THE EFFECT OF PROLONGED CORTISONE TREATMENT ON NORMAL RABBITS AND ON THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS

by

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P R E F A C E

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INTRODUCTION

The progress made by science for humanity has brought with it an aging population and attending this an ever increasing incidence of arteriosclerosis. This disease is beginning to play a serious role in the world so that it is now the commonest cause of death in the older age group. It therefore becomes imperative to deal with the problem. Many advances have already been made in this field of experimental research. Of these the importance of the serum lipid interrelationships in the genesis of experimental atherosclerosis have been repeatedly emphasized. A great step forward was taken when it was found that experimental cholesterol atherosclerosis in rabbits could be inhibited by rendering them diabetic with "alloxan"[61,62,65] or by injecting them intravenously with the detergent Tween 80[67,68,69]. This inhibition was accompanied by a particular lipid pattern. The same pattern was observed following the administration of Cortisone clinically[50,51,52,53,54] and in experiments independent of those on atherosclerosis[60]. This suggested that Cortisone might also inhibit the development of atherosclerosis. An experiment was undertaken to study this effect with the hope that it might lead to further understanding of the disease.

REVIEW OF THE LITERATURE.

1. Historical Background[1]

As far back as Aristotle, descriptions of bone in the heart of certain animals suggested arteriosclerosis and it is somewhat surprising that he did not note these findings in his comparative studies of the human body; nor was this analogy made until the sixteenth century anatomists encountered calcified aortic valves. Although the ancient papyrus did not report the disease, its presence in the XVIII Dynasty was established by Ruffer[2] in his gross and microscopic studies of Egyptian mummies. A step forward was taken by Crell in 1740 when he described the tophaceous (atheromatous) nature of the lesions which subsequently hardened. He associated this condition not merely with senescence but also the "intemperate youth". Boerhaave named the hardening and constriction of the vasa vasorum responsible for the subsequent degeneration of the vessel proper. An important contribution made by Morgagni from his studies of morphological and clinical correlations was the association of "ossification" of the coronary arteries with angina pectoris. Hodgson described the common atherosclerotic lesions clearly and located them in the intima. He advanced the view that this was a true disease and not a natural process of aging. Carl Rokitansky speculating on the pathogenesis of the disease took the stand that an endogenous product from the blood mass became deposited on the intima with subsequent hypertrophy, atheroma and ossification. A few years later Virchow introduced the primary imbibition theory by postulating

that through some constitutional dyscrasia substances passed from the blood stream into the intima which loosened the ground substance and led to further degenerative changes. Thoma (1883) felt that the intimal thickening was a compensatory response to medial degeneration which in its turn was due to nutritional disturbances of age. He was supported by Marchand (1904) who also stressed the influence of elevated blood pressure. Oskar Klotz (1911) dealt with the concept of "medial arteriosclerosis" in which he stressed the importance of infections and stress and strain. Since this time great strides were taken toward more modern advances in arteriosclerosis which will be discussed as they occur in the pages that follow.

2. Definition of the Term Atherosclerosis.

The state of confusion begins at once with the term "Arteriosclerosis" which had been coined by Lobstein[1] and means merely hardening of the arteries (Gr. skleros = hard). William Boyd[3] considers this an "omnibus term" which carries a variety of non-inflammatory arterial diseases which may or may not be related to each other:
1. atherosclerosis - a patchy lipoidal degeneration of the intima;
2. medial calcification or Mönckeberg's Degeneration; 3. diffuse arteriolar sclerosis. Hueper[4] uses it also as a collective term for the degenerative and sclerosing arterial diseases. Atheroma (Gr.Athere = mush) was for a long time used to denote any closed sac filled with gruel-like material from which Marchand[1](1904) devised the name "Atherosclerosis".to include the lipoidal, degenerative and sclerotis changes which occur in the atheromatous type of arteriosclerosis. Although he

considered Mönckeberg's medial calcification and arteriosclerosis related, he treated them separately because they occurred in different portions of the arterial tree. The term does no longer include syphilitic affections of the vascular tree, mycotic aneurysms, physiologic narrowing, thromboangiitis obliterans, periarteritis nodosa and rheumatic fever.

Since "atherosclerosis" did not prove to be a true definition from a clinical or etiological point of view it was deemed wiser to consider it as a descriptive morphological term to denote the characteristic thickenings of the intima in which lipids were deposited, while the term atheroma was reserved for an atherosclerotic lesion in which the centre was a grumous mixture of lipid material and necrotic tissue debris[5].

3. The Morphology of Atherosclerosis in Man.

The Fatty Streaks: At six months of age fatty streaks appear in the aorta frequently and become, according to Zinserling[6], constant after four years, increasing in size and number with advancing age. Since he found that the localization of these streaks corresponded to those of artieriosclerosis he considered them an early stage of that disease. Ribbert[7], who contested this, stated that the fatty spots were independent lesions due to a semiphysiological deposition of lipid material which could be reabsorbed, whereas atheromatous lesions were distinctly pathological from the beginning since they were accompanied by the development of new fibrous tissue and necrosis. However, the absence of fibrosis and necrosis should not rule out a pathological lesion and it is now generally accepted[5,6,8] that the fatty

spots are the early atherosclerotic lesions which may retrogress or develop into more advanced forms. For, in the common atheromatous aorta a variety of lesions[9] appear together as various stages of the same process. Since the least involved areas present the simplest lesions, they are assumed to be the earliest and since they closely resemble the fatty streaks the latter are likewise considered.

Grossly[9,5] the fatty flecks appear as small round or oval, slightly elevated, greyish or yellowish spots, a few mm. in diameter. They coalesce to form larger irregular plaques and longitudionally orientated streaks. Ophüls found them most commonly in the valves of the left side of the heart, at the base of the aorta, in the sinuses of Valsalva or just above them where their long axis usually runs transversely, while others found them more commonly along the posterior thoracic aorta with a tendency to arrange themselves between and around the intercostal orifices. Thence they might continue into the abdominal aorta.

Some of the fatty flecks or streaks retrogress leaving a grossly normal intima or tiny fibrous plaques free from lipids. The greater the thickness of the plaque the less likely is the lipid to be removed, though some resorbed plaques of moderate size may be found.

Those which develop further accumulate more lipid material and become covered by smooth connective tissue caps. As time goes on the central areas most rich in lipids break down to form a necrotic yellow atheromatous lesion which shines through the thickened grey translucent fibrous covering which protrudes into the lumen. This necrotic

process spreads in all directions to coalesce with adjacent lesions in various stages of development or break through the intimal surface spilling its grumous contents into the blood stream or through the fragmented internal elastic membrane into the media. The ragged defect becomes covered with thrombus material although necrotic remnants always remain in the depths or the surface may be sealed by the development of a calcareous plate. In these stages the abdominal aorta is usually more involved than the thoracic and in advanced cases the process extends into the iliac arteries, to the base of the aorta and even into the proximal parts of its larger branches.

Microscopically[5], the earliest lesions or fatty streaks show an increase and swelling of the intimal metachromatic ground substance within which are scattered fine droplets of sudanophilic lipid material which may also be scattered alongside the subjacent elastic lamellae. The intracellular deposits occur in globular macrophages which becoming filled with lipd appear as "foam cells" and in spindle-shaped or stellate mesenchymal cells which are best seen in sections cut parallel to the surface. The lining endothelial cells seldom contain any stainable lipid. As these develop further a moderate diffuse fibrosis of the intima occurs which is thicker at the site of the gross lesion. This fibrous tissue from the very beginning undergoes a particular type of necrosis which breaks the fibrillated collagen into fine pale basophilic granules (hematoxylin stain). This may possibly be calcareous material. It occurs in a patchy fashion usually in the deeper layers of the intima. This change may appear prior to the fatty degenerations. The amount of

lipid material varies and bears no relation to the amount of fibrous tissue but as the latter is increased in the superficial parts, lipid-filled foam cells are increased in the deeper parts. There are also present many stellate connective tissue cells filled with minute fat froplets. The fatty change usually starts in the musculo-elastic layer but on occasion may be more conspicuous in the superficial layers of the intima. Many of the lipid-filled foam cells degenerate into an atheromatous mass of finely granular necrotic debris through which are scattered fat droplets of varying size and precipitated cholesterol crystals. Surrounding the necrotic zone are large numbers of foam cells, a few stellate connective tissue cells and lymphocytes. The necrosis extends to involve the internal elastic membrane which becomes fragmented and allows the atheromatous process to invade the media, at first superficially, but in far advanced cases, deeply.

The cap of proliferating fibrous tissue with its increased amounts of metachromatic ground substance(13) undergoes a hyaline degeneration and fibrinoid necrosis(14). This covering becomes destroyed in the wake of the degenerative process which discharges its contents into the blood stream. Thrombi attempt to cover the ragged ulcer in whose depths are found remnants of necrotic debris and cholesterol crystals, while in other areas a calcareous plate may be formed to seal the lesion. Thus the variegated picture of atherosclerosis is developed.

4. <u>The Nature of the Cellular Elements</u>.

<u>The Foam Cells</u>: Particularly attractive in the picture of atherosclerosis are the foam cells. They have also been called(15) lipophages,

lipoid cells, xanthoma cells or cholesterol phagocytes. These[16] are round or polygonal with a small round deeply staining centrally placed nucleus around which the narrow rim of pale basophilic cytoplasm in the smaller cells becomes voluminous, reticular and foamy as the cells enlarge. Duff feels certain that they are macrophages which have ingested lipids, for they resemble in both form and function the large monenuclear phagocytes in the animal body and take up colloidal dyes as do other macrophages. Duguid[17] believes that they are wandering macrophages which are normally present in the arterial intima prior to the appearance of lipids upon which they increase in number by multiplication and phagocytose the lipid material. Others[18,19] think that they represent desquamated fat-laden phagocytes of the reticulo-endothelial system which enter the blood stream either actively or passively and penetrate the lining endothelium to lodge in the subendothelial layers. Hueper[4], finding foam cells in the blood stream, offered to support this view. Altschul[15] maintains that the endothelial cells develop into foam cells. He describes their presence in the lining endothelium and their amitotic division and migration to the subendothelial layer where they actively or passively take up lipoids. He speaks of "dedifferentiation", in which the endothelial cells become stellate mesenchymal connective tissue cells and fibroblasts which settle in the amorphous ground substance. In tissue cultures[20] endothelium has been shown to ingest particulate matter and to migrate and become identical with the mesenchyme. This Altschul feels is equivalent to dedifferentiation in arteriosclerosis[15,21]. Hematoidin, hemosiderin, fat droplets and cho-

lesterol crystals have been demonstrated within endothelial cells as evidence of their phagocytic activity while others would attribute this to irritation phenomena. McClung[22] states that an endothelial cell which is not phagocytic may become so if damaged, against which Altschul argues that damage does not exclude phagocytosis nor is it necessary for phagocytosis. Some[16] believe that all intimal foam cells are macrophages or fibroblasts and that foam cells of so-called endothelial origin are degenerated rather than phagocytic elements. The branched cells are usually considered to be derived from fibroblasts although others state they arise from endothelial cells[21].

5. The Lipid Composition of Atherosclerotic Lesions in Man.

Atheromatous aortae have been shown to contain increased amounts of total cholesterol, phospholipids and fatty acids which rose progressively with the severity of the lesions[23,24]. Windaus[25] showed that the cholesterol ester values in atheromatous aortas were 20 - 26 times as much as normal aortas, while the free cholesterol was 6 - 7 times as much. From analyses made on the intima and inner portions of the media Schoenheimer[26] reported that the cholesterol esters comprised 10% of the total lipids in normal aortas and rose to 60% in those with atherosclerosis while the average amount of free cholesterol remained constant. Lehnherr[23] from his studies added that the changes in diabetic atherosclerotic aortas were similar but more marked. Meeker and Jobling[24] analyzed the atherosclerotic plagues which they had removed from the intima. Compared to normal sections similarly prepared they found that the phospholipid proportion was constant; fatty extract and

total cholesterol increased with the severity of the lesions; the rise in the ester cholesterol was greater than that of the free cholesterol except, in the more severe lesions, when the free cholesterol was greatly increased. They[27,28,29] attributed this release of free cholesterol to a splitting of the cholesterol esters. In noting that the lipid composition in the normal intima and in the early atheromatous plaques corresponds to their proportions in the blood[30,31] very persuasive evidence was introduced to suggest that the lipids enter the aorta by a method of non-selective infiltration.

6. Experimental Cholesterol Atherosclerosis in Rabbits.

Origin and Development:[32] While studying the effects of unnatural foods such as meat, milk and eggs in rabbits Ignatowski in 1908 discovered lesions in the aorta which resembled human atherosclerosis. These were attributed to the protein in the diet until Stuckey and Wesselkin were able to demonstrate that the fatty substances were responsible. Approaching the problem more closely, Anitschkow (1912) together with Chalatow (1913) showed that the feeding of pure cholesterol dissolved in oil produced the characteristic lesions of experimental atherosclerosis in rabbits. When the work of previous investigators was examined in the light of this finding, it was seen that those who attributed the resultant lesions to protein or those who incriminated the toxic effects of staphylococci injections or alcohol poisoning had at the same time, fed diets containing cholesterol. Wacker and Hueck added further to the role played by cholesterol. They showed that the lesions were produced when cholesterol was fed in solid form with the ordinary

food and that this was accompanied by a hypercholesterolemia which was largely due to the cholesterol ester fraction.

Since that time the many cholesterol feeding experiments which were conducted produced lesions in rabbits which approached those of human atherosclerosis so closely as to warrant their comparisons. Duff[16] has chosen to call this experimental disease of the arteries "Experimental Cholesterol Arteriosclerosis".

Methods of Production: In the earlier studies substances rich in cholesterol[16,33] such as egg yolks, brain tissue, liver or hydrous wool fat were mixed with the ordinary food or administered in liquid form by means of a stomach tube. When cholesterol alone was shown to be effective, it was used mixed with the food or in oily solution via stomach tube. Wacker and Hueck[34] showed that solid cholesterol would produce experimental atherosclerosis but that a larger dose and longer period of time was required (1.25 gm. per day for at least 5 months). However, dry cholesterol[29] mixed with the daily food has been more successfully used. A daily dose of 1.0 gm. for at least 60 days was required before well-marked gross lesions appeared. The cholesterol dissolved in ether is mixed with the food and then the ether is allowed to evaporate. However, if administered in an oily solution the lesions appear much sooner (e.g. 0.75 gm. of cholesterol per day dissolved in 15 cc. of corn oil mixed with the daily food per os will produce definite atherosclerotic lesions in 40 days). Because of the objection sometimes encountered on the part of the rabbit to take the unnatural food, the stomach tube has been used, but then again a longer time is required and the danger of perforation

of the oesophagus or aspiration pneumonia create their own disadvantages which, however, should be reduced to a minimum with skill. Whatever method is used, a certain amount of time must elapse before the lesions are demonstrable. Otherwise, the larger the dose of cholesterol, the more prominent will be the lesions. The microscopic foam cells and lipoid deposits may appear one month before the gross lesions, the earliest of which require about six weeks and the more obvious ones from three to six months of cholesterol feeding(5). Hueper attributes this "incubation period" in the early stages of cholesterolemia to the ability of the liver cells and the suprarenal cortical cells to store excess cholesterol until their absorptive power is exhausted when the histiocytes take over this function. Schürmann and MacMahon noting that connective tissue phagocytes take up lipids late postulate that the common endothelium of lymph and blood vessels create a "blood-tissue barrier" whose resistance breaks down in time to allow substances from the blood to invade the subendothelial tissues. In an attempt to produce earlier lesions Klotz(35) used I. V. injections of a colloidal solution of cholesterol in 5% sodium oleate. This work has been recently undertaken again using sodium stearate(36) with which extensive lesions appear in 36 days; injections of oxidized cholesterol(37) will work still more rapidly and it is claimed that suspensions of cholesterol in deproteinized rabbits' serum(38) will at once deposit cholesterol in the intima of the rabbit's aorta. These experiments await further development.

