.B., Oser, B.L., and Summerson, W.H.: Practical Physiological Chemistry, Philadelphia, The Blakiston Co. 12th edition, 1947, Chap. 23.

Fisk, C.H., and Sunbarrow, Y.: The Colorimetric Determination of Phosphorus. J. Biol. Chem., 66: 375, 1925.

Fatty Acids:

Method by Stoddard and Drury modified by Man and Gildea.

Micromethods - Cont'd.

References:

Stoddard, J.L., and Drury, P.E.: A Titration Method for Blood Fat.
J. Biol. Chem., 84: 741, 1929.

Man, E.B., and Gildea, E.F.: A Modification of the Stoddard and Drury Titrametric Method for the Determination of the Fatty Acids in Blood Serum.
J. Biol. Chem., 99: 43, 1932.

Formula used in the calculation of Fatty Acids of Neutral Fat:

$$N.F. = F.A. - \left[(P \times 0.58) + \frac{(E \times 10)}{386}\right]$$

N.F. = Neutral fat in milliequivalents

F.A. = Fatty Acids in milliequivalents

P. = Phospholipids in mgms.%

E. = Cholesterol Ester in mgms.%

Average values for normal rabbits:-

M - 41 - 70)	Free cholesterol	6.9 mgms.%
)		
O - 18 - 29)	Total cholesterol	53.35 "
	Phospholipids	9.65 "
	Fatty Acids	12.27 m.eq.
	Neutral Fat	4.88 "
L - 23 - M - 24)	Free cholesterol	25.7 mgms.%
)		
Q - 6 - 9)	Total cholesterol	71.75 "
	Phospholipids	10.13 "
	Fatty Acids	17.46 m.eq.
	Neutral Fat	10.41 "

Micromethods - Cont'd.

Digitonin solution (in alcohol) was used in the determination of normal values for free and total cholesterol on sera of Q 6 - Q 9. Previously, digitonin in aqueous solution was used. The results, however, are comparable with those of the previous groups.

GROUP I

NORMAL

Animal No.	Sex	Weight Initial	Weight Final	Cholesterol consumed gms.	Cholesterol eyes	Lipemia	Cholesterol Free mgm.%	Cholesterol Total mgm.%	Cholesterol Ester mgm.%	Phospholipids mgm.%	Fatty Acids m.eq.	Neutral Fat m.eq.
0-57	M		2040	0	0	-	9	52	43	9.9	10	3.16
M-23	M		2592	0	0	±	24	55	31	12	31	23.24
M-24	M		2570	0	0	-	22	48	26	7.6	10	5.02
L-23	M		4130	0	0	-	39	90	51	12.25	22.5	14.08
L-24	M		3740	0	0	±	34	100	66	11.25	22.5	14.27

GROUP II

1 WEEK

Animal No.	Sex	Weight Initial	Weight Final	Cholesterol consumed gms.	Cholesterol eyes	Lipemia	Cholesterol Free mgm.%	Cholesterol Total mgm.%	Cholesterol Ester mgm.%	Phospholipids mgm.%	Fatty Acids m.eq.	Neutral Fat m.eq.
0-18	M	1857		3.75	0	-	8	70	62	11	15	7.01
			1931		0	-	6	64	58	10.1	15	7.64
0-21	M	2085		5		-	3	50	47	8.4	12.5	6.41
			2238		0	-	69	310	241	15.5	25	9.76
0-22	M	1984		4.90		-	3	30	27	11.25	12.5	5.28
			2116		0	-	26	156	130	17.2	22.5	9.15
0-23	M	2024		4.94		-	3	30	27	9.5	10	3.79
			2271		0	-	30	254	224	14.5	25	10.80
0-24	M	2185		6		-	6	64	58	12.3	15	6.37
			2251		0	-	40	270	230	14.6	17.5	3.07