Morphology: Experimental cholesterol atherosclerosis lipids are first deposited[32,16] in the cells of the reticulo-endothelial system in the liver, spleen, lymph nodes and bone marrow. When these and the suprarenal cortex become filled, the fatty deposits appear in the arteries, the heart valves, the veins; in the skin, subcutaneous tissue and tendons; in the interstitial tissue of the kidneys and in the eyes. In the aorta[16] the earliest gross lesions occur in the arch as minute yellowish white opaque spots, elevated slightly above the normal intima. These tend to be disposed about the mouths of the vessels arising from the aorta or just above the aortic valve ring. At first, the fatty flecks fade off imperceptibly into the surrounding intima, but as they increase in size they become more sharply outlined, rounded or irregularly-shaped and protrude into the luman as glistening yellowish-white nodules. As they increase in number they spread onto the posterior aspect of the thoracic aorta and gather about the mouths of the intercostal arteries, but may also be found on the anterior and lateral aspects. The lesions progress to involve the abdominal aorta and the nodules coalesce to form large irregular plaques with a warty surface which may impinge upon the patency of the lumen, although, in still later stages, when the elasticity of the vessel has become greatly reduced, it undergoes dilatation, particularly in the arch. The main trunk of the pulmonary artery may, early in the disease, also show nodular intimal thickenings. When the lesions in the aorta become well-established, isolated plaques appear in its larger branches (namely, the innominate, common carotid, subclavian, common iliac and femoral arteries)

where they are usually at the points of bifurcation. Later, when the lesions spread into the smaller branches of the arterial tree, the coronary arteries are frequently involved[39].

The earliest microscopic change in the intima is described as a swelling of the subendothelial metachromatic ground substance[40], contrary to the views of earlier observers who maintained that the alteration in the ground substance was secondary to the lipoid deposits. Arguments were then held to determine the primary site of deposition of the lipoids. Some claimed that in the earliest lesions anisotropic lipoid material was present in the subendothelial cells but not in the ground sugstance[18]. Others[41,42] observed it first in the endothelial and subendothelial cells but still not in the ground substance. Duff[40] presents very persuasive evidence that the earliest lipid deposits occur in the swollen subendothelial ground substance whence they are subsequently taken up by the prolifering cellular elements[45]. From his observations he feels certain that extracellular lipid material precedes that of any intracellular lipid. Indeed, the interstitial lipid deposits may become abundant with very little cellular proliferation.

In experiments, at present underway in this laboratory[43], rabbits were fed cholesterol in oil and microscopic lesions of the ninth day were examined. In vertical sections, intracellular sudanophilic lipid deposits were found in the subendothelial layer, while a smaller quantity of anisotropic cholesterol crystals appeared in the deeper intima. (Comments on any intercellular lipid material were reserved at this stage in respect of technique and artefact). In later lesions anisotropic ma-

terial began to accumulate in greater quantity in the subendothelium layers while sudanophilic droplets were deposited intracellularly as well.

In experiments currently conducted in this laboratory[44], large surface areas of the aortic endothelium of cholesterol-fed rabbits were stained with toluidine blue. Microscopic lesions of the fourth day disclosed sudanophilic droplets in the intercellular ground substance but lipoid material was not demonstrated within the cells.

However, the later microscopic lesions may vary considerably. Thus the prominent feature of the intimal thickenings may be the ground substance, the foam cells or the fibroblastic reaction[40]. As the lesions develop they nearly always contain a large number of cells. Of these, the foam cell is the most conspicuous and is in form and function apparently identical with that described in atherosclerotic lesions of human arteries. Duff[16] feels that they are macrophages since they resemble the large mononuclear phagocytes elsewhere in the animal body and actively take up lipids and colloidal dyes. But he admits also the possibility that they may arise from wandering mononuclear phagocytes present in the intima prior to the appearance of lipoids. Others still claim that these macrophages migrate from the media - yet foam cells do not accompany the occurrence of anisotropic lipid deposits in the media. The fat-laden cells may become so prominent as to mask the extracellular lipoids. Scattered about between the foam cells are smaller spindle-shaped or stellate cells which are indistinguishable from fibroblasts and are therefore identified as such. These increase in number when the lesions are advanced. The lining endothelial cells play roles

which differ according to the various observers. Anitschkow believed that they merely formed a single thin layer of flat cells as a lining for the blood vessels. Others, having noted fat granules in the lining cells, attributed to them a more active role. But this was considered with some reservation since the lining endothelium in the normal rabbit's aorta is difficult to see and easy to wipe off while preparing the material.

With the advent of many cells in the intima the ground substance may become still more abundant. The lipoid material may be scattered in finely-divided granules or diffusely spread through the ground substance. The foam cells become greatly filled with sudanophilic fat droplets which are also contained in many of the mesenchymal connective tissue cells. A large portion of the fatty material is anisotropic, consisting chiefly of cholesterol and its esters.

As the lesions develop[5,16,40] further the foam cells in the deeper layers disintegrate, scattering their fatty granules amidst necrotic debris. In this way atheromatous lesions are formed. The fibroblasts proliferate considerably in the deeper intimal layers. The fibroblasts may proliferate to a lesser degree in the superficial intimal layers to form a little fibrous cap for the necrotic lesions. Many foam cells are still scattered freely about but tend also to surround the "pool" of free fatty substances.

The process extends to involve the underlying elastic laminae which shows splitting and the formation of many new elastic fibrils. Eventually the necrotic and lipid material may break through the elastica.

to invade the media which itself shows degenerative fatty changes and areas of focal necrosis which may be independent of the intimal changes.

7. The Serum Lipids in Atherosclerosis.

The term "lipid" or lipoid will be used to include those substances which in their chemical, physical and solubility properties resemble the true fats, which are included also[16, 72].

Whatever role the lipids may play in the genesis of atherosclerosis, the fact still remains that the development of atherosclerosis in the rabbit[5,16,40,65,99], guinea-pig, chicken[46,47,48] and dog[49,78] is associated with a sustained dietary elevation of the blood cholesterol[98]. Clinically an increased severity of atherosclerosis is found in those conditions which are associated with hypercholesterolemia, such as diabetes mellitus, essential xanthomatosis, myxedema and in the nephrotic stage of nephritis. Steiner reported that in a large percentage of cases with atherosclerosis there is a significant hypercholesterolemia[100], although others have questioned this[101]. Morrison found that the blood cholesterol levels rose above 260 mg% in 68% of patients under 60 years of age with coronary occlusion[102]. Hypercholesterolemia was demonstrated more particularly in young men[103] and women[104] with coronary heart disease. Gubner and Ungerleider suggest that a marked elevation of blood cholesterol predisposes to the development of atherosclerosis in humans while low levels seem to offer some protection[98,105].

In experimental cholesterol atherosclerosis in rabbits all the lipid constituents in the blood are elevated, particularly the total, ester and free cholesterol. The phospholipids were elevated to a lesser

degree than the total cholesterol[5,10,28,29,65,99]. Although the absolute levels of the individual lipids varied considerably, their proportions presented a similar pattern. The greatest elevation was in the total serum cholesterol, for which the cholesterol ester was largely responsible, while that in the free cholesterol was considerably less. These lipid elevations appeared soon after the beginning of cholesterol-feeding. As feeding was continued, the concentration of all the lipids increased so that the ratio of total cholesterol to the phospholipids also increased. The neutral fat rises also, but again to a lesser degree than the ester or total cholesterol. Essentially the same pattern was observed in rabbits fed cholesterol in oil or pure cholesterol, in cholesterol-fed birds[79] and in dogs fed cholesterol and thiouracil[78]. Although hypercholesterolemia admittedly plays a role in experimental cholesterol atherosclerosis, the nature of this role is not obvious. Whether it is merely an association of atherosclerosis or more directly related to the genesis of the disease remains to be disclosed. Furthermore, the interrelationship of the various lipid fractions appear to be more important than the level of hypercholesterolemia in itself. Particular emphasis has been placed on the phospholipids and the total cholesterol/phospholipid ratio[78,79,80]. Ahrens and Kunkel[80] maintain that the deposition of lipid compounds are not determined by absolute levels of total lipid concentration or of any single lipid fraction. They emphasize particularly the importance of the ratio of cholesterol to phospholipid and the ability of high concentrations of phospholipids to "solubilize" the hydrophobic cholesterols and neutral

fats and hold them in colloidal dispersion. A decreased cholesterol to phospholipid ratio will result in a deranged colloidal state of the serum lipids which permits the deposition of cholesterol in the intima. This feature will be discussed again later when its relationship in the lipemia of Cortisone is considered.

By repeated injections of polyvinyl alcohol and other macromolecular substances Hueper was able to produce lesions which were morphologically like those of experimental cholesterol atherosclerosis except that the lesions contained the foreign substance injected instead of the lipids. He termed this disease "Macromolecular Atherosclerosis".

More recent studies suggested that the size and physical state of the lipid molecules may be even more important in the genesis of atherosclerosis than the concentration or interrelationship of the serum lipids. Gofman, Jones et al. (63,115) used this approach to the study of atherosclerosis. They submitted to ultracentrifugal flotation the serum lipids of normal persons of various ages and those of patients with coronary artery disease, diabetes mellitus and other states commonly associated with premature and more severe degress of atherosclerosis. By this method they were able to identify and quantitate groups of molecules of various densities as they undergo flotation. A study of the various macromolecular complexes disclosed that one class of molecules in particular appeared to be related to the atherosclerosis in humans. These included at least three species which migrate with rates between 10 and 20 units expressed in terms of flotation rates Sf (= Svedberg units). Each of the three species contained approximately 30% of chole-

sterol by weight. The class of molecules with rates between 3 - 8 S_f units carried a major fraction of the serum cholesterol and did not appear related to atherosclerosis. In 230 males with myocardial infarction 91% showed the presence of S_f 10 - 20 molecules as compared with approximately 50% in normal controls. This suggested that these macromolecules are in some way associated with the development of atherosclerosis. Since they were also found in 40% of 226 normal males between twenty and thirty years of age, the possibility that these are the persons more readily destined to develop atherosclerosis was entertained. Although with higher serum cholesterol levels there is a tendency toward higher concentrations of S_f 10 - 20 molecules, it is not possible to predict from the serum cholesterol value in the individual patient the concentration of S_f 10 - 20 molecules. A group of men and women placed on a low fat, low cholesterol diet showed consistent trends toward lower concentrations of the S_f 10 - 20 molecules over a period of weeks to months. Moreton[117] found that there were much greater numbers of lipid macromolecules in the serum following ingestion of a fatty meal than in normal fasting plasma or after fat-free meals. From this observation he postulated that a cumulative effect of this phenomenon might be the underlying cause of intimal lipid deposition in human atherosclerosis. He suggests further that due to the increase in size of the lipid particles they are retained by the barrier of the internal elastic membrane. However, his theories are as yet not supported by evidence, whereas Gofman and his collaborators have observed a correlation between the presence of increased numbers of S_f 10 - 30 molecules and an increase in the severity of experimental cholesterol atherosclerosis.

Although the relative importance of the role which the giant lipoprotein molecules may play in the pathogenesis of atherosclerosis is not apparent they more than suggest that a derangement of the lipids is an important factor.

8. The Influence of Lipotropic Agents on Experimental Cholesterol Atherosclerosis.

Studies of the effects of lipotropes on experimental cholesterol atherosclerosis have produced contradictory results. Steiner[107] reported that choline tended to delay the appearance of gross atherosclerotic lesions in the aorta but did not prevent its eventual appearance. Bauman and Rusch[108], however, from their experiments reported that there was no effect on the blood cholesterol and atherosclerosis in the rabbit, even with large amounts of choline. Himsworth[109] also failed to produce any inhibition on the development of atherosclerosis in cholesterol-fed rabbits. However, Kesten and Silbowitz[112] produced suggestive evidence that soybean lecithin or choline in equivalent amounts will delay the development of experimental cholesterol atherosclerosis. Morrison and Rossi[113] were also able to inhibit the development of atherosclerosis in cholesterol-fed rabbits by feeding them choline for a period of 92 days. With larger doses the lesions were prevented in a larger number of animals at the same time. Regression of atherosclerotic lesions in cholesterol-fed rabbits treated with choline have also been reported[110,111]. This occurs more readily when the initial blood cholesterol is not very high. Regression of lesions in these cases is accompanied by normal levels of blood cholesterol.

9. The Influence of Surface-Active Agents on Experimental Cholesterol Atherosclerosis.

Kellner and Correll(67,68) studied the relationship of blood lipids to the development of experimental cholesterol atherosclerosis by using the surface-active agents Tween 80 and Triton A 20. Chelesterol-fed rabbits were given repeated intravenous injections of Tween 80 and Triton A 20. This produced a hyperlipemia which was characterized by a greatly elevated serum total cholesterol over and above that of the cholesterol-fed control rabbits, but which was accompanied by a proportionate or even greater rise in the serum phospholipids. This was further accompanied by an inhibition of the development of experimental cholesterol atherosclerosis as compared to the cholesterol-fed control rabbits in whom more severe degree of atherosclerosis was accompanied by a decreased phospholipid to cholesterol ratio. Duff and Payne(69) conducted similar experiments in which they also observed in rabbits injected intravenously with the detergent Tween 80 a rise in the serum cholesterol above that of normal and a proprotionate increase in the lipid phosphorus, while the fatty acids were not greatly elevated. The rise in the total serum cholesterol was due less to the ester than the free fraction - a reversal of the situation found in the cholesterol-fed controls. This was accompanied by an inhibition or minimal degree of atherosclerosis. They stressed the importance of the interrelationship of the serum lipid fractions and of their relationship to the serum proteins. For, in the lipemic sera of the rabbits treated with Tween 80, the greater proportion of serum lipids was "readily extractable" (i.e. unbound or only loosely bound to the serum proteins). This suggested that loosely-linked serum protein-lipid bonds

were conducive to lipid deposition in the intima. However, the increased proportion of loosely-bound or unbound serum lipid-protein complexes was present in the sera of Tween 80 injected rabbits whether atherosclerosis developed or not. Therefore, other conditions must also determine the intimal lipid deposition. In cholesterol-fed rabbits treated with oral Tween 80 the rise in total serum cholesterol was accompanied by elevations of the other lipids. But the ratios of total cholesterol/lipid phosphorus: neutral fat corresponded to those of the control rabbits. The rise in the serum total cholesterol was due more to the ester fraction than the free cholesterol. This was associated with a degree of atherosclerosis like that of the control animals.

The mechanism whereby the surface-active agents induce the hyperlipemia is not clearly understood. These agents may act directly by increasing the capacity of the plasma to hold lipids in stable emulsion, or they may interfere with enzyme systems which are essential for the intermediary metabolism of fats, or by accelerating the synthesis of cholesterol or retarding its degradation.

The mechanism whereby the surface-active agents injected intravenously in rabbits modifies the development of atherosclerosis is also not known. Whether the presence in the blood of the surface-active agents themselves are responsible for the inhibition of experimental cholesterol atherosclerosis or whether they exert their effect through the elevated serum phospholipids is yet not certain. But the evidence in favour of the importance of the elevated phospholipids is strong. For it has been shown in vivo and in vitro that elevated phospholipids exert a "stabilizing"

influence on lipid emulsions in hyperlipemic blood[80,119]. Also atherosclerosis in cholesterol-fed rabbits was consistently accompanied by a decreased phospholipid to cholesterol ratio. A similar derangement has been observed in chickens[121] and in humans[98,118] with clinical evidence of atherosclerosis.

10. The Effect of Diet on Atherosclerosis.

Many[98] observations and studies have been made in man to determine the effect of diet on the development of atherosclerosis. Since the importance of cholesterol in this condition has attracted much attention dietary restriction of cholesterol was attempted. Where cholesterol itself was restricted little or no effect was produced on the level of the blood cholesterol. Since fat was found to be important not only in cholesterol synthesis but in cholesterol absorption as well[119], in that absorption of cholesterol occurs only in the presence of fatty acids, the effects of alterations of the fat in the diet were studied. The administration of fat alone resulted in a hypercholesterolemia which was parallel to the increase of blood fatty acids while on a fat-free diet cholesterol could be recovered quantitatively in the feces[124,125]. These findings reflected the importance of the fatty acids for intestinal absorption of cholesterol. Rabinowitch[126] maintains that high fat diets in diabetics favour and low fat diets retard the appearance of hypercholesterolemia and arteriosclerosis. Significant decreases in blood cholesterol have been observed with the Kempner rice diet[127], which has a very low fat content of approximately 5 gm. But there is little or no effect on blood cholesterol unless

dietary restriction of fat is extreme.

Cholesterol levels[98] in the blood may be altered by the increased degradation of cholesterol in the intestines by bacterial flora alkaligens faecalis and Escherichia Coli. Cholesterol may thus be reduced to cholestenone, at which stage the process is still reversible. However, it may go on, finally, to coprosterol which is a non-reversible degradation product. The pathway taken by cholestenone depends on factors in the g. i. tract and on the diet. A high protein diet was found to favour the predominance of coliform organisms and the degradation of cholesterol. Studies on the American population have indicated that their higher fat diet is associated with higher cholesterol levels in the blood and a greater incidence of arteriosclerosis than in peoples with very low fat diets. However, these differences attend extreme differences in the fat content of the diet, so that moderate restrictions would be of little value in lowering the blood cholesterol levels. Firstbrook[95] stressed the importance of weight loss and under nutrition in the inhibition of atherosclerosis in rabbits but he considered this in relation to other factors which are associated with the development of arteriosclerosis.

11. The Influence of Endocrines.

It has been observed clinically that hypothyroidism is frequently associated with an increased degree of severity of atherosclerosis while patients with hyperthyroidism are relatively free from this disease. Leary[128] suggested that a derangement of thyroid function altered the intimal ground substance in preparation for the deposition of cholesterol.

In experimental cholesterol atherosclerosis in animals Dauber et al [129] found that dessicated thyroid decreased the severity of atherosclerotic lesions in cholesterol-fed chicks, as compared to controls. Horlick and Havel[130] were unable to produce atherosclerosis in rats by feeding propylthiouracil to cholesterol-fed rats. Steiner and Kendall[49] were able to produce atherosclerosis in dogs by feeding large doses of cholesterol and thiouracil.

Bruger and his collaborators[131] found that in cholesterol-fed rabbits the thyrotropic hormone of the anterior pituitary produced increased deposits of cholesterol in the aortae of rabbits. They failed to alter the development of atherosclerosis with desoxycorticosterone acetate[132]. However, using testosterone propionate and estradiol dipropionate, there was a decreased deposition of cholesterol in the aorta of the female. When the gonads were removed this effect was also removed[133]. No effects were produced in male rabbits with testosterone or estradiol[134]. Chaikoff et al[79] and Horlick and Katz[135] found that diethylstilbestrol would produce atherosclerosis in the aortae of cockerels without cholesterol feeding. Kellner and Correll[136] found that cholesterol-fed, adrenalectomized rabbits continued to develop atherosclerosis if maintained on DCA, suggesting, therefore, that the other adrenal hormones were not essential for the development of atherosclerosis.

12. Cortisone as Related to Atherosclerosis.

Many clinical observations and experimental studies revealed that Cortisone produced well-defined changes in the serum lipids. In the sera of patients receiving prolonged Cortisone therapy Adlersberg and his associates[50,51,52,53] noted that there was a consistent elevation of total serum cholesterol, esterified cholesterol, and phospholipids. The level of hypercholesterolemia appeared to be directly related to dosage: it developed more frequently in prolonged than in shorter courses; withdrawal of dosage resulted in the return of cholesterol to control levels, even when hypercholesterolemia was maintained for a long time; fluctuations of the serum cholesterol occurred with changes in dosage, and hypercholesterolemia developed more slowly in patients in whom there was a low serum cholesterol control level. They stated that there was a decided "parallelism" between the changes in serum cholesterol and phospholipids, in that hyperphospholipidemia regularly accompanied hypercholesterolemia and a drop in serum phospholipids almost always occurred when the serum cholesterol decreased. It is worthy to note here that the "parallelism" between the serum cholesterol and phospholipids described by Adlersberg is not a true one geometrically or mathematically. More correctly the rise in serum phospholipids is concomitant, rather than parallel, with that of the serum cholesterol. The significance of this point will be considered shortly. These hyperlipemic states were grossly manifested by opalescence of the sera and this frequently occurred even when serum neutral fat was at a very low level. The turbidity of "fasting sera" was

observed often at normal levels of serum neutral fat. They thought that this might possibly be due to the production of a lipid of decreased solubility, or of macromolcular particles, or to an altered ratio between the free and conjugated lipoproteins.

Further studies[54] of the interrelations of the serum lipids in patients treated with Cortisone were made in which three ratios were determined: Free/total cholesterol; total cholesterol/phospholipids; and free/total cholesterol:phospholipids. These disclosed consistent alterations: - there was a definite lowering of the free/total cholesterol; there was a less marked rise in the total cholesterol/phospholipid ratio (largely due to a disproportion in the elevation of total serum cholesterol to serum phospholipid); and a marked and consistent reduction of the free/total cholesterol:phospholipid.

This suggested that a change in the rate of esterification of cholesterol and synthesis of the phospholipids was responsible for the altered interrelationships of the serum lipids. These results were in keeping with other clinical and experimental findings that lipid metabolism is disturbed in adrenal hypercorticalism. For in Cushing's syndrome there is a characteristic sustained elevation of serum cholesterol and phospholipids with an abnormal distribution of body fat. This has been found to occur frequently in patients treated over long periods of time with adrenal cortical hormones.

In animal experimentation it was shown that fatty infiltration of the liver and ketonuria produced by various means could be prevented by adrenalectomy[55,56]. Also, in the rabbit[57], adrenal homotransplantation resulted in hypercholesterolemia.

Rich et al.[58] studied the ability of Cortisone to induce lipemia in albino rabbits who were given 7.5 gm. of Cortisone for 16 days. Spectrophotometric turbidity readings were in keeping with the visual estimations of the grossly turbid sera. Chemical analysis of the hyperlipemia disclosed that both the total fatty acids and cholesterol were elevated, but were not accompanied by a lowering of the plasma proteins, while the livers showed a considerable deposition of fat droplets. The simple explanation which they offered for the lipemia was that the excess cortical hormone mobilized excess fat from the depots. Other studies[59], although in mice, suggested that mobilization of fat for the liver depends at least in part upon exogenous or endogenous adrenocortical hormones.

In view of his findings and the assumed relationship between hypercholesterolemia and atherosclerosis, Adlersberg[51] suggested the possible development of premature atherosclerosis in patients treated with Cortisone. And this idea he supported further by the observations that atherosclerosis does frequently develop prematurely in patients with Cushing's syndrome.

Kobernick and More[60], while studying the effect of Cortisone on tissue lesions produced by foreign serum proteins in rabbits, observed changes in carbohydrate metabolism and serum lipids, which accompanied by "hydropic changes" in the islets of Langerhans, bore a strong resemblance to a true diabetic state. They reported an increase in the serum lipid phosphorus, total and free cholesterol, and fatty acids of neutral fat which they stated presented a pattern similar to that described by

Payne and Duff[61] in alloxan diabetes in the rabbit, but reaching somewhat higher levels. This would suggest that Cortisone might have an inhibitory effect on experimental cholesterol atherosclerosis similar to that produced by alloxan diabetes.

Although, in experimental cholesterol atherosclerosis, we recognize the importance of hypercholesterolemia per se, its effect seems to depend upon the relative proportions of the other serum lipid fractions. It would be wise, therefore, at this stage, to examine these relationships in the hyperlipemia produced by alloxan diabetes and by Cortisone, since these states have suggested the hypothesis upon which the present investigation was undertaken.

In alloxan diabetes the expected development of atherosclerosis in rabbits fed cholesterol was inhibited[62,65,64]. Still more surprisingly this occurred in the presence of extremely high levels of hypercholesterolemia. However, it was later found that his hypercholesterolemia was accompanied by a concomitant and marked elevation of the serum phospholipids and neutral fat[65]. As a corollary to this, it was observed that in those diabetic rabbits in which the serum phospholipids and neutral fat were not markedly elevated in the presence of hypercholesterolemia, atherosclerosis developed. Also, if the diabetic rabbit was treated with insulin, the serum lipid ratios became like those of the cholesterol-fed control rabbits and the inhibitory effect on atherosclerosis was withdrawn[66]. Consistent with these findings were those obtained by the intravenous injections of the detergents[67,68,69] Tween 80 and Triton A 20 to cholesterol-fed rabbits. There was a considerable rise in the serum chole-

sterol which was paralleled by an increase in the phospholipids, whereas the rise in the fatty acids of neutral fat was moderate. In keeping with these findings, atherosclerosis was again strikingly inhibited. Conversely, in those rabbits which developed lesions, the phospholipid to cholesterol ratio tended to be decreased. With further respect to these relationships it is worthy of note that a decreased phospholipid to cholesterol ratio is frequently observed in young patients with coronary artery occlusion[70,71] and in those diseases which are associated with hyperlipemia and excessive atherosclerosis such as diabetes mellitus, hypothyroidism and hypercholesterolemic xanthomatosis.

Further study[61] of the absolute lipid levels of alloxan diabetic rabbits disclosed that all the serum lipid fractions were elevated but not to the same degree. Relative to their normal proportions, the increment in the fatty acids of neutral fat was greatest. This also occurs in the hyperlipemia of diabetes in man. The rise in lipid phosphorus was least while that of the total serum cholesterol was intermediate. The free cholesterol was more responsible for the elevation of the total cholesterol than was the ester fraction. Determinations made of the "readily extractable" portions of the lipids showed that in the hyperlipemia of alloxan diabetic rabbits the increase of lipids was largely in those fractions not closely bound with the serum proteins. In the diabetic rabbit there was a loss of body weight concomitant with the elevation of serum lipids, while the appetite was maintained. In view of this, Payne and Duff postulated that the hyperlipemia was due to mobilization of fat from the tissue fat depots and related to the severity of the diabetes.

It would seem, therefore, that, although hypercholesterolemia is a sine qua non for the development of experimental cholesterol atherosclerosis, hypercholesterolemia per se is not alone responsible. Both clinical and experimental evidence would indicate that in the presence of hypercholesterolemia the development or inhibition of atherosclerosis further depends upon the colloidal stability of the serum lipids; and in turn, the colloidal stability of the serum lipids depends on the proper interrelationship of the various lipid components and on their affiliation with the serum proteins.

In the normal sera of humans[73,74] and animals[75] there are wide variations in the absolute levels of the individual constituents. However, the various lipid components maintain a constant interrelationship with each other and a close association with the serum proteins. The blood remains clear when its colloidal stability is thus maintained[72]. In normal sera the ratio of total cholesterol to lipid phosphorus[76] and of free to total cholesterol[77] tends to remain fixed. When the total lipid content in the rabbit[75] is elevated within the normal range the individual lipid constituents rise relatively so that the normal ratios are maintained.

The importance of the phospholipids has been emphasized in this regard. As a group they are more soluble in water than the other lipids[72]. This emulsifying property enables them to form stable suspensions or colloidal solutions, thus they may play an important part in keeping the hydrophobic colloid cholesterol in suspension.

However, it seems that the ability of the phospholipids to act as stabilizers of the other serum lipids depends upon the constancy of its ratio to the total cholesterol. Davidson et al.found that in normal dog the molar ratio of cholesterol to phospholipid was approximately 1:1[78]. In the serum of dogs fed cholesterol and thiouracil, all the lipids were elevated, but not proportionally, so that the molar ratio of total cholesterol to phospholipid became 5:1. In cholesterol-fed rabbits[29]and birds[79]a similar lipid pattern was obtained. There was an increase in all of the lipid fractions. The greatest rise was in the total cholesterol for which the ester cholesterol was largely responsible. There was a lesser rise in the neutral fat and phospholipids so that the ratios between total cholesterol, neutral fat and phospholipids were increased as compared to normal controls. Associated with this type of deranged lipid pattern was the development of atherosclerosis. In more recent experimental studies Ahrens and Kunkel[80] have produced further evidence to support the view that the phospholipids stabilize the other serum lipids. With a decrease in the phospholipid/cholesterol ratio there is a decrease in the colloidal stability of the serum lipids and with that is associated the deposition of lipoids in the intimal cells.

We find a corollary support to these views if we return again to the experimerts discussed earlier in this section. Closer examination of the hyperlipemic patterns of the cholesterol-fed alloxan-diabetic rabbits[61,65]and of the cholesterol-fed rabbits injected intravenously with the detergents Tween 80 and Triton A 20[69] reveals a phospholipid/cholesterol ratio which approaches that of the normal "stable" sera.

Associated with these serum lipid patterns was the inhibition of atherosclerosis. The Cortisone-induced hyperlipemia in rabbits, described by Kobernick and More[60], presented a lipid pattern similar to that of the alloxan-diabetic rabbit. In fact, with the higher lipid levels, seen in the later stages of the experiment, the phospholipid to cholesterol ratio approached the ratio of "stable" normal sera more closely than that in the alloxan-diabetic rabbit. It seemed reasonable to assume, therefore, that Cortisone might inhibit the development of experimental cholesterol atherosclerosis.

Two publications have come to my attention since this investigation was undertaken. Etheridge and Hoch-Ligeti[137] studied the lipid deposition in aortas in younger age groups following Cortisone and adrenocorticotropic hormone administration. Thirty-four cases were selected under twenty-one years of age. Most of them had died of leukemia. They were compared with a control group which included twelve cases of leukemia who received similar treatment with X-ray irradiation and anti-folic compound. An apparent increase of lipid deposition was noted in the intima of the aortae in those patients under eleven years of age. The results in the older age groups were equivocal, since lipid deposition was found so frequently in the aorta of the controls. However, in view of the many uncontrolled variable factors the results of this study could not be evaluated.

More recently Cook[138] and his co-workers reported the effects of Cortisone and DCA on total serum cholesterol, lipoproteins and atherosclerosis in the rabbit. In cholesterol-fed rabbits they reported that

Cortisone did not significantly affect the levels of serum lipoproteins, cholesterol concentration, or degree of atherosclerosis, as compared to controls. In view of the high absolute mean value obtained for the serum total cholesterol, it would be interesting to know the relative rise of the phospholipids and neutral fats. However, their results must be considered with the greatest reservation since examination of their data disclosed that the observations and statistical determinations were made on only three Cortisone cholesterol-fed animals and since the arbitrary grading of gross atherosclerotic lesions is too crude to determine accurate differences.

PART II

REPORT OF EXPERIMENT.

1. Experimental Procedures.

It was proposed to conduct an experiment which would determine the effect of Cortisone on the development of atherosclerosis in the rabbit. The morphological and metabolic studies which were undertaken were those which suggested to yield information concerned with the pathogenesis of atherosclerosis.

The Animals: The experimental animals selected were litter mates of pedigreed, New Zealand albino rabbits, which were young, thriving and long-eared. Both sexes were represented, but the animals in each litter were of the same sex. These prerequisites were chosen to control hereditary differences and to insure as hardy a constitution as possible to meet the demands which the experiment might make upon the animal. Their age on arrival ranged from ten to sixteen weeks and their weight from 1.5 to 3.0 kilograms. They were housed in separate metal cages, given a diet of Miracle Rabbit Pellets (prepared by the Ogilvie Flour Mills Co., Ltd.), and water ad libitum. During this sixteen-day period of acclimatization the animals were bled on two occasions in order to establish a base line control of their serum lipids, blood sugars and hemoglobins, with which abnormal values might later be compared. These are shown in Table III.

Animal Groups: Seventy-two of the more robust rabbits were chosen for the experiment. These consisted of nine sets of quadruplets

and twelve sets of triplets. These were arranged in four groups which were to be treated simultaneously, to control factors which the time and season might influence, particularly since hormonal mechanisms were being considered.

Group I received cholesterinized food and the vehicle of Cortisone.

Group II " " " " and Cortisone.

Group III " normal food and Cortisone.

Group IV " normal food and the vehicle of Cortisone.

Group II was the essential experimental group.

Group I served as a cholesterol control for group II.

Group III served as a Cortisone control for group II.

Group IV served as a normal control for groups I, II and III.

Each animal in Group I was compared with a litter mate in Group II and in Group III. In addition there were nine normal control animals in Group IV. These were also litter mates of rabbits in each of the other three groups. Although both sexes were represented, the litter mates in each set were of the same sex. In this manner sex differences were controlled.

A housing plan was arranged to facilitate working conditions and identification of the animals, each rabbit being represented by its litter mate in a comparable cage location. A schematic diagram of this plan is shown in Table II.

Feeding Regime: To control as far as possible any influences which diet might bear upon the development of atherosclerosis each animal was given 100 gm. of food per day per os, an amount found through

experience in this laboratory to be sufficiently nourishing for healthy young rabbits. The cholesterinized food given to Groups I and II was allotted in such a manner as to accommodate certain difficulties which were anticipated. Since the experiment was originally planned to run ninety days, it was felt that a dose of 1.0 gm. of cholesterol per day might produce a degree of atherosclerosis severe enough to mask the expected influence of Cortisone. To allow atherosclerosis to develop to a moderate or marked degree over a period of sixty to ninety days, it was decided arbitrarily to use 0.75 gm. of cholesterol as a daily dose for each rabbit, for six days of the week. Another problem to be considered was that rabbits finding the cholesterol diet distasteful frequently refrain from eating it[16]. Therefore the daily dose of cholesterol was included in only 35 gm. of normal food. This was given early in the morning with the hope that through the length of the day the animal would become hungry enough to eat such a small amount of food, even though it was not so palatable; thus the intake of cholesterol was better assured. In the late afternoon the remaining 65 gm. of normal food was given to Groups I and II to bring the total daily food consumption up to 100 gm. for each rabbit. 100 gm. of normal food was given each morning to Groups III and IV. The daily intake of cholesterol and of normal food was calculated and recorded. The total cholesterol and normal food intake was determined at the end of the experiment and listed in Table X.