GROUP III

2 WEEKS

Animal No.	Sex	Weight Initial	Weight Final	Cholesterol consumed gms.	Cholesterol eyes	Lipemia	Cholesterol Free mgm.%	Cholesterol Total mgm.%	Cholesterol Ester mgm.%	Phospholipids mgm.%	Fatty Acids m.eq.	Neutral Fat m.eq.
0-25	M	1640		8.88		-	6	60	54	10.1	12.5	5.24
			1990		0	-	100	330	230	13	20	6.50
0-26	F	1995		9.80		-	25	54	29	12.25	17.5	3.65
			2180		0	-	60	168	108	12	17.5	7.74
0-27	F	1816		10		-	6	64	58	8.9	15	4.69
			2090		0	+	175	790	615	23	42.5	13.23
0-29	M	1976		10		-	27	90	63	8.3	15	8.56
			2324		0	-	60	202	142	12.25	17.5	6.72

GROUP IV

3 WEEKS

Animal No.	Sex	Weight Initial	Weight Final	Cholesterol consumed gms.	Cholesterol eyes	Lipemia	Cholesterol Free mgm.%	Cholesterol Total mgm.%	Cholesterol Ester mgm.%	Phospholipids mgm.%	Fatty Acids m.eq.	Neutral Fat m.eq.
M-41	M	1907		15		-	50	168	118	10.7	12.5	3.29
			2370		0	±	135	366	231	22.6	32.5	13.42
M-42	F	1578		16		-	10	60	50	10.1	10	2.85
			2260		0	-	150	588	438	21.5	32.5	8.68
M-43	F	1758		15		-	3	50	47	9.3	12.5	5.89
			2285		0	±	50	140	90	10.5	17.5	9.08
M-44	M	1543		16		-	3	50	47	9.3	7.5	0.89
			2230		0	±	260	860	600	24.5	40	6.35
M-45	F	1827		15		-	7	54	47	9.7	7.5	0.65
			2370		0	-	32	80	48	7.5	12.5	6.51

GROUP V

3 WEEKS

Animal No.	Sex	Weight Initial	Weight Final	Cholesterol consumed gms.	Cholesterol eyes	Lipemia	Cholesterol Free mgm.%	Cholesterol Total mgm.%	Cholesterol Ester mgm.%	Phospholipids mgm.%	Fatty Acids m.eq.	Neutral Fat m.eq.
Q-6	M	3108		19.6			10	30	20	8.1	12.5	7.28
			3550		0	+++	309	1240	931	28	40	0
Q-7	M	3195		19.95			27	70	43	10.9	15	7.57
			3318		0	++	480	1800	1320	51	60	27.02
Q-8	M	2636		9.81			27	110	83	9.5	13.75	6.09
			2469		0	++	175	656	481	25.5	35	7.81
Q-9	F	2630		19.85			23	70	47	9.5	12.5	5.77
			2749		0	+++	355	1000	645	16.1	47.5	12.13

GROUP VI

4 WEEKS

Animal No.	Sex	Weight Initial	Weight Final	Cholesterol consumed gms.	Cholesterol eyes	Lipemia	Cholesterol Free mgm.%	Cholesterol Total mgm.%	Cholesterol Ester mgm.%	Phospholipids mgm.%	Fatty Acids m.eq.	Neutral Fat m.eq.
M-46	M	1688		20.29		-	7	34	47	8.5	8.75	3.13
			2321		0	-	280	380	400	23.5	32.5	8.51
M-47	M	1531		20		-	15	54	39	9.3	8.75	2.35
			2130		0	-	7	70	63	11.5	17.5	8.75
M-49	F	1572		17.7		-	15	50	35	7.5	7.5	2.24
			2000		0	-	10	40	30	7.5	17.5	12.37
M-50	M	2052		21.0		-	25	88	63	9.9	10	2.89
			2530		0	-	226	588	362	25	27.5	3.62