Another dietary feature which was critically considered in this experiment was the effect which oil might bear on the blood and tissue

lipids in those animals receiving cholesterol. For it had become the preference of this laboratory to prepare the cholesterinized food with oil, since the latter enhanced the development of atherosclerosis[16,32]. Therefore, to avoid introducing any more extraneous factors in one group than another, it was decided not to use oil[29,34]. A solution of cholesterol in ether was sprinkled evenly over the normal food which was thinly spread in enamel trays. This was rapidly stirred to assure even distribution of the cholesterol on the food, which was then allowed to stand until all the ether had evaporated. To allow for loss along the sides of the containers during this preparation 0.80 gm. of cholesterol was used per 100 gm. of food, instead of 0.75 gm. It was found practical to use 16 gm. of cholesterol dissolved in 150 cc. of ether for 684 gm. of food. Therefore it was assumed that 35 gm. of this preparation contained 0.75 gm. of cholesterol. This was the daily dose used for each rabbit in Groups I and II. The daily ration of the 7th day consisted of 100 gm. of normal food to each rabbit in every group and this was given on the evening of the 6th day.

Cortisone Administration: Cortisone was given to Groups II and III. To control any effects which might be attributed to the vehicle in which Cortisone was suspended, a preparation of the vehicle was obtained and this was given in the same manner and comparable dose to Groups I and IV. The Cortisone and vehicle were given intramuscularly via a tuberculin syringe in which small amounts of the fluids could be measured more accurately.

In short-term experiments[60,81], there was a high mortality

rate within a period of thirty days in rabbits receiving 20 mg. of Cortisone intramuscularly per day. Since a longer-term experiment was undertaken it was necessary to reduce the dose of Cortisone to levels which would produce definite metabolic changes in the animal but which would, nevertheless, allow them to survive long enough for atherosclerosis to develop grossly to a moderate or marked degree. Recently, Adlersberg[82] reported that there were definite elevations of the serum lipids in rabbits given Cortisone in doses identical to, or slightly higher than, the therapeutic doses given to man[50,51,53,83,84,85]. Therefore, the dose decided upon was 3 mg. per kilo body weight per day. The animals were weighed weekly and the dose of Cortisone or vehicle was adjusted accordingly. A daily record was kept of the Cortisone administration and at the end of the experiment the total Cortisone received by each animal was determined as shown in Table XI. The injections were given in the thigh muscles. The overlying skin was first cleansed with 70% alcohol and throughout the experiment no inflammatory or traumatic complications resulted from this procedure.

Animal Weights: The animals were weighed on arrival and weekly every Wednesday thereafter. At autopsy the final weight and carcass weight were taken and the per cent weight gain or loss was calculated for each animal thus: -

$$\frac{\text{Final Weight - Initial Weight}}{\text{Initial Weight}} \times 100 =$$

± per cent weight change of initial weight.

(+ = weight gain; - = weight loss).

See Table XII.

Bleeding Schedule: During the period of acclimatization all the animals were bled on two occasions for determinations of serum lipids, blood sugars, electrolytes and hemoglobins, in order to establish a base line with which abnormal values could later be compared. Since it was not feasible to bleed all the animals on the same day, they were divided arbitrarily into two groups "A" and "B" as shown in Table II. This was planned in such a way that litter mates of groups I, II and III were not broken up. On the fourth experimental day the rabbits in group "A" were bled for determination of serum lipids, blood sugar, hemoglobin and serum electrolytes; on the fifth experimental day the rabbits in group "B" were bled also for these determinations. Then groups "A" and "B" were bled on alternate weeks for serum lipids. Weekly blood sugar determinations were made since they were to be used as an indication of the metabolic state of the animal[60,81]. A final bleeding was performed prior to autopsy for serum lipids, blood sugars, electrolytes and hemoglobins. Blood was taken for electrolyte studies on fewer occasions than for the other chemical analyses. The sera for these were stored in a "deep-freeze".

The animals selected for groups "A" and "B" are shown in Table II. The bleeding schedule is shown below: -

BLEEDING SCHEDULE

Experimental Day	Animal Groups	Chemical Determinations	
-10		Serum Lipids Blood Sugar Hemoglobin Electrolytes	Determinations for a Normal Base Line
-9		Serum Lipids Blood Sugar Hemoglobin Electrolytes	
-3		Serum Lipids Blood Sugar Hemoglobin Electrolytes	
-2		Serum Lipids Blood Sugar Hemoglobin Electrolytes	
(Mon.) 4	"A"	Serum Lipids Blood Sugar Hemoglobin Electrolytes	
(Tues) 5	"B"	Serum Lipids Blood Sugar Hemoglobin Electrolytes	
(Mon.) 11	"A"	Serum Lipids Blood Sugar Hemoglobin	
(Tues) 12	"B"	Blood Sugar	
(Mon.) 18	"B"	Serum Lipids Blood Sugar Hemoglobin	
(Tues) 19	"A"	Blood Sugar	

The above schedule was continued till the end of the experiment.

Blood was obtained by nicking the central artery of the ear with a sharp razor blade. Approximately 10 cc. were taken for lipid chemistry, 5 cc.for electrolytes, $^{1}/_{10}$ of a cc. was drawn up into a micro-pipette for blood sugar estimation and 2 ml. were drawn up in a specially calibrated pipette for the hemoglobin determination.

Chemical Determinations: Determinations were made of the total and free serum cholesterol by a modified Schoenheimer-Sperry method(86). From the values obtained the ester cholesterol was readily derived by merely subtracting the free from the total cholesterol.

The serum phospholipids were extracted with a 3:1 95% alcohol-ether solution. From a 10 cc. aliquot of the resultant filtrate lipid phosphorus determinations were made by a modified method of Youngburg(87). A 25 cc. aliquot of the same filtrate was used for the determination of the total fatty acids. These were o btained by the method of Stoddard and Drury(88) as modified by Man and Gildea(89).

Values for the total and free cholesterol, phospholipids and fatty acids were first obtained as colorimetric galvanometer readings. By applying these to their respective standard curves the cholesterol readings were converted to mg.%, the phospholipids to mg.% and the fatty acids to M.Eq./Litre.

The fatty acids of neutral fat were estimated by the formula of Peters and Man(76):

Fatty acids of neutral =

$$\text{Total fatty acids} - \left[\left(\frac{\text{cholesterol ester} \times 10}{386}\right) + \left(\text{lipid phosphorus} \times 0.58\right)\right]$$

expressed in milli-equivalents per litre.

The blood sugars were determined by a modified Folin's micro method[90,91,92,93,94]. The colorimetric values obtained were converted to mg.% by applying them to a standard sugar curve.

The sera obtained for the electrolyte estimations were carefully sealed and stored in a "deep-freeze" for future use.

Autopsy Technique: An autopsy was performed on each animal. When a rabbit died during the course of the experiment the litter-mates were sacrificed at the same time (except those for the first few mortalities which were allowed to survive until the experiment was terminated). Most of the animals were killed by intravenous nembutal while a few received a blow on the head. Final bleedings were performed for lipid and sugar determinations. The total body weight and carcass weight were taken. (Carcass weight = body weight - the intestinal tract, urinary bladder, spleen and pancreas).

Weights were recorded of the pituitary, adrenals, spleen, liver, kidneys, lungs, heart, brain. Sections of the following tissues were placed in a 10% formalin solution to be kept in stock for future use: adrenal, spleen, kidney, liver, lung, heart, diaphragm, gastrocnemius muscle, pancreas, skin, thymus, gall-bladder, brain. A knee joint was exposed and fixed in 10% formalin. The entire pituitary and sections of the above tissues, except the knee-joint, were also fixed in a 1/4 solution of "pure" formalin/Zenker base for 24 hours,following which the tissues were washed for 24 hours with freely-running tap water, then put into iodine alcohol in preparation for the H.P.S. stain (Haemalum, Phloxin and Saffron).

One-half adrenal and an aliquot of spleen and liver were placed in absolute alcohol for chemical determinations of their lipid content. The last half of adrenal was placed in a dry test tube for future determination of the ascorbic acid content.

The aorta was severed immediately below the heart valves and opened. A small vertical section of its uppermost portion was taken for microscopic examination. This was fixed in a 5% lead subacetate solution for 18-24 hours after which it was washed under freely running tap water for 24 hours. Then it was placed in absolute alcohol in preparation for the toluidine blue stain in order to demonstrate the ground substance.

Schematic diagrams were made of the degree of atherosclerosis observed in the intima. The adventitia was carefully stripped and the remaining intima and media immersed in absolute alcohol for chemical analysis of its lipid content.

2. Results.

When we consider each of the four animal groups as a whole, some striking characteristics and differences were manifested during the course of the experiment.

General Considerations: Throughout the entire experiment all the normal control animals (Group IV) thrived. They presented no problems, were well-adjusted to their new environment and cooperative during experimental procedures. They maintained a good appetite and weight-gain and suffered no mortality until the animals were sacrificed for autopsy. There were no changes produced which could be attributed to the "vehicle" administration.

Many members of Group I - the cholesterol-fed control rabbits - showed a distaste for the cholesterinized food during the first few days of the experiment and refrained from eating it. Several of these animals lost appetite also for the normal food, which was given separately at the end of the day, while a few animals cleverly learned to avoid the cholesterinized food in the morning and await the normal food, which they ate freely. However, most of the animals in this group readjusted to the unnatural diet in a few days and by the end of the first week all the rabbits ate their entire daily ration as heartily as their litter mates in Group IV. Very infrequently, thereafter, did these animals leave any food uneaten.

The cholesterol-fed animals grew and gained in weight at a rate similar to that of the normal control rabbits. They presented clinically a state of good health but for the few exceptions described below.

There were two spontaneous deaths in Group I (cholesterol-fed). W-23 died at the end of thirty-three experimental days. She had refused her food on the previous day and became drowsy and finally comatose. At autopsy a patchy bilateral consolidation of both lungs was found. V-52 died at the end of fifty-five days. This rabbit was the only one with whom rapport was difficult to establish. He was highly strung, nervous and uncooperative. He was poorly groomed since he suffered throughout the entire experiment from diarrhoea which was treated with frequent intramuscular injections of 125 mg. of streptomycin. This at first improved his condition considerably so that until the last two weeks prior to his death he maintained his appetite and weight. Then these decreased rapidly as his diarrhoea increased so that his final weight showed a 17.6% loss over his initial weight. At autopsy his extreme emaciation was accompanied grossly by a marked degree of pyonephrosis.

Particularly during the first two weeks of the experiment many animals suffered from varying degrees of diarrhoea which would respond well to one or two intramuscular injections of 125 mg. of streptomycin. On a few occasions, early in the experiment, all of the animals were injected with streptomycin. This was done for two reasons: one was for a prophylactic measure in an attempt to keep the disease from spreading; the other was to maintain the experiment under control and not introduce any more extraneous factors in one animal than in another. However, this procedure became impractical and had to be abandoned . Thereafter the rabbits were treated for diarrhoea individually and as time went on the condition appeared less frequently.

The Cortisone cholesterol-fed rabbits (Group II) manifested some interesting clinical features soon after the first injection of Cortisone. If they felt any distaste for the cholesterinized food they did not show it but ate all their rations with greater appetite than their normal control litter mates. In addition they developed behaviour patterns which were more readily obvious when compared with Groups I & IV. The increased appetite and personality changes were similarly manifested by the Cortisone control animals (Group III). They were more active and interested in their appearance and surroundings. This state of "well-being" continued for two weeks following which the rabbits in Groups II and III began to lose weight and appetite and became listless.

The first mortality in the Cortisone groups occurred on the thirteenth experimental day when a Cortisone control rabbit, V-90, died. On the twenty-first day of the experiment W-18 of Group II died. Following this the mortality rate in both Contisone Groups II and III rose rapidly. The manner of death in these animals seemed similar. For a day or several days prior to death the rabbit would lose appetite, become listless, drowsy and finally semi-comatose. At autopsy, the adrenals were strikingly reduced in size, the liver larger and fatty in appearance and the kidneys enlarged and pale, suggesting a biochemical death.

By the end of the third experimental week, the rabbits of Groups II and III had lost significantly in weight while their litter mates in Groups I and IV were gaining. In an attempt to keep the litter mates comparable it was decided to increase the food intake of Groups II and III

that their weights might be maintained. Therefore, each Cortisone treated animal was given an extra ration of 50 gm. of normal food per day. Except for those animals who became too ill to eat, this increase in diet did increase the body weights but not to their former levels or to those of their litter mates in Groups I and IV. Since their poor appetites did not seem to warrant further increase in diet, it was necessary to resort to other measures to maintain their weight and prolong their life,, for the condition of the Cortisone-treated rabbits suggested that they would not survive for the length of time necessary for gross atherosclerotic lesions to develop. Therefore the dose of Cortisone was reduced gradually to avoid withdrawal symptoms and increased again to one-half of the original dose. At this level they still showed the effects of Cortisone in that the blood was lipemic and t he urinalyses positive for sugar and acetone. (The blood sugars were not greatly elevated above those of their litter mates in Groups I and II, and only rarely was a blood sugar elevated to diabetic levels = 300 mg%). The animals now showed more promise of survival. From the forty-fourth experimental day onward, as each animal died, his litter mates in the other groups were sacrificed at the same time, so that comparable studies could be made according to the procedures outlined in the preceding section of this report.

Detailed charts were made. Table I shows a list of the animals according to their sex, experimental number, experimental group, and the number of experimental days the animal survived. From this list we can see those animals which can be comparably studied. With the aid of this

chart it can be seen in the housing plan, as arranged in Table II, how readily the animals in each group could be identified and compared with their litter mates in each of the other groups. This arrangement proved to be of great value throughout the performance of the experiment.

Table X shows the total food consumption and cholesterol consumption for each rabbit at the end of the experiment. This is arranged so that the groups and litter mates can be compared. It is important to note that the differences are due not only to the length of time which the animal lived but also to the fact that from the fourteenth experimental day onward Groups II and III received an increase of 50 gm. of normal food per day, above that of Groups I and IV, in an attempt to maintain the body weight. The total cholesterol intake was approximately the same in those litter mates of Groups I and II who survived the same length of time.

A similar chart for the total Cortisone and vehicle administration was drawn up in Table XI so that the animals in each group and the litter mates in each of the four groups can be compared. Here again it must be recalled that the differences in the total amount of Cortisone or vehicle given was not only due to the period of survival (which information is also shown in the chart) but to the fact that the Cortisone was given in mg. per kilo body weight as described in section 1. of this report (Experimental procedures). The change of dose which was discussed above in this section, under general considerations, will be mentioned again here to show the absolute dose values. The Cortisone was given in mg. per kilo body weight thus: -

Experimental Day:	4	22	25	28	39	41
Dose of Cortisone in mg. per kilo Body Weight:	3	2	$1\frac{1}{2}$	$\frac{1}{2}$	1	$1\frac{1}{2}$

Thus, although the total amount received by each animal varied and the dose was altered, each rabbit really received a comparable amount of Cortisone since it was given according to the body weight.

Weekly blood sugars were determined on every rabbit according to the schedule outlined in the previous section on experimental procedures. The values were arranged in Table XIII so that the groups and litter mates could be compared according to the experimental day, and to the dose of Cortisone. Although the blood specimens were taken every Monday and Tuesday morning, since the rabbits were allowed to eat their food ad libitum, fasting blood sugars were obtained only if the rabbit had not been eating for the larger part of the day prior to obtaining the blood. Again, the animal may have eaten the greater part of his daily ration just before the blood was drawn. Then his blood sugar would be at its highest level. So that the range of values obtained in the normal animals and the experimental animals had to be taken into consideration from this point of view. The normal blood sugar levels, as determined in all the animals on the two occasions during the period of acclimatization and of the normal control animals (Group IV) during the experiment, ranged from 77 mg% to 185 mg% with mean values of 101 - 122 mg%. The blood sugar levels of the cholesterol-fed rabbits fell within these ranges also. The Cortisone cholesterol-fed rabbits and the Cortisone

control rabbits showed blood sugar levels which were elevated when compared with those of their normal control litter mates and with the cholesterol-fed litter mates. But these elevations were not very high and, in fact, fell within the normal range as shown in both the individual and mean values. Rarely a level of 200-250 mg% was reached and only one level of 398 mg% was obtained in a semi-comatose rabbit (V-69 of Group III) who died the following day. Since in the rabbit the normal blood sugar range allowed is greater than that in the human and since only one frankly diabetic level (i.e. above 300 mg%) was obtained, although a few approached slightly diabetic levels, (200-250 mg%), the individual differences and mean differences as shown in Tables XIII and XIV were not considered a sufficiently good indication of the metabolic state of the individual animal. With higher doses of Cortisone used in previous experiments the blood sugar level appeared to be a very useful guide to the metabolic state of the rabbit since their values rose to diabetic levels and were considerably different than those of the control animals.

One can only say, therefore, that in the present experiment, the blood sugar in the Cortisone animals tended to rise slightly above those of the cholesterol-fed and normal control rabbits. This became more evident when litter mates were compared. But the trend is shown also in the mean values. As the dose of Cortisone was lowered this tendency was withdrawn and as the dose of Cortisone was again increased, the tendency of the blood sugars to rise was again increased.

It may be noteworthy that the daily injections of Cortisone were given after all the blood specimens had been taken, so that the

level of Cortisone in the animal body would be at its lowest at the time of bleeding and with that the blood sugar might be correspondingly lower. In retrospect it would be interesting to know if the blood sugars would have been significantly elevated if taken shortly after the injections of Cortisone. This may very well be possible - so that the same dose of Cortisone used in this experiment may have produced higher levels of blood sugar than those actually obtained.

Those animals who survived during the second month of the experiment were on two occasions placed in metabolism cages (again litter mates were studied at the same time). Urinalyses in both Cortisone groups disclosed the presence of sugar in the urine which varied from a trace (frequently) to three plus (occasionally) according to Benedict's Qualitative and Quantitative methods[97]. The cholesterol and normal control rabbits did not show the presence of sugar in the urine. Also both Cortisone groups (II and III) showed varying amounts of albumen in the urine which was only infrequently seen in the normal and cholesterol-fed animals. These findings when present reflected the effect of Cortisone.

Table XII shows the body weights according to animal groups, litter mates and experimental day. The weights were taken at weekly intervals and the final weight was taken just prior to autopsy. The per cent weight change and carcass weight were derived as described in section 1 of this report. Groups I and IV showed a 10 to 30% (approx.) weight gain which was more closely comparable in litter mates. The weight gain was changed to a loss in V-52, who suffered from diarrhoea and anorexia. A few animals who had a high initial weight (approx. 3.0 kilograms)

showed a slight weight gain or loss (+8.7%, +2.1%, +0.2%, -5.3%). The animals who were receiving Cortisone suffered almost invariably a weight loss which varied considerably in the different rabbits from -4.1% to 21.9%. When the food was increased and the dose of Cortisone reduced, in an attempt to increase their weight, these animals began to regain their initial weight. Some even showed a final increase which, however, was minimal except in a few cases which rose from 10 to 15% (approx.)

The possibility that this weight loss in the Cortisone rabbits fed cholesterol might influence the development of atherosclerosis was considered.

The Cortisone-treated rabbits and the cholesterol-fed rabbits treated with Cortisone constantly had lower body weights than the corresponding litter mates (with the exception of those mentioned above). This weight change was invariably associated with hypercholesterolemia and, in Group II, with a lesser degree of gross atherosclerosis in the aorta (as compared with the cholesterol-fed controls).

These findings correspond to those observed by Firstbrook[95] who concluded that relative undernutrition or weight loss leads to partial inhibition of the development of atherosclerosis.

Experiments in this laboratory[96] were undertaken to study the effects of undernutrition on the development of atherosclerosis in the rabbit. These showed that undernutrition was associated with loss of weight, higher levels of hypercholesterolemia and a decreased severity of gross aortic atherosclerosis than in the control animals. No correlation was found between the state of nutrition or degree of weight

loss or weight gain and the severity of atherosclerosis when the serum cholesterol levels were not taken into account[96]. At present there is no conclusive evidence that weight loss inhibits the development of atherosclerosis. In this experiment it may be that the weight changes are factors which are merely constantly associated with nutrition, hypercholesterolemia, the dose of Cortisone and the degree of atherosclerosis, in view of which weight changes, in themselves, are not necessarily the direct cause of the experimental results.

Serum Lipid Studies: Examination of the serum lipids revealed several interesting results. These showed consistencies and trends in the different experimental groups which suggested certain associations in the genesis of experimental cholesterol atherosclerosis in the rabbit.

In all three experimental groups (Cortisone rabbits fed cholesterol, cholesterol control rabbits and Cortisone control rabbits) there were elevations of all the serum lipid fractions. However, the absolute levels and proportionate rise of the lipid constituents were different in each group. These hyperlipemic states were accompanied by a grossly visible lipemia in both the Cortisone and cholesterol-fed rabbits which varied from a mild to marked degree in the individual rabbits of each group but did not present any striking differences between the groups.

The serum total cholesterol in the cholesterol-fed control rabbits (Group I) rose to abnormally high levels soon after cholesterol-feeding was begun. On the fourth experimental day these values ranged from a normal level of 40 mg% to a peak of 460 mg%. Four of these were well within the normal range, five closely approached or were bordering

on abnormal levels (110 - 150 mg%) while the remaining twelve in that group were above the normal limits. On subsequent bi-weekly determinations these values rose considerably showing in most cases a moderate to marked elevation (200 - 700 mg%) and on a number of occasions levels above 1000 mg% were reached. Rarely a value which fell within normal limits was obtained. There were wide individual variations in the total serum cholesterol levels. Those animals who attained a high serum total cholesterol level early tended to increase and maintain those high levels, while those animals who developed a slight or moderate elevation of the total cholesterol increased their levels accordingly. Yet on subsequent bleeding days, in each animal, levels both above and below those of the previous week were observed, although cholesterol feeding was continued daily. These alterations might be related to the time of bleeding in relation to the time of cholesterol consumption or to the degree of weight change. Table IV a, b, c, and d show the serum total cholesterol levels of the rabbits in each group according to the experimental day. In each of these tables the litter mates are in a comparable sequence.

The mean values of the serum total cholesterol levels of each bleeding day were obtained and the standard error of the mean was calculated. These are shown in Table III. The normal mean value of the cholesterol-fed rabbits (Group I) was 64.7 mg%. This rose rapidly to 191.7 mg%. on the fourth experimental day and to 431 mg%. one week later. Thereafter Group I maintained markedly elevated mean levels of serum total cholesterol until the experiment was terminated at sixty days, with a peak of

606 mg% on the twenty-fifth experimental day. From the experience of this laboratory this pattern of hypercholesterolemia is considered sufficient to produce a mild to moderate degree of experimental cholesterol atherosclerosis in rabbits in forty-five to sixty days.

The cholesterol-fed rabbits injected with Cortisone (Group II) showed elevations of the serum total cholesterol considerably and almost consistently lower than those in the cholesterol-fed control rabbits. This difference was more consistent when litter mates were compared and was also clearly demonstrated by the mean values of the total serum cholesterol levels of these two groups. Graphs 1 to 11 show these features in comparable litter mates while Graph 12 is similarly drawn up from the mean values of the animals in each group. On each bleeding day the mean value of the serum total cholesterol of Group II rose less rapidly and to a much lower absolute level than that of Group I. This pattern was consistent until day forty-five - forty-six of the experiment when the mean serum total cholesterol of Group II was 425 mg% as compared to 325 mg% in Group I. Several explanations may be offered for this change. In Group I the number of values used for the mean determination was eleven with a standard error of $\pm$72.7, while the mean value of 425 mg% in Group II at that time was derived from only six figures with a standard error of $\pm$163.7. Other factors, however, may be responsible for this. For at this stage of the experiment the dose of Cortisone had been reduced, and associated with this was a more rapid rise in the serum total cholesterol levels of the Cortisone cholesterol-fed rabbits which approached more closely, and in some cases even surpassed, the

levels of their cholesterol-fed litter mates. This appeared as a "compensatory rise" of serum cholesterol attending the withdrawal of Cortisone. Actually this elevation of the serum total cholesterol in Group II toward and above those levels in Group I appeared as a delayed reaction. For upon the first reduction of the dose of Cortisone there was frequently a fall in the total serum cholesterol below that of previous levels, and occasionally a slight to moderate rise above previous levels. Subsequently, when the Cortisone was increased again to 1/2 the original dose almost all the animals in Group II showed a general rise in the serum total cholesterol.

The serum total cholesterol levels of the Cortisone control rabbits (Group III) rose slowly and to lower levels than those of their cholesterol-fed Cortisone litter mates. In fact, until the twenty-fifth day of the experiment their levels fell within the normal range. Then they rose gradually to levels slightly above normal. Only one animal reached a peak of 346 mg% while on a few occasions normal values were still obtained during the last thirty days of the experiment. The mean values showed the same trend with a gradual rise to a final level of 198.9 mg%.

The serum cholesterol levels of the normal control litter mates remained within normal limits throughout the experiment.

Again these patterns may be seen in Graphs 1 to 11 where they can be compared to those of their litter mates in the other experimental groups, while Graph 12 shows their mean values.

The elevation of the serum total cholesterol in the cholesterol

control rabbits was always due to a greater rise in the serum cholesterol ester than in the free cholesterol. With higher absolute values of serum total cholesterol the proportionate rise of the ester fraction resulted in a greater difference between the levels of the ester and free cholesterol. In the cholesterol-fed rabbits injected with Cortisone the elevation of ester cholesterol was also greater than that of the free cholesterol, except on several occasions when the free cholesterol rose above that of the ester. These are shown clearly in Graphs 1, 2, 3 and 11. The reversal of the proportions of the free to ester cholesterol occurred even more frequently in the Cortisone control litter mates. This pattern begins to approach that of the alloxan diabetic rabbit[61] in which the elevation of the total cholesterol was due much more to an increase in the free cholesterol than to an increase in the ester fraction. Although in the alloxan diabetic rabbit the serum total cholesterol reached much higher levels than those of the Cortisone treated rabbits in this experiment, the change in the free to ester ratio occurred also in those alloxan diabetic rabbits which showed a lesser rise or no increase in the total cholesterol.

Tables XV and XVI show the ratios of the mean values of the serum cholesterol fractions in each of the four groups throughout the experiment.

In each experimental group the elevation of the serum total cholesterol was accompanied by an elevation of the serum phospholipids. The proportionate rise of the serum phospholipids to that of the serum total cholesterol was not the same in the three groups. The different total cholesterol to phospholipid ratios were due to the difference in

the absolute elevations of the serum total cholesterol since the phospholipid elevations in the three groups were similar. With alteration of the dose of Cortisone there were fluctuations in the lipid phosphorus levels but the elevation above the normal levels was maintained. The lipid phosphorus as related to the other serum lipids is shown in Graphs 1 to 11 where the experimental groups can be compared according to their litter mates, while Graph 12 shows that the mean values obey similar phsopholipid patterns. Closer studies of these ratios, as seen in Table XVII, show that in Group I (cholesterol-fed control) the total cholesterol rose out of all proportion to the serum phospholipids. In Group III (the Cortisone control rabbits) the phospholipids ran a course more closely parallel to that of the total cholesterol so that the total cholesterol/phospholipid ratio closely approached that of normal rabbit sera, while the total cholesterol to phospholipid ratio of the Cortisone cholesterol-fed rabbits was between that of Group I and Group II.

It may be noteworthy to mention here that the alloxan diabetic and alloxan diabetic cholesterol-fed rabbits present serum total cholesterol to phospholipid ratios which appear to be similar to those described above, even though the absolute lipid values were much higher in the alloxan rabbits. So that, for purposes of comparison, if we substitute the alloxan diabetic rabbit for the Cortisone rabbit, and the alloxan diabetic cholesterol-fed rabbit for the Cortisone cholesterol-fed rabbit, and compare them with cholesterol-fed and normal controls, a comparable pattern of total cholesterol to phospholipid ratios is obtained. That is, the alloxan diabetic rabbit shows a total cholesterol

to phospholipid ratio which closely approaches that of normal, and this ratio is approached less closely by the alloxan-diabetic cheolsterol-fed rabbit. It is possible that with higher doses of Cortisone the lipid patterns of the Cortisone cholesterol-fed rabbits may resemble more closely those of the alloxan-diabetic cholesterol-fed rabbits, in absolute values as well as in proportionate values. For the lipid patterns observed by Kobernick and More[60] in rabbits with higher doses of Cortisone were more similar to those of the alloxan-diabetic rabbits. The importance of these observations will be discussed further.

A study of the total serum fatty acids and fatty acids of neutral fat produced additional facts which may prove to have an important bearing on the problem of the genesis of atherosclerosis. As all the other lipid constituents in each of the three experimental groups rose, the total serum fatty acids and fatty acids of neutral fat were also increased. But they presented a strikingly interesting disproportion to the other lipid fractions in their respective experimental group and to each other. The data of the fatty acids has been arranged to show these features in Tables III, VIII, IX and XVIII.

The serum total fatty acids of the Cortisone cholesterol-fed rabbits and their Cortisone control litter mates rose rapidly and steeply above the normal control base line, while in the cholesterol-fed controls the elevation was only moderate and roughly one-third that of the Cortisone groups, in which the levels of fatty acids were to all intents and purposes the same. These lipid patterns were consistently obtained, and even moreso when litter mates were compared. Graph 17 also demon-

MEAN VALUES OF SERUM FATTY ACIDS

Serum Lipid	Experm. Group	Experimental Day								
		-10	4	11	18	25	30-32	38-39	45-46	49-60
Total	I	10.2	10.1	17.1	18.2	24.5	13.4	22.0	11.8	17.2
Fatty	II	8.7	14.4	38.2	41.5	73.1	30.4	29.0	45.0	42.5
Âcids	III	8.05	12.7	31.4	46.1	65.5	31.8	28.9	70.8	67.8
M.Eq./L	IV	7.5	5.3	6.5	5.6	5.9	6.3	5.3	5.8	5.1
Fatty	I	4.58	3.19	2.91	3.48	8.43	3.63	6.24	2.56	2.78
Acids	II	5.02	8.32	26.52	31.62	60.26	23.47	18.41	27.07	38.23
of Neutral	III	4.23	8.71	27.06	40.70	56.48	25.93	20.55	59.04	53.24
Fat	IV	3.56	2.7	4.00	2.20	2.85	1 case	2.18	2.26	0.97
M.Eq./L.										

strates these features as they are observed by the mean values. Comparison of Tables VIII and IX will show that the elevations in the serum total fatty acids are relative to the fatty acids of neutral fat, both presenting the same picture - a moderate increase in the cholesterol-fed rabbits and much greater elevation in the Cortisone cholesterol-fed rabbits and their Cortisone control litter mates. Indeed in the latter the fatty acids of neutral fat showed a tendency toward levels still higher than those of the Cortisone cholesterol-fed rabbits.

In view of the striking difference between the fatty acids in the Cortisone rabbits fed cholesterol and the cholesterol-fed control rabbits the possibility arises that these lipids may play an important role in the genesis of experimental cholesterol atherosclerosis. It became, therefore, prerequisite to submit these values to statistical analyses to determine their significance. The "T-test" was applied to the mean values of the serum total fatty acids of the Cortisone cholesterol-fed rabbits and their cholesterol-fed controls of each bleeding day. Using the formula

$$T = \frac{V_1 - V_2}{\sqrt{(SV_1)^2 + (SV_2)^2}}$$

the values were found to be very significant.when the probability of error was derived as shown on the following page.

At this point certain fatty acid lipid patterns observed in the alloxan-diabetic rabbits[61,65] and in rabbits injected intravenously with the detergent Tween 80[67,68,69,114] are brought to mind.

T-TEST AS APPLIED TO THE MEAN VALUES OF THE TOTAL FATTY ACIDS OF GROUPS I AND II

Formulae	-10	4	11	18	25	30-32	38-39	45-46	49-60
V_1	10.18	10.14	17.14	18.20	24.50	13.41	22.00	11.75	17.16
V_2	8.7	14.41	38.23	41.50	73.13	30.42	29.00	45.00	42.45
SV_1	± 2.0	± 0.7	± 2.0	± 2.7	± 4.1	± 1.8	± 5.0	± 2.2	± 1.8
SV_2	± 0.7	± 1.3	± 4.8	± 6.2	±14.4	± 4.3	± 7.2	± 7.1	±13.0
T	0.698	2.885	4.056	3.447	3.248	3.650	0.799	4.475	1.926
$N_1 + N_2$	39	35	21	20	18	17	17	15	31
D. F.	37	33	19	18	16	15	15	13	29
P ≐ (probability)	0.5	0.01	0.001	0.001	0.01	0.001	0.4	0.001	(0.05)?

V_1 - mean value of total fatty acids of Group I - cholesterol control.

V_2 - mean value of total fatty acids of Group II - Cortisone cholesterol-fed rabbits.

SV_1 & SV_2 - standard error of the mean values

N - number of animals.

D. F. - degrees of freedom.

P - probability (approx.)