GROUP VII

5 WEEKS

Animal No.	Sex	Weight Initial	Weight Final	Cholesterol consumed gms.	Cholesterol eyes	Lipemia	Cholesterol Free mgm.%	Cholesterol Total mgm.%	Cholesterol Ester mgm.%	Phospholipids mgm.%	Fatty Acids m.eq.	Neutral Fat m.eq.
M-51	M	1718		25.91		-	15	54	39	9.9	10	3.25
			2365		0	-	80	316	236	17.6	27.5	11.19
M-52	F	1697		25.92		-	15	54	39	9.5	8.75	2.23
			2260		0	-	64	316	252	18.75	27.5	10.10
M-53	F	1897		26		-	7	60	53	11.5	12.75	4.71
			2400		0	-	90	296	206	16.9	25	19.66
M-54	M	1849		22		-	10	60	50	9.5	12.5	5.69
			2445		0		20	80	60	20.7	33	19.45

GROUP VIII

6 WEEKS

Animal No.	Sex	Weight Initial	Weight Final	Cholesterol consumed gms.	Cholesterol eyes	Lipemia	Cholesterol Free mgm.%	Cholesterol Total mgm.%	Cholesterol Ester mgm.%	Phospholipids mgm.%	Fatty Acids m.eq.	Neutral Fat m.eq.
M-56	M	1811		24.88		-	5	70	65	5.4	12.5	7.69
			2681		1	±	244	624	380	20	35	13.56
M-57	F	1598		28.93		-	15	148	133	7	8.75	1.25
			2521		1	++	313	1508	1195	37.5	55	2.24
M-58	M	1983		30		-	30	130	100	8.4	13.75	6.29
			2683		tr	-	70	356	286	19	29.2	10.74
M-59	M	1717		29.70		-	10	60	50	9.9	12.5	5.46
			2600		0	+	178	600	422	21.5	32.5	9.1
M-60	M	1729		29.91		-	30	86	56	11.5	17.5	9.38
			2686		1	++	440	928	488	26	45	17.36
M-61	M	1781		29.97		-	24	62	38	12	12.5	4.56
			2766		0	±	440	908	468	20	35	11.28

GROUP IX

7 WEEKS

Animal No.	Sex	Weight Initial	Weight Final	Cholesterol consumed gms.	Cholesterol eyes	Lipemia	Cholesterol Free mgm.%	Cholesterol Total mgm.%	Cholesterol Ester mgm.%	Phospholipids mgm.%	Fatty Acids m.eq.	Neutral Fat m.eq.
M-62	F	1641		34.96		-	5	20	15	4.25	12.5	9.65
			2686		0	-	104	428	324	18.75	27.5	8.24
M-63		1924		30.19		-	15	48	33	7.5	10	4.8
			2383		2	+++	504	2060	1556	52	85	14.53
M-64	M	1915		33.93		-	25	118	93	9	5	0
			2735		tr	-	72	306	234	16.6	30	14.32
M-65	F	1749		33.86		-	5	20	15	7.5	10	5.26
			2511		0	-	55	296	241	13.8	27.5	15.85

GROUP X

8 WEEKS

Animal No.	Sex	Weight Initial	Weight Final	Cholesterol consumed gms.	Cholesterol eyes	Lipemia	Cholesterol Free mgm.%	Cholesterol Total mgm.%	Cholesterol Ester mgm.%	Phospholipids mgm.%	Fatty Acids m.eq.	Neutral Fat m.eq.
M-66	M	1842		38.23		-	7	20	13	8.5	10	4.73
			2845		1	+	52	280	228	15	27.5	12.89
M-67	M	1894		39.65		-	10	20	10	7.2	7.5	3.17
			3073		0	+	134	540	406	21.5	27.5	4.52
M-68	M	1775		40		-	10	54	44	6.8	12.5	7.42
			2985		1	-	52	240	188	12.5	17.5	5.48
M-69	M	1555		37.31		-	7	14	7	5	10	6.84
			2400		tr	++	152	500	348	15.2	22.5	4.70
M-70	F	1660		39.71		-	10	54	44	10.4	13.75	6.58
			2920		1	+++	400	1580	1180	22.2	55	11.55

APPENDIX C

TECHNIQUES.

APPENDIX C

AORTA: Zenker-formol fixation.

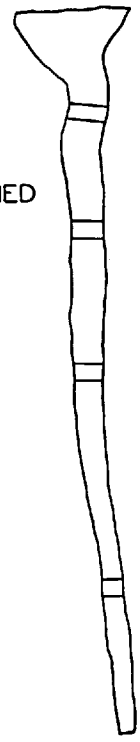
Figs. 1 - 4

Illustrations of levels at which sections were taken and method of fixation.

SECTIONS FOR ZENKER-FORMOL FIXATION

GROUPS I IV

AORTA NOT OPENED

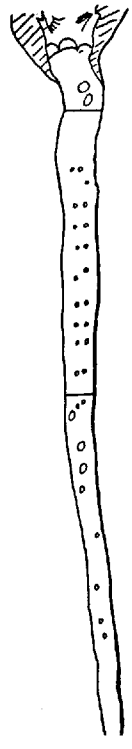


1

SECTIONS FOR ZENKER-FORMOL FIXATION

GROUPS II III V

AORTA ROLLED

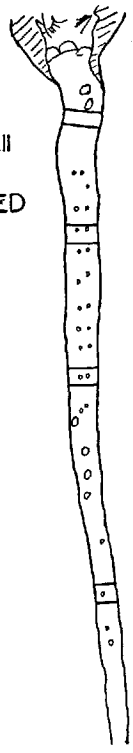


2

SECTIONS FOR ZENKER-FORMOL FIXATION

GROUPS VI VII VIII

AORTA OPENED

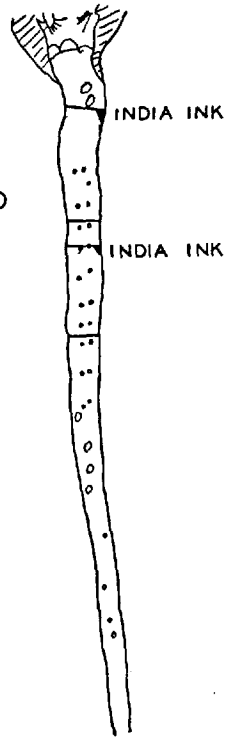


3

SECTIONS FOR ZENKER-FORMOL FIXATION

GROUPS IX X

AORTA OPENED



4

FIXATIVES USED IN EXPERIMENT:

Zenker Formol (stock solution)

Saline Formol 10%

Formol 10%

1. Bouin (modification of Berger)

Picric acid sat. sol.....	88 cc.
Formol pure	330 cc.
Trichloracetic acid 10%	26.5 cc.

2. Picroalcohol-Chloroform

Picric acid aqueous sat. sol.....	2 parts
Alcohol Abs	1 part
Chloroform	1 part

3. Du Bosq - Brazil (modification of Berger)

Alcohol 70%	300 cc.
Picric acid	2 gm.
Formol (neutral) pure	100 cc.
Trichloracetic acid 10%	6 cc.

5. Bouin (stock solution)

6. Formol-chloralhydrate-alcohol

Formol (neutral).....	15 cc.
Chloralhydrate	15 cc.
Alcohol 70%	300 cc.

8.

Alcohol	100
Chloroform	60
Formol (neutral) ..	60
Acetic acid	30

9.

Alcohol Abs	270 cc.
Trichloracetic acid 10% ..	40 cc.
Formol (neutral)	90 cc.

11.

Alcohol	75 cc.
Trichloracetic acid (in alcohol)10%	75 cc.
Chloroform	75 cc.
Mercury bichloride	5 gm.

Fixatives used - Cont'd.

12. Du Bosq - Brazil

Alcohol 80%	300 cc.
Formol (neutral)	120 cc.
Picric acid	2 gm.
Trichloracetic acid 10%	30 cc.