In the alloxan-diabetic rabbits and in the alloxan-diabetic rabbits fed cholesterol the fatty acids of neutral fat showed a rise which was much greater in proportion to that of the total cholesterol. when compared to cholesterol-fed control rabbits or "alloxan-recovered" rabbits. Also, relative to normal proportions, the fatty acids were increased more than the phospholipids. This pattern of lipid proportions was accompanied by inhibition of atherosclerosis even in the presence of marked hypercholesterolemia. Except for the absolute levels of the hypercholesterolemia, the fatty acid patterns in the present experiment resemble those of alloxan-diabetic and alloxan-diabetic cholesterol-fed rabbits.

In cholesterol-fed rabbits injected intravenously with Tween 80 (polyoxyalkylene sorbitan mono-oleate) the serum cholesterol rose to higher levels than in the cholesterol-fed controls. This was accompanied by a markedly elevated lipid phosphorus which paralleled the total cholesterol as compared to the decreased phospholipid/total cholesterol ratio in the controls. However, the fatty acids of neutral fat were not greatly elevated[69]. In this last respect the rabbits injected with intravenous Tween 80 differed from both alloxan-diabetic rabbits described above and the Cortisone injected rabbits reported herein. Each of these experiments were accompanied by inhibition of experimental cholesterol atherosclerosis (which finding is established in the former two, but not definitely in the latter). This suggests that the fatty acids of neutral fat do not play as important a role as the phospholipids, the latter being consistently and proportionately elevated in all three types of inhibition experiments. But neither

does it rule out the possibility that the fatty acids of neutral fat are important in the genesis of experimental cholesterol atherosclerosis, particularly since it has been suggested that Tween 80 may produce an inhibitory effect on the development of experimental cholesterol atherosclerosis independent of the changes produced in the serum lipids. To add to this thought Ahrens[80], in his studies, found that the serum neutral fats were considerably elevated in cholesterol-fed rabbits injected intravenously with Tween 80.

3. Discussion.

In cholesterol-fed rabbits injected with Cortisone certain lipid patterns were obtained which were associated with a modification of the development of experimental cholesterol atherosclerosis. These patterns are in many ways comparable to those of other experiments which produced inhibitory effects on experimental cholesterol atherosclerosis in rabbits. Of these, the importance of the serum phospholipids is again stressed. In the Cortisone treated rabbits the phospholipid increment was more proportionate to that of the serum total cholesterol than in cholesterol-fed controls. This was associated with a lesser degree of atherosclerosis in the Cortisone cholesterol-fed rabbits than in their cholesterol-fed litter mates. This association has been more strikingly demonstrated in the alloxan-diabetic cholesterol-fed rabbit and in the cholesterol-fed rabbit injected intravenously with the detergent Tween 80, in both of which the inhibition of the development of atherosclerosis was definitely established. The mechanism whereby these similar effects and associations are produced is far from clear and may be quite different in the three types of experiments. Tween 80, via its detergent qualities, may improve the state of colloidal suspension of the hyperlipemic sera and thus inhibit the deposition of lipids in the intima, or it may exert its effect largely through the elevated serum phospholipids. It is also possible that Tween 80 may in itself in some as yet unknown way be responsible for the inhibition. The lipid patterns of the Cortisone-treated rabbits fed cholesterol bore a closer resemblance to those in the alloxan-diabetic rabbit in that the total fatty acids

and the fatty acids of neutral fat were in both markedly elevated, in addition to the other lipid fractions. Since Cortisone produces a diabetic state similar to that in the alloxan-diabetic rabbit, it is possible that the inhibitory effect of Cortisone on experimental cholesterol atherosclerosis is through this diabetic state. Even if this were so, the mechanism whereby Cortisone inhibits the development of atherosclerosis would not be clarified, since the relationship of alloxan diabetes to the inhibition of atherosclerosis is far from clear. It may be that Cortisone by altering the carbohydrate metabolism causes an increased combustion of fat[123]. For this purpose the fat depots would be mobilized and their depletion would be manifested by a loss of weight, such as was demonstrated in the Cortisone-treated rabbits. The appearance of increased amounts of lipids in the serum would be a reflection of this altered metabolism. Cholesterol esters are formed in the intestine as a vehicle for fatty acid absorption and transport and are brought to the liver where they are transferred to choline phosphatids (i.e. lecithin) in preparation for further fat metabolism. Since the liver occupies a key position in the regulation of cholesterol metabolism Cortisone may induce its effect through its influence on this organ by altering the turnover rate of liver phospholipids. For the livers of Cortisone-treated rabbits become larger and paler and filled with glycogen.

It is possible that Cortisone may exert its influence by interfering with the intestinal absorption or by increasing the excretion of cholesterol, thus giving rise to the lower absolute levels of serum cholesterol seen in the Cortisone cholesterol-fed rabbits in this experiment.

However, further study of these mechanisms are beyond the scope of this work.

Having acknowledged the presence of those serum lipid relations which are shown as important factors in the inhibition of experimental cholesterol atherosclerosis, we are still faced with three grave problems in this experiment: the absolute levels of serum cholesterol, the reduced number of animals, and the weight change. First we must reconsider the absolute levels of the serum total cholesterol. Although hypercholesterolemia per se is not as important in the development of experimental cholesterol atherosclerosis as the serum lipid proportions, it must, nevertheless, be present in sufficient levels in order to produce atherosclerotic lesions at all, irrespective of the lipid interrelationships. Reviewing the data in this light, according to previous observations in this laboratory, from the absolute levels of cholesterol we can estimate, roughly, that within forty-five to sixty days an expected degree of atherosclerosis from "one to two plus" would develop in the Cortisone rabbits fed cholesterol. If that is the case,this development was modified in these rabbits, since none developed lesions much larger than a trace or low one plus. When the aortae of litter mates were compared the Cortisone cholesterol-fed rabbits consistently showed a lesser degree of atherosclerosis than their cholesterol-fed litter mates. These differences are definitely shown in the litter mates V-64 - V-65, V-88 - V-89, W-5 - W-6, W-9 - W-10 (diagram 1) in which the cholesterol-fed rabbits have developed a degree of "two plus" atherosclerosis compared to minimal lesions in the Cortisone cholesterol-fed litter mates. Of the remaining litter mates

which survived long enough for atherosclerosis to develop, V-77 and V-79 were both refractory (which phenomenon in itself shows the importance of using litter mate controls in these experiments). The differences between the litter mates V-52 - V-53, V-67 - V-68, V-71 - V-72, V-84 - V-85, V-97 - V-98, W-1 - W-2, W-29 - W-30 (diagrams 2 and 3) showed also a lesser degree of atherosclerosis in the Cortisone cholesterol-fed animals than in their cholesterol-fed litter mates. However, the significance of the difference between these rabbits was greatly reduced by the fact that only slight lesions were being compared to minimal lesions. Nevertheless, consistency was maintained in that no Cortisone cholesterol-fed rabbit developed any more than a "one plus" atherosclerosis and no cholesterol-fed rabbit developed more atherosclerosis than his cholesterol control litter mate. The importance of using litter mates repeats itself in these observations as well as in those of the serum lipid patterns.

It still remains to decide whether the difference in the absolute serum total cholesterol levels alone would be sufficient to cause the differences in degree of atherosclerosis which were described above. This possibility cannot be denied since we have been reduced to comparing such small differences in so few animals. However, the possibility that Cortisone may inhibit the development of experimental cholesterol atherosclerosis is still suggested, since the Cortisone cholesterol-fed rabbits developed phospholipid and fatty acid (or neutral fat) patterns which are considered important in the inhibition of atherosclerosis. If this experiment could be conducted over a longer period of

time with sufficiently high doses of Cortisone this possibility might be more definitely established.

The problem which the weight loss introduces in this experiment has previously been discussed. Although Cortisone may produce weight loss independent of the inhibition of atherosclerosis, the relationship of weight loss to inhibition of atherosclerosis may be more closely related[95,120].

This report has repeatedly emphasized the importance of the interrelationship of the serum lipids and their relationship to the serum proteins, rather than the absolute levels of the total serum cholesterol, in the genesis of experimental cholesterol atherosclerosis. Of the various lipid constituents the phospholipids seem to be particularly important in their relationship to the serum total cholesterol, for, when they were proportionately increased with the cholesterol elevations, there was an inhibition of atherosclerosis irrespective of the absolute levels of the cholesterol. This influence has been attributed to the emulsifying property of the hydrophilic phospholipids which enables them to "solubilize" the hydrophobic cholesterol[80] and maintain a state of colloidal suspension. When the phospholipid to cholesterol ratio is reduced the colloidal state of the lipids is deranged so that the cholesterol is not kept as much in suspension, but becomes deposited in the intima. The influence of the elevated phospholipids on the colloidal state of hyperlipemic sera has been frequently referred to as "stabilizing" since this state was accompanied by inhibition of the development of atherosclerosis. However, it is important to note

that hyperlipemic states are not in the true sense of the word "stable" even though the lipid ratios may approach those of normal sera. But with regard to atherosclerosis hyperlipemic states are referred to as being "stable" or "instable" insofar as they are associated with inhibition or potentiation of the deposition of lipids in the intima.

It should be noted also that factors other than the phospholipids influence the solubility of lipids and the stability of their colloidal states in body fluids. Cohn[122] and his collaborators have shown that in human plasma almost all the lipid is in combination with an alpha and a beta globulin - approximately 25% as an alpha and 25% as a beta lipoprotein. The beta lipoprotein is under certain conditions soluble in aqueous media despite the fact that it is composed largely of lipid. It is probable that these lipoproteins greatly influence the solubility and colloidal stability of the blood lipids and therefore play an important role in the genesis of atherosclerosis. This field awaits further investigation.

SUMMARY AND CONCLUSIONS

An experiment was undertaken to study the morphological and metabolic effects of prolonged treatment with Cortisone on Experimental Cholesterol Atherosclerosis in the Rabbit. Control measures were taken to validate as much as possible the experimental results. Pedigreed rabbits were arranged in groups so that litter mates could be compared.

Group I received Cholesterinized food

Group II received Cholesterinized food and Cortisone

Group III received Cortisone

Group IV served as a normal control.

From this study a number of striking and consistent results were obtained which suggested persuasively that Cortisone inhibits the development of experimental cholesterol atherosclerosis in rabbits. Of these, the proportionate elevations of the serum phospholipids and neutral fats to the total cholesterol, in the Cortisone treated cholesterol-fed rabbits, were the most outstanding. The lipid patterns were in many respects like those which were found to be associated with the inhibition of atherosclerosis in other experiments, namely the alloxan-diabetic cholesterol-fed rabbit[61,62,65] and the cholesterol-fed rabbit injected intravenously with the detergent Tween 80[67,68,69]. However, the inhibitory effects of the cholesterol-fed rabbit treated with Cortisone could not be properly established since the high mortality rate shortened the experimental term and reduced the number of comparable animals below significant values.

The possible interference of other factors which escaped the bounds of control were considered, particularly that of weight change.

It was also suggested that with an adequate dose of Cortisone over a longer period of time the possibility that Cortisone may inhibit the development of experimental cholesterol atherosclerosis in rabbits might be more definitely established. The importance of the interrelationships of the serum lipids is further emphasized.

Studies of the morphology and chemical compositon of the aortae and other tissues are still underway.

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TABLE I

INDEX OF EXPERIMENTAL ANIMALS

Sex of litter mates	Animal number	Experim. Group	Total Experim. Days.
Male litter mates	V-52	I	55
	V-53	II	56
	V-54	III	56
	V-55	IV	56
Male litter mates	V-56	I	61
	V-57	II	29
	V-58	III	24
	V-59	IV	61
Female litter mates	V-60	I	61
	V-61	II	25
	V-62	III	40
	V-63	IV	61
Male litter mates	V-64	I	57
	V-65	II	57
	V-66	III	57
Male litter mates	V-67	I	55
	V-68	II	55
	V-69	III	55
	V-70	IV	56
Female litter mates	V-71	I	44
	V-72	II	44
	V-73	III	41
Female litter mates	V-74	I	61
	V-75	II	24
	V-76	III	61
Female litter mates	V-77	I	60
	V-78	-	Rejected
	V-79	II	60
	V-80	III	60
Female litter mates	V-81	I	61
	V-82	II	41
	V-83	III	39
Male litter mates	V-84	I	55
	V-85	II	55
	V-86	-	Rejected
	V-87	III	55
Male litter mates	V-88	I	57
	V-89	II	57
	V-90	III	13
Male litter mates	V-91	-	All Rejected
	V-92	-	
	V-93	-	
Female litter mates	V-94	I	61
	V-95	II	24
	V-96	-	Rejected
Male litter mates	V-97	I	45
	V-98	II	45
	V-99	III	45
	V-100	IV	45
Female litter mates	W-1	I	49
	W-2	II	49
	W-3	III	49
	W-4	IV	49
Male litter mates	W-5	I	46
	W-6	II	46
	W-7	III	46
	W-8	IV	46
Female litter mates	W-9	I	54
	W-10	II	54
	W-11	III	54
	W-12	IV	54
Female litter mates	W-13	I	57
	W-14	II	29
	W-15	III	57
Male litter mates	W-16	-	Rejected
	W-17	I	61
	W-18	II	21
	W-19	III	26
Male litter mates	W-20	I	56
	W-21	II	24
	W-22	III	55
Female litter mates	W-23	I	33
	W-24	II	29
	W-25	III	26
Male litter mates	W-26	-	all rejected
	W-27	-	
	W-28	-	
Female litter mates	W-29	I	61
	W-30	II	60
	W-31	III	60
	W-32	IV	61

TABLE II

SCHEMATIC HOUSING DIAGRAM OF ANIMAL GROUPS

	I	II	III	IV
	W-23	W-24	W-25	
B	V-88 V-94 W-13 W-17 W-20	V-89 V-95 W-14 W-18 W-21	V-90 V-96 W-15 W-19 W-22	
B	V-71 V-74 V-77 V-81 V-84	V-72 V-75 V-79 V-82 V-85	V-73 V-76 V-80 V-83 V-87	
A	W-1 W-5 W-9 W-29 V-64	W-2 W-6 W-10 W-30 V-65	W-3 W-7 W-11 W-31 V-66	W-4 W-8 W-12 W-32
A	V-52 V-56 V-60 V-67 V-97	V-53 V-57 V-61 V-68 V-98	V-54 V-58 V-62 V-69 V-99	V-55 V-59 V-63 V-70 V-100
	Cholesterol + Vehicle	Cholesterol + Cortisone	Normal Food + Cortisone	Normal Food + Vehicle