16. Aojama for Golgi Apparatus.

17. Osmic acid for Golgi.

18. Osmic acid for fat.

GELATIN IMBEDDING METHOD

After fixing in formalin 10% the tissue is washed and then placed in:-

1. Gelatin sol. 5% in an incubator at about 37°C. - 24 hrs.
2. Gelatin sol.10% in an incubator at about 37°C. - 12 - 16 hrs.
3. Imbed in gelatin sol. 10%
4. Place in a refrigerator for a few hours until the gelatin hardens
5. Cut blocks
6. Put in formol 10% for several hours (to make the gelatin insoluble in the water. They can be left in this solution indefinitely)
7. Rinse the block in the water
8. Trim down to the tissue
9. Section by dry freezing in CO_2.

Sections as thin as 5 micra have been obtained by this procedure.

Zwemer's "Glychrogel" Mounting medium is primarily intended for mounting frozen sections:-

Glycerin	20 cc.
Gelatin granulated	3 gm.
Chrome alum 0.2	gm.
Dist. water	80 cc.

1. Dissolve chrome alum in 30 cc. of dist.water (heat)
2. Dissolve gelatin in 50 cc. of dist.water (heat)
3. Combine the glycerin with the gelatin solution while it is still warm
4. Add the warm chrome alum sol.
5. Mixx thoroughly
6. Filter
7. Add a bit of camphor as a preservative

Keep the bottle well stoppered to prevent evaporation.
To use, warm the solution in an oven at about 30° - 37°C.

ESTER WAX EMBEDDING MEDIUM

The tissues were fixed using the following:-

1. Formol 10%
2. Transferred to cellosolve 100% - 24 hrs.
3. Cellosolve 50% and Ester wax 50% - 24 hrs.
4. Cellosolve 20% and Ester wax 80% - 24 hrs.
5. Cellosolve 100% - 24 hrs.
6. Cellosolve 100% - 24 hours
7. Embedded in cellosolve 100%

Blocking:

1. Two cold metal L pieces were placed on a glass slide 3" X 1".
2. Ester wax (20°C.) was poured into the mould of two metal L pieces.
3. Specimen transferred from the molten wax in the embedding oven to the mould so that it layed on the skin of congealed wax on the glass slide and in an angle of one of the L pieces rather than in the centre.
4. Slide and mould containing molten wax transferred to a petri dish of cold water.
5. Holes which formed by the cooling of the wax filled in.
6. Slide and metal L pieces removed after cooling.

Trimming:

1. Block placed in warm water for a few seconds
2. Trimmed quickly.

Ester Wax Embedding Medium - Cont'd.

Section Cutting:

Using a freshly honed knife, sections were cut very slowly. For thick sections the blocks could be cut with the microtome the same day they were made. However, when thin sections (3-6 μ) were required, it was found that leaving the block for two or three days gave a more satisfactory result.

Flattening of sections was treated in the same way as paraffin sections (in gelatin water and on the warm plate)

Staining:

Routine H.P.S. was used. The sections were cleared in cellosolve and mounted in permount.

SCARLET RED - SHARLACH RED - SUDAN IV (for frozen sections)

1. Dip for an instant in 70% alcohol
2. Stain in the scarlet red sol 2 - 5 mins.
3. Wash quickly in 70% alcohol
4. Wash in water
5. Stain in Alum Hematoxylin 1/2 - 2 mins.
6. Wash thoroughly in water 3 - 5 mins.
7. Mount in glycerin or glycerin jelly.

Results:

Nuclei blue, fat orange to red; cholesterol less brilliantly red; normal myelin unstained; and fatty acids unstained.

1. Scarlet Red Solution:

Scarlet red (Sudan IV) ..... 1 gm.
Alcohol 70% .....50 cc.
Acetone C.P. .....50 cc.

Keep in tightly stoppered bottle.

2. Alum Hematoxylin:

Hematoxylin ..... 1 gm.
Ammonium or Potassium Alum ...20 gm.
Dist. water .....400 cc.
Thymol ..... 1 gm.

The solution will be ripened in about 10 days. It will keep for 2 - 3 months.

3. Farrant's Gum Glycerin:

Glycerin .....50 cc.
Dist. water .....50 cc.
Gum arabic .....50 gm.
Arsenious acid or Thymol .....

Sudan Green and Sudan Blue: The technique is identical to that when using Sudan IV, substituting the Sudan Green or Blue for the Scarlet Red solution.

SUDAN BLACK FOR FAT STAIN

1. Wash frozen sections in 70% alcohol
2. Place in Sudan Black for 20 mins.
3. Rinse twice in alcohol 50%
4. Wash in tap water
5. Mount in corn syrup

If slides are kept permanently cover slip is sealed with paint after all air bubbles have been carefully expelled.

Sudan Black..... 1 gm.
Alcohol abs. .....70 cc.
Dist. water .....30 cc.

Mounting medium:

Corn syrup ..... 1 part
Dist. water ..... 2 parts
Thymol ..... a few crystals

Warm before using

OSMIC ACID STAIN FOR FAT:

Fixation:- Fix in 10% Formalin - 24 hrs.

1. Cut frozen section and place in dist. water.
2. Place section in 1% osmic acid - 24 hrs.
3. Wash thoroughly in tap water 6 - 12 hrs.
4. Absolute alcohol for several hours.
5. Wash in dist. water.
6. Mount in Glycerin Jelly.

JENNER'S STAIN FOR BLOOD

Toluol

Alcohol

Alc. Iodine 1% - 15 mins. (f. Z.F.)

Rinse in dist. water

Hyposulphite

Wash in tap water - 10 mins.

Rinse in abs. alcohol

Jenner's stain - 7 mins.

Abs. alcohol to remove excess of stain

Transfer quickly to dist. water

Giemsa's stain - 1/2 hr. (1 drop of Giemsa's stain and 1 cc. of dist. water)

Rinse in H_2O (to remove all excess of stain)

Abs. alcohol - 2 changes

Toluol - 2 changes

Permount

Jenner's stain:

Jenner's powder..... 0.5 gm.
Methyl alcohol ..... 100 cc.

Giemsa's stain:

Stock solution (Coleman and Bell Co.)

VAN GIESON VERHOEFF'S (for elastic tissue)

1. Hydrate paraffin sections
2. Stain 5 - 10 hrs. (over night) in following:-

5% Hematoxylin in abs. alcohol.....	4 cc.
10% Ferric chloride	3 cc.
10% Sod. or Potassium Iodine	5 cc.
50% Alcohol	50 cc.

Mix just before using

3. Rinse in dist. water
4. Differentiate in 2% Ferric chloride (5 - 10 mins)
5. Wash in tap water
6. Alcohol 95% - 20-30 sec.
7. Wash in tap water - 5 mins. or longer
8. Van Gieson's picro-fuchsin - 2 mins.
9. Wash and pass rapidly through alcohols to xylol.

Van Gieson's acid fuchsin 1% aqueous -	5 cc.
Picric acid sat. aqueous sol	100 cc.

TRICHROME

1. Hydrate paraffin section
2. Iron alum 5% at 45° - 50° - 15 mins. (room temperature to 24 hours)
3. Rinse in dist. water
4. Regaud's Hematoxylin at 45° - 50° - 15 - 30 mins.
5. Rinse in abs. alcohol
6. Differentiate with picric acid at 50° - fast
7. Rinse in abs. alcohol
8. Wash in tap water 15 mins. or longer
9. Ponceau 10 mins to 1 hr.
10. Rinse in 1% acetic acid
11. Phosphomolybdic acid 1% 45° - 50° - 15 mins to 1 hour or longer for cytological detail
12. Rinse with dist. water
13. Aniline blue or fast green -

    concentrated sol. 5 - 15 mins.
    dilute sol. 1/2 - 1 1/2 hr.

14. Rinse with dist. water - fast
15. Phosphomolybdic acid 1% 1/2 - 1 min. (for aniline blue slides only)
16. Acetic acid 1% - 2 mins.
17. Abs. alcohol
18. Toluol and mount in Balsam (for fast green - salycylic balsam)

Trichrome - Cont'd.