TABLE III

MEAN VALUES AND STANDARD ERROR OF THE MEAN VALUES OF THE SERUM LIPIDS

Serum Lipid	Expm. Group	Experimental Day -10 M.	-10 S.E.	4 M.	4 S.E.	11 M.	11 S.E.	18 M.	18 S.E.	25 M.	25 S.E.	30-32 M.	30-32 S.E.	38-39 M.	38-39 S.E.	45-46 M.	45-46 S.E.	49-60 M.	49-60 S.E.
Total Cholesterol mg%	I	64.7	±12.0	191.7	±27.2	431.1	±69.5	446.0	±81.4	606.0	±113.1	366.0	±77.8	461.0	±105.2	325.2	± 72.7	598.1	±74.5
	II	51.1	± 6.0	113.5	±10.4	265.2	±55.6	209.0	±79.6	268.0	± 68.8	117.0	± 9.9	193.0	± 64.7	425.6	±163.7	229.6	±33.0
	III	51.1	± 4.7	46.0	± 4.4	59.8	± 9.9	108.2	±14.9	126.0	± 25.0	109.7	±22.2	135.1	± 20.7	193.0	± 22.1	198.9	±26.2
	IV	39.5	± 7.1	35.8	± 6.1	34.0	± 5.0	55.6	±15.5	39.2	± 7.0	128 -	1 case*	50.0	± 8.8	77.0	± 14.5	153.3	±25.5
Ester Cholesterol mg%	I	49.9	± 7.3	145.0	±20.2	328.8	±53.3	329.0	±56.1	457.9	± 85.3	275.1	±66.1	324.8	± 70.9	241.5	± 56.2	433.7	±58.2
	II	42.0	± 4.7	89.5	± 8.3	192.7	±40.4	137.0	±56.8	167.8	± 40.6	65.7	± 6.4	127.8	± 49.8	259.2	± 99.5	143.0	±25.2
	III	41.4	± 2.9	35.3	± 4.0	32.8	± 6.6	57.6	± 6.6	58.7	± 11.7	58.7	±14.2	76.7	± 16.0	107.1	± 8.1	81.9	± 7.1
	IV	29.1	± 7.7	30.8	± 5.6	24.8	± 4.2	37.5	±12.4	36.8	± 14.3	(113-	1 case)*	36.7	± 10.9	58.2	± 14.1	(110.0	±24.7)?)
Free Cholesterol mg%	I	14.8	± 5.9	46.3	± 7.3	102.3	±16.7	118.0	±26.4	148.1	± 28.8	90.9	±14.3	136.3	± 39.9	83.7	± 17.3	164.4	±17.6
	II	9.2	± 1.6	24.5	± 2.6	72.6	±15.9	73.0	±23.0	100.9	± 25.9	59.0	±13.5	66.0	± 17.5	166.4	± 67.8	92.1	±16.2
	III	11.5	± 1.4	10.7	± 1.1	27.0	± 6.2	50.7	±10.5	67.3	± 18.9	51.0	± 8.4	58.4	± 11.8	85.8	± 33.3	117.0	±23.3
	IV	10.9	± 3.2	5.0	± 0.9	9.2	± 1.7	6.5	± 0.3	13.0	± 4.5	(15.0	± 0.0)*	13.3	± 5.1	18.8	± 5.5	43.5	± 3.2
Phospholipids mg%	I	5.2	± 0.7	7.5	± 0.4	10.8	± 1.3	12.2	± 2.0	13.2	± 2.0	9.9	± 0.9	15.5	± 3.1	9.3	± 1.3	10.2	± 1.1
	II	4.9	± 0.3	6.9	± 0.4	11.4	± 0.9	10.9	± 1.5	15.2	± 2.4	10.5	± 0.8	12.1	± 1.4	17.4	± 4.3	10.2	± 2.1
	III	5.1	± 0.2	5.7	± 0.4	7.5	± 0.8	10.7	± 1.8	12.5	± 2.3	9.9	± 0.9	10.9	± 1.1	15.5	± 3.3	14.6	± 2.5
	IV	4.7	± 0.5	3.6	± 0.2	4.0	±	4.2	± 0.2	3.6	± 0.2	4.0	± 0.8	3.6	± 0.4	3.6	± 0.4	3.9	± 0.5
Total Fatty Acids M.Eq./L.	I	10.2	± 2.0	10.1	± 0.7	17.1	± 2.0	18.2	± 2.7	24.5	± 4.1	13.4	± 1.8	22.0	± 5.0	11.8	± 2.2	17.2	± 1.8
	II	8.7	± 0.7	14.4	± 1.3	38.2	± 4.8	41.5	± 6.2	73.1	± 14.4	30.4	± 4.3	29.0	± 7.2	45.0	± 7.1	42.5	±13.0
	III	8.5	± 0.3	12.7	± 1.2	31.4	± 4.5	46.1	± 9.6	65.5	± 17.9	31.8	± 3.9	28.9	± 5.0	70.8	± 17.3	67.8	±18.9
	IV	7.5	± 0.8	5.3	± 0.5	6.5	± 0.7	5.6	± 0.4	5.9	± 0.4	6.3	± 0.6	5.3	± 0.3	5.8	± 0.9	5.1	± 0.1

Mean values of Serum Fattv Acids of Neutral Fat are included in the text.
* 1 case only.

Formulae used in computing the Standard Deviations and Standard Errors of the Mean Values.

$$S = \sqrt{\frac{Ev^2 - \bar{v}Ev}{N - 1}}$$

where: S - standard deviation
E - sum of
v - variable
$\bar{v}$ - mean of the variables
N - number of variables.

$$S\bar{v} = \frac{S}{\sqrt{N-1}}$$

where: $S\bar{v}$ - standard error of the mean
S - standard deviation
N - number of variables.

TABLE IV-a

SERUM CHOLESTEROL - TOTAL - mg%.

Group I - Cholesterol

Animal No.	Experimental Day														
	-10	4	11	18	25	32	38	39	45	46	50	53	56	57	60
V-52	36	150	590		188			560							
V-56	(222)?	460	406		480			76				140			230
V-60	40	114	578		1190		1062					1050			660
V-67	48	208	400		726			124				518			
V-97	52	118	114		632			210	90						
W-1	60	162	198		150			154			220				
W-5	90	320	904		1190			920							
W-9	140	210	340		526			400				490			
W-29		232	480		820			300				590			474
V-64	36	134	502		930		720					972		1362	
V-71	26	400		380	190										
V-74	44	40		650		220				450					710
V-77	52	190		70		278				154					150
V-81	62	406		890		670				470					660
V-84	44	200		284		180				140					
V-88	40	180		406		246				394				670	
V-94	54	30		626			546			780					820
W-13		258		584		626				430				700	
W-17		64		182		182				216					350
W-20	54	110		388		526				128			598		
W-23		40	230		250										
Dose Cortisone - mg/kilo Body Wt.		0													

TABLE IV-b

SERUM CHOLESTEROL - TOTAL - mg%.

Group II - Cholesterol + Cortisone.

Animal No.	Experimental Day														
	-10	4	11	18	25	32	38	39	45	46	50	53	56	57	60
V-53	(3)?	90	150		196			300				200	368		
V-57	54	218	710		844										
V-61	52	104	230												
V-68	26	54	136		168			124				310			
V-98	60	110	356		190			620	474						
W-2	36	118	250		140		254				210				
W-6	48	96	180		224			182		972					
W-10	60	144	230		320			90				278			
W-30	40	52	82		182			40				46			100
V-65	48	200	414		50		64					346	120		
V-72	72	140		(60)	460										
V-75	130	114		188											
V-79	80	104		80		110				182					258
V-82	48	140		146		140									
V-85	26	50		114		124				310					
V-89	40	30		60			70			190				290	
V-95	48	118		182											
W-14		154		340											
W-18		140		76											
W-21	53	114		844											
W-24	48	94	180		182										
Dose Cortisone - mg/kilo Body Wt.	0	3			1	1/2		1							

TABLE IV-c

SERUM CHOLESTEROL - TOTAL - mg%.

Group III - Cortisone

Animal No.	Experimental Day											
	-10	4	11	18	25	32	38	39	46	53	56	60
V-54	20	20	20		82			176		118	224	
V-58	60	62	30									
V-62	60	48	82		268		160					
V-69	20	12	70		162			80		346		
V-99	48	68	120		100			110				
W-3	40	60	60		136			76				
W-7	62	64	70		186		200		160			
W-11	80	72	36					160		274		
W-31	86	46	70		100			46		140		162
V-66	48	62	40		60			208		216		
V-73	60	46		82	40							
V-76	60	36		170		118			190			140
V-80	48	52		68		86			258			170
V-83	68	34		52		68						
V-87	36	24		140		68			250			
V-90	36	20										
V-96	52											
W-15		80		128		118			150			
W-19	54	52		146								
W-22	60	36		124		200			150			
W-25	60	26		64								
Dose Cortisone - mg/kilo Body Wt.	0	3			1	1/2		1				

TABLE IV-d

SERUM CHOLSTEROL - TOTAL - mg%.

Group IV - Normal Control

Animal No.	Experimental Day												
	- 10	4	11	18	25	32	38	39	45	46	53	56	60
V-55	54	30	30		30			82			112	106	
V-59	10	48	40		24		56						118
V-63	20	68		36	56					100			
V-70	52	16	46	40	52			36			130	136	
V-100	40	30	20		34			50	46				
W-4	36	24		20						90			
W-8	68	40	34	118				24		30			
W-12				70			52			82	128		
W-32	36	30		50		128				114			100

TABLE V-a

SERUM CHOLESTEROL - ESTER - mg%.

Group I - Cholesterol

Animal No	Experimental Day														
	-10	4	11	18	25	32	38	39	45	46	49	53	56	57	60
V-52	31	119	453		145			485							
V-56	118	344	308		365			58				114			157
V-60	33	88	431		865		746					825			457
V-67	38	161	336		566			69				368			
V-97	45	92	88		495			169	72						
W-1	57	119	138		132			109			125				
W-5	80	255	689		917			505							
W-9	120	138	256		356			285				348			
W-29		170	360		642			215				408			324
V-64	18	104	385		695		520					751		1027	
V-71	25	300		282	147										
V-74	41	33		443		130				345					459
V-77	42	140		60		178				99					91
V-81	49	296		627		520				333					463
V-84	37	150		222		105				106					
V-88	31	138		326		196				303				507	
V-94	47	24		456			412			613					585
W-13		198		445		499				285				522	
W-17		56		139		147				151					245
W-20	36	90		290		426				108			464		
W-23		30	173		170										

TABLE V-b

SERUM CHOLESTEROL - ESTER - mg%.

Group II - Cholesterol + Cortisone.

Animal No.	Experimental Day														
	-10	4	11	18	25	32	38	39	45	46	49	53	56	57	60
V-53	(3)?	63	86		76			175				145	248		
V-57	47	178	524		519										
V-61	37	77	165												
V-68	23	47	108		93			79				130			
V-98	53	90	251		115			481	349						
W-2	35	110	182		102		137				150				
W-6	43	68	135		174			105		567					
W-10	51	113	185		205			64				163			
W-30	27	47	72		112			23				18			62
V-65	41	153	285		12		39					301	79		
V-72	54	114		36	315										
V-75	100	89		123											
V-79	71	78		27		62				102					124
V-82	35	108		96		59									
V-85	23	38		80		76				173					
V-89	33	23		37			47			105				153	
V-95	38	84		117											
W-14		116		211											
W-18		117		48											
W-21	42	92		595											
W-24	41	74	127		123										
Dose Cortisone - mg/kilo Body Wt.	0	3			1	1/2		1							

TABLE V-c

SERUM CHOLESTEROL - ESTER - mg%.

Group III - Cortisone.

Animal No.	Experimental Day											
	-10	4	11	18	25	32	38	39	46	53	56	60
V-54	17	10	13		75			101		54	109	
V-58	55	53	22									
V-62	45	35	48		82		69					
V-69	17	8	15		71			46		97		
V-99	39	53	75		41			60				
W-3	31	50	45		41			28				
W-7	47	47	17		126		77		98			
W-11	56	62	21					95		80		
W-31	66	31	44		47			36		67		100
V-66	38	49	28		20			178		77		
V-73	40			62	25							
V-76	50	24		70		63			90			56
V-80	30	34		33		38			116			97
V-83	50	28				40						
V-87	33	17		52		33			140			
V-90	26	13										
V-96	47			32								
W-15		62		69		59			102			
W-19	44	43		87								
W-22	50	28		69		119			97			
W-25	47	23		44								
Dose Cortisone - mg/kilo Body Wt.	0	3			1	1/2		1				

TABLE V-d

SERUM CHOLESTEROL - ESTER - mg%.

Group IV - Normal Control

Animal No.	Experimental Day												
	-10	4	11	18	25	32	38	39	45	46	53	56	60
V-55	44	25	22		23			70			67	66	
V-59	9	43	25		14		48						88
V-63	10	58		30						93			
V-70	37	12	36		37			?(1)			92	101	
V-100	33	27	13		24			47	31				
W-4		17		13						49			
W-8	42	37	28		86			17		20			
W-12				64			37			59	75		
W-32	29	27		43		113				97			45

TABLE VI-a

SERUM CHOLESTEROL - FREE - mg%.

Group I - Cholesterol

Animal No.	Experimental Day														
	-10	4	11	18	25	32	38	39	45	46	49	53	56	57	60
V-52	5	31	137		43			75							
V-56	104	116	98		115			18				26			73
V-60	7	26	147		325		316					225			203
V-67	10	47	64		160			55				150			
V-97	7	26	26		137			41	18						
W-1	3	43	60		?(18)			45			95				
W-5	10	65	215		273			415							
W-9	20	62	84		170			115				142			
W-29		62	120		178			85				182			150
V-64	18	30	117		235		200					221		335	
V-71	1	100		98	43										
V-74	3	7		217		90				105					251
V-77	10	50		10		100				55					59
V-81	13	110		263		150				137					197
V-84	7	50		62		75				54					
V-88	9	43		80		50				91				163	
V-94	7	6		170			134			167					235
W-13		60		139		127				145				178	
W-17		8		43		35				65					105
W-20	18	20		98		100				20			134		
W-23		10	57		80										

TABLE VI-b

SERUM CHOLESTEROL - FREE - mg%.

Group II - Cholesterol + Cortisone

Animal No.	Experimental Day														
	-10	4	11	18	25	32	38	39	45	46	49	53	56	57	60
V-53	(0?)	27	64		120			125				115	120		
V-57	7	40	186		325										
V-61	15	27	65												
V-68	3	7	28		75			45				180			
V-98	7	20	105		75			139	125						
W-2	1	8	68		38		117				60				
W-6	5	28	45		50			77		405					
W-10	9	31	45		115			26				115			
W-30	13	5	10		70			17				28			38
V-65	7	47	129		38		25					45	41		
V-72	18	26		34	145										
V-75	30	25		65											
V-79	9	26		53		48				80					134
V-82	13	32		50		81									
V-85	3	12		34		48				137					
V-89	7	7		23			23			85				137	
V-95	10	34		65											
W-14		38		129											
W-18		23		28											
W-21	10	32		249											
W-24	7	20	53		59										
Dose Cortisone - mg/kilo Body Wt.	0	3			1	1/2		1							

TABLE VI-c

SERUM CHOLESTEROL - FREE - mg%.

Group III - Cortisone.

Animal No.	Experimental Day											
	-10	4	11	18	25	32	38	39	46	53	56	60
V-54	3	10	7		7			75		64	115	
V-58	5	9	8									
V-62	15	13	34		186		91					
V-69	3	4	55		91			34		249		
V-99	9	15	45		59			50				
W-3	9	10	15		95			48				
W-7	15	17	53		60		123		62			
W-11	24	10	15					65		194		
W-31	20	15	26		53			10		73		62
V-66	10	13	12		40			30		139		
V-73	20			20	15							
V-76	10	12		100		55			100			84
V-80	18	18		35		48			142			73
V-83	18	6		20		28						
V-87	3	7		88		35			110			
V-90	10	7										
V-96	5											
W-15		18		59		59			48			
W-19	10	9		59								
W-22	10	8		55		81			53			
W-25	13	3		20								
Dose Cortisone - mg/kilo Body Wt.	0	3			1	1/2		1				

TABLE VI-d

SERUM CHOLESTEROL - FREE - mg%.

Group IV - Normal Control

Animal No.	Experimental Day													
	-10	4	11	18	25	32	38	39	45	46	49	53	56	60
V-55	10	5	8		7			12				45	40	
V-59	1	5	15		10		8					(45)?		30
V-63	10	10		6	4					7				(35)?
V-70	15	4	10		15			(35)?				38	35	
V-100	7	3	7		10			3	15					
W-4		7		7						41	(59)?			
W-8	26	3	6		32			7		10				
W-12				6			15			23		53		
W-32	7	3		7		15				17				55

TABLE VII-a

SERUM PHOSPHOLIPIDS - mg%.

Group I - Cholesterol

Animal No.	Experimental Day																		
	-10	-3	-2	4	11	18	25	30	32	38	39	45	46	49	53	55	56	57	60
V-52	4.0			6.1	8.9		6.5				31.6								
V-56	15.2		11.9		12.5			11.9			6.3				7.5				8.0
V-60	4.1		8.3		12.7		22.5			25.2					16.6				16.8
V-67	4.5		7.0		10.0		13.8				9.5				13.6				
V-97	3.7	4.9		7.2	4.8		10.9				6.1	10.9							
W-1	3.5	3.6		9.2	6.8		6.5				7.5			5.7					
W-5	4.7		6.9	11.0	20.4		16.2				26.0								
W-9			6.2	8.6	10.4		10.3				13.4				8.3				
W-29			4.9	8.6	12.9		22.5				11.6				8.3				10.3
V-64			4.3		9.5		15.0			18.0					14.0			16.0	
V-71	5.5	7.8				14.8			8.7										
V-74	4.8	4.1		6.1		21.4			9.0				10.6						18.2
V-77	4.4	4.4		6.4		5.9			8.9				6.0						7.2
V-81	5.2		3.1	11.3		21.4			13.8				10.7						2.6
V-84	4.0			6.0		9.6			7.6				4.7			6.0			
V-88	4.4			6.1		7.4			5.9				6.2					7.2	
V-94	4.7			5.6		16.3			14.5				17.3						14.1
W-13	3.6		5.8	9.3		11.3			13.3				13.8						
W-17	5.0		5.9	5.9		5.3			6.3				6.8						7.3
W-20	5.5		7.3	7.3		8.6			9.2				5.7				5.9		
W-23			6.3	5.9	9.3		7.5												

TABLE VII-b

SERUM PHOSPHOLIPIDS - mg%.