Regaud's Hematoxylin:

Hematoxyline ..... 1 gm)
Dist. water ..... 80 cc.) heat
Alcohol Abs ..... 10 cc.
Glycerin ..... 10 cc.

Ponceau:

Ponceau 1%..... 4 parts
Fuchsine acid 1% ..... 1 part
Fuchsine S 1% ..... 1 part

Aniline Blue:

Aniline blue ..... 3 gm.)
Dist. water ..... 100 cc.) heat

When cool add -

Acetic acid ..... 2 cc.

Filter

LAIDLAW FOR RETICULUM STAIN

1. Hydrate paraffin sections
2. Alcohol Iodine 1% - 5 mins
3. Rinse in dist. water
4. Hyposulphite 1% - 5 mins
5. Wash
6. Permanganate 0.25% - 2 mins
7. Rinse
8. Oxalic acid 5% - 5 mins
9. Rinse in dist. water
10. Wash in tap water - 10 mins
11. Wash in 3 changes of dist. water for 5 - 10 mins
12. Rio Hortiga Sol. 45°C. (heat in paraffin oven) - 5 mins
13. Rinse in dist. water
14. Formol 1% - 5 mins (changing solution several times)
15. Rinse in dist. water
16. Gold Chloride 1/500 - 10 mins
17. Oxalic acid 5% - 5 mins
18. Hyposulphite 5% - 5 mins
19. Tap water - 10 mins
20. Rinse in acetic acid 1%
21. Ponceau - 5 mins
22. Acid phosphomolybdic 1% - 5 mins
23. Light Green 1/2000 - 2 mins
24. Acetic acid 1% - 2 mins
25. Abs. Alcohol, Toluol, Mount in Balsam

Laidlaw for Reticulum Stain - Cont'd.

RIO HORTIGA SOLUTION:

Dissolve 10 gm. silver nitrate in 20 cc. of dist. water, add 200 cc. of aqueous sat. solution of Lithium carbonate. The resulting brownish precipitate is decanted 6 times, then is dissolved in strong ammonia water, which is added slowly drop by drop, shaking continually. It is better to filter out a few undissolved granules than to run the risk of adding too much ammonia. The resulting solution is made up to 100 cc. with distilled water and filtered before use. The used solution can be filtered and used a dozen times or more.

CADMIUM CHLORIDE FORMOL (GOLGI APPARATUS)

1. Fix small pices of tissue in

   Cadmium chloride..... 1 gm)
   Neutral formol ..... 15 cc.) for 3 - 4 hours.
   Dist. water ..... 85 cc.)

2. Rinse quickly in two changes of dist. water

3. Silver nitrate 1.5% 10 - 15 hrs. at 22°C.

4. Rinse quickly in two changes of dist. water (preferably in a dark room)

5. Transfer to the reducing solution for 5-10 hrs -

   Hydroquinone ..... 1 gm
   Neutral formol ..... 15 cc.
   Dist. water ..... 85 cc.
   Sod. sulphite ..... 0.1 - 0.15 gm.

6. Wash thorough in tap water

7. Upgrade

8. Imbed

9. Section

   Hydrate paraffin section
   Tone in Gold chloride 1/500 - 1/2 to 1 hr.
   Fix in Hyposulphite 5% - 15 mins.
   Wash thoroughly in tap water
   Counterstain in Trichrome.

OSMIC ACID FOR GOLGI APPARATUS, SJÖVALL.

1. Fix small pieces in 10% formalin - 24 hrs.
2. Wash in dist. water overnight
3. Transfer to osmic acid 2% - 6 hrs, leave in darkened cupboard at room te
4. Wash in dist. water for several hrs.
5. Dehydrate
6. Imbed in hard wax
7. Section
8. Mount

Unstained: fats black, Golgi apparatus and mitochondria black.

In the osmic acid stain for fat the tissues are kept in the solution of osmic acid 2% for 24 hours only. Otherwise the technique is identical to that above.