Group II - Cholesterol + Cortisone

Animal No.	Experimental Day																	
	-10	-3	-2	4	11	18	25	30	32	38	39	45	46	49	53	56	57	60
V-53	2.7			5.9	13.4		16.4				16.6				8.8	11.0		
V-57	4.6		6.2		16.8		28.5											
V-61	4.1			8.0	13.6													
V-68	4.1			4.5	9.2		12.6				10.8				24.4			
V-98	4.8			4.3	11.2		13.8				16.2	12.4						
W-2	3.7	5.5		6.7	10.0		9.4							8.0				
W-6	5.1		4.2	8.0	10.1			10.0			14.0		24.4					
W-10	5.2		6.2	8.8	8.5		18.5				9.2				15.8			
W-30	4.2		4.4	4.3	6.4			12.1			9.7				5.6			3.5
V-65	4.6		8.0		14.6		7.5			8.3					2.1	3.5		
V-72	6.5	6.8		7.6		6.8			11.4									
V-75	5.1	5.1		8.0		13.8												
V-79	10.5			5.9		10.7			8.8				12.2					15.8
V-82	5.9	5.9		7.0		10.6			13.6									
V-85	2.4			4.7		8.3			9.3				29.0					
V-89	4.5	4.5		6.6		7.5			8.0				9.2				13.8	
V-95	4.8	6.7		6.4		10.0												
W-14	4.4		6.5	10.2		14.5												
W-18	4.8		4.0	7.4		6.5												
W-21	5.0		5.6	8.6		20.6												
W-24	5.7		6.3	7.3	11.0		15.2											
Dose Cortisone - mg/kilo Body Wt.				3			1	$\frac{1}{2}$			1	$1\frac{1}{2}$						

TABLE VII-c

SERUM PHOSPHOLIPIDS - mg%.

Group III - Cortisone

Animal No.	Experimental Day															
	-10	-3	-2	4	11	18	25	30	32	38	39	46	53	55	56	60
V-54	3.1		2.7		2.4		4.6				10.6		7.6		9.9	
V-58	4.0			5.6	5.9											
V-62	5.5		3.9		9.3		22.5			18.2						
V-69	4.8		3.6		10.1		14.5				9.4		30.0			
V-99	4.1	5.5		6.4	11.1		11.0				11.2					
W-3	5.7		7.0	7.4	8.8		15.6				10.4					
W-7	5.0		8.0	8.1	9.3		9.8			11.3		9.0				
W-11	5.7		9.3	7.0	5.5			15.0			12.0		22.0			
W-31	6.9		3.5	2.9	8.9		9.7				7.4		10.6			12.0
V-66	3.7		3.9		5.8			9.8			7.6		18.4			
V-73	6.3	6.0		5.6		6.2			5.7							
V-76	7.3	7.3		5.1		17.6			10.9			23.6				13.0
V-80	5.2	5.2		4.9		8.1			10.7			23.8				5.0
V-83	5.1			3.8		5.9			7.0							
V-87	4.3			5.7		15.0			10.0			19.0		17.4		
V-90	4.1	2.5		6.2												
V-96	5.1	6.3														
W-15	5.6		5.1	5.3					11.0			8.2				
W-19	5.7		5.9	7.3		12.8										
W-22	5.6		7.5	5.7		9.0			9.2			9.1				
W-25	4.7		8.1	4.1	4,9											
Dose Cortisona - mg/kilo Body Wt.				3			1	1/2			1	1 1/2				

TABLE VII-d

SERUM PHOSPHOLIPIDS - mg%.

Group IV - Normal Control

Animal No.	Experimental Day															
	-10	-3	-2	4	11	18	25	32	38	39	45	46	49	53	56	60
V-55	3.7			2.8	2.1		3.0			2.7				4.3	2.5	
V-59	3.4		3.5		4.0		3.5		4.8					2.3		3.5
V-63	5.9		5.5			4.6		2.0				4.2				7.2
V-70	4.4	5.0			3.7		3.8			4.0				3.7	3.1	
V-100	3.4	3.5		3.7	2.2					3.2	3.4					
W-4	3.8		5.8	4.1		3.7		3.4				2.9	2.5			
W-8	5.9		5.7	3.8	4.0		3.9			3.5		3.3				
W-12	7.9		9.7			4.4		5.6				4.8		5.2		
W-32	4.1		3.4	3.8		4.2		4.9				2.7				4.8

TABLE VIII-a

SERUM TOTAL FATTY ACIDS - M. Eq./L.

Group I - Cholesterol

Animal No.	Experimental Day																	
	-10	-3	-2	4	11	18	25	30	32	38	39	45	46	49	53	55	56	57	60
V-52	7.5			7.5	23.75		7.5				45								
V-56	42.5		22.5		21.25			7.5			10				7.5				12.5
V-60	7.25		8.75		20.0		40.0			35					22.5				22.5
V-67	7.5		7.5		16.25		26.25				11.25				17.5				
V-97	7.5	5.0		12.2	7.5		17.5				6.25	5							
W-1	12.5	6.25		15	13.75		13.75				7.5			10					
W-5	10.0		10.0	12.5	30.0		30.0				45								
W-9			5.0	10.0	10.0		20.0				15				13.75				
W-29			5.0	12.5	17.25		45.0				20				17.25				13.75
V-64	7.5		5.0		16.25		32.5			25					25.00			35	
V-71	7.5	7.25				17.25			7.5										
V-74	8.75	7.25		12.5		3125			12.5				13.75						27.5
V-77	10.0	5.0		7.5		5.0			15.0				6.25						6.25
V-81	8.75		5.0	10.0		27.5			18.75				15.0						18.75
V-84	7.5	7.25		7.5		16.25			11.25				6.25			10			
V-88	6.25	7.25		7.5		13.75			7.5				8.75					15	
V-94	7.25			8.75		27.25			25.0				20.0						27.25
W-13	7.5		7.5	12.5		17.5			18.75				25.0					20	
W-19	8.75		7.5	6.25		11.25			8.75				11.25						7.5
W-20	8.75		11.5	12.5		15.0			15.0				6.25				13.75		
W-23			7.5	7.5	12.5		12.5												

TABLE VIII-b

SERUM TOTAL FATTY ACIDS - M. Eq./L.

Group II - Cholesterol + Cortisone.

Animal No.	Experimental Day																	
	-10	-3	-2	4	11	18	25	30	32	38	39	45	46	49	53	56	57	60
V-53	7.25			17.50			80				65.0				43.50	30.00		
V-57	7.25		12.50		55.00		160											
V-61	10.00			17.50	67.50													
V-68	7.25			8.75	35.00		55				32.5				150.00			
V-98	8.75			7.50	35.00		70				32.5	30						
W-2	7.25	5.00		8.75	35.00		40							30				
W-6	10.00	8.75		22.50	32.50						23.0		65.00					
W-10	7.50		6.25	18.75	25.00		70				22.5				55.00			
W-30	7.25		6.20	10.00	17.25			35.00			15.00				23.50			5.00
V-65	11.25		17.50		45.00		40			12.5					6.25	8.75		
V-72	8.75	7.50		15.00		28.75			30.00									
V-75	10.00	10.00		20.00		45.00												
V-79	22.50			12.50		42.50			22.50				47.50					65.00
V-82	7.50	7.50		12.50		25.00			47.50									
V-85	7.50	7.25		5.00		32.50			22.50				50.00					
V-89	8.75	8.75		20.00		28.75			25.00				32.50				50	
V-95	7.25	7.50		10.00		43.75												
W-14	5.00		10.00	27.50		62.50												
W-18	7.25		5.00	12.50		23.75												
W-21	7.25		7.50	12.50		82.50												
W-24	7.25		6.25	15.00	35.00		70											
Dose Cortisone - mg/kilo Body Wt.				3			1	1/2			1	1 1/2						

TABLE VIII-c

SERUM TOTAL FATTY ACIDS - M. Eq./L.

Group III - Cortisone

Animal No.	Experimental Day															
	-10	-3	-2	4	11	18	25	30	32	38	39	46	53	55	56	60
V-54	5.00		7.50		7.50		8.75				21.25		16.25		21.25	
V-58	7.25			11.25	20.00											
V-62	11.25		12.50		45.00		145.00			45						
V-69	11.25		7.5		57.50		75.00				27.50		200			
V-99	7.25	5.00		15.00	35.00		60.00				25.00					
W-3	7.50		8.75	17.50	45.00		90,00				37.50					
W-7	7.50		7.50	17.50	30.00		40.00			55		32.5				
W-11	10.00		7.50	20.00	20.00			45.00			17.50		105			
W-31	7.25		6.25	6.25	32.50		40.00				13.75		30			37.5
V-66	7.25		5.00		21.25			27.50			17.50		75			
V-73	8.75	7.25		10.00		20.00			15.00							
V-76	5.00	8.75		10.00		97.50			45.00			103.5				32.5
V-80	8.75	8.75		12.50		32.50			37.50			110.0				55.0
V-83	7.50	7.25		5.00		17.50			16.25							
V-87	7.50	5.00		20.00		76.25			35.00			103.5		105		
V-90	8.75	5.00		7.50												
V-96	7.50	7.50														
W-15	8.75		7.50	13.75		31.25			37.50			30.0				
W-19	7.50		7.50	17.25		55.00										
W-22	10.00		11.25	11.25		54.50			27.50			45.0				
W-25	7.50		10.00	8.75		30.00										
Dose Cortisone - mg/kilo Body Wt.				3			1	1/2			1	1 1/2				

TABLE VIII-d

SERUM TOTAL FATTY ACIDS - M. Eq./L.

Group IV - Normal Control

Animal No.	Experimental Day															
	-10	-3	-2	4	11	18	25	32	38	39	45	46	49	53	56	60
V-55	7.25			3.75	6.25		6.25			5.00				5.00	5	
V-59	5.00		5.00		6.25		5.00		5					5.00		5
V-63	7.25		5.00			6.25		6.25				5				5
V-70	7.50	7.50			6.25		6.25			6.25				5.00	5	
V-100	8.75	5.00		5.00	8.75					5.00	10					
W-4	7.25		6.25	6.25		5.00		5.00				5	5			
W-8	5.00		6.25	5.00	5.00		6.25			5.00		5				
W-12	12.50		11.50			6.25		7.50				5		6.25		
W-32	7.25		6.25	6.25		5.00		6.25				5				5

TABLE IX-a

SERUM FATTY ACIDS OF NEUTRAL FAT - M. Eq./l L.

Group I - Cholesterol

Animal No.	Experimental Day											
	-1o	4	11	18	25	32	38	39	46	49	53	60
V-52	4.40	0.88	6.85					14.14				
V-56	30.60		6.25					4.80			0.30	6.80
V-60	4.00		1.43		4.50		1.07					1.00
V-67	3.92		1.75		3.58			3.96			0.10	
V-97	4.18	5.94	2.42									
W-1	7.95	6.62	6.28		6.63			0.28		3.46		
W-5	5.2		0.35					16.82				
W-9		1.44			4.78							
W-29		3.10	0.43		25.27			7.73			1.85	
V-64			0.75		5.80		1.13					
V-71	3.66			1.35								
V-74	4.91	8.15		7.37		3.93						5.10
V-77	6.31	0.17		0.05		5.19			0.19			
V-81	4.48								0.17			5.25
V-84	4.22	0.11		4.90		4.13			0.80			
V-88	2.90	0.45		1.00								
V-94	3.30	5.23		5.94								4.20
W-13		1.99							9.62			
W-17		1.38		4.55		1.25			3.44			
W-20	4.63	5.94		2.49					1.15			
W-23		3.30	2.62	3.70								

TABLE IX-b

SERUM FATTY ACIDS OF NEUTRAL FAT - M. Eq./L.

Group II - Cholesterol + Cortisone

Animal No.	Experimental Day													
	-10	4	11	18	25	32	38	39	45	46	53	56	57	60
V-53	5.60	12.45			68.53			50.87			36.2	17.20		
V-57	3.30		31.70		130.06									
V-61	6.70	10.87	55.33											
V-68	4.26	4.92	27.00		45.29			24.15			132.5			
V-98	4.60	2.67	22.00		59.02			10.64	13.76					
W-2	4.19	2.00	24.49		31.86					21.51				
W-6	5.93	16.14	23.20					12.18		36.21				
W-10	3.16	10.72	15.31		53.99			15.54			41.6			
W-30	4.11	6.28	11.69					8.80			19.8			1.40
V-65	7.50		29.12		35.29		6.69					4.75		
V-72	3.58	7.70		23.92										
V-75	4.41	13.00		33.81										
V-79	14.57	7.08		35.60		15.80				37.76				52.60
V-82	3.19	5.60		16.41		38.07								
V-85	5.45	1.32		25.63		16.53				28.72				
V-89	5.29	15.57		23.39						24.48			38	
V-95	3.49	4.11		34.92										
W-14		18.57		48.63										
W-18		5.17		18.71										
W-21	3.26	5.13		55.19										
W-24	2.88	8.86	25.31		58.01									
Dose Cortisone - mg/kilo Body Wt.		3			1	1/2		1	1 1/2					

TABLE IX-c

FATTY ACIDS OF NEUTRAL FAT - M. Eq./L.

Group III - Cortisone

Animal No.	Experimental Day											
	-10	4	11	18	25	32	38	39	46	53	56	60
V-54	2.80	1.48	5.76		4.11			12.53		10.45	12.75	
V-58	3.50	6.64	16.03									
V-62	6.98		38.36		129.78		32.41					
V-69	8.01		51.31		64.76			20.81		180.10		
V-99	3.86	9.93	26.66		52.54			16.95				
W-3	3.38	11.90	38.83		79.94			30.77				
W-7	3.38	11.68	24.16		31.04		46.40		24.76			
W-11	5.24	14.34	16.25					8.04		90.10		
W-31	1.54	3.75	26.16		33.17			8.52		22.20		27.9
V-66	4.17	17.13						8.49		62.30		
V-73	4.06			14.80								
V-76		6.48		85.49		37.07			87.47			23.8
V-80	4.98	8.82		26.95		30.32			93.20			49.6
V-83	3.24	2.08		13.27		11.11						
V-87	4.16	16.26		66.20		28.35			88.87			
V-90	5.70	3.57										
V-96	3.32											
W-15		9.07				29.59			22.66			
W-19	3.05	11.91		45.35								
W-22	5.46	7.22		47.51		19.12			37.29			
W-25	3.55	5.75		26.06								
Dose Cortisone - mg/kilo Body Wt.		3			1	1/2		1	1 1/2			

TABLE IX-d

FATTY ACIDS OF NEUTRAL FAT - M. Eq. /L.

Group IV - Normal Control

Animal No.	Experimental Day												
	-10	4	11	18	25	32	38	39	45	46	53	56	60
V-55	4.00		4.48		3.95			1.59			0.80	1.75	
V-59	2.80		3.30		2.64		0.96						0.7
V-63	3.60			2.77						0.19			
V-70	3.99		3.22		3.09			3.92			0.50	0.70	
V-100	5.93	2.20	7.11					1.88	7.2				
W-4		3.40		2.53						2.03			
W-8	0.49	1.84	1.90		1.72			2.56		2.58			
W-12				1.99				0.67			1.35		
W-32	4.12	3.35		1.49		0.52		0.89					1.0

TABLE X

TOTAL FOOD CONSUMPTION - gm.

Group I.				Group II.				Group III			Group IV		
Animal No.	Norm. Food	Chol.	Exp. day	Animal No.	Norm. Food.	Chol	Exp. Day.	Animal No.	Norm. Food	Exp. Day	Animal No.	Norm. Food	Exp. Day
V-52	5111	31	55	V-53	6735	31	56	V-54	5624	56	V-55	5600	56
V-56	6095	38	61	V-57	3397	16	29	V-58	2746	24	V-59	5100	61
V-60	6033	37	61	V-61	2631	14	25	V-62	4551	40	V-63	6100	61
V-67	5278	32	55	V-68	7354	34	55	V-69	6100	55	V-70	5600	56
V-97	4488	28	45	V-98	6170	27	45	V-99	5650	45	V-100	4600	45
W-1	4835	30	49	W-2	6650	30	49	W-3	6408	49	W-4	4900	49
W-5	4511	27	46	W-6	6200	28	46	W-7	5787	46	W-8	4600	46
W-9	5394	33	54	W-10	6436	30	54	W-11	5705	54	W-12	5400	54
W-29	5600	32	61	W-30	6255	27	60	W-31	7368	60	W-32	5871	61
V-64	5612	35	57	V-65	6684	30	57	V-66	6662	57			
V-71	4365	27	44	V-72	4670	23	44	V-73	3211	44			
V-74	5683	34	61	V-75	2750	14	24	V-76	6913	29			
V-77	5648	35	60	V-79	8273	37	60	V-80	7496	60			
V-81	6008	36	61	V-82	4862	23	41	V-83	5150	39			
V-84	5402	32	55	V-85	6600	32	55	V-87	7439	55			
V-88	5656	34	57	V-89	5867	27	57	V-90	1300	13			
V-94	5322	33	61	V-95	2613	13	24	V-96	-	-			
W-13	5568	34	57	W-14	3508	17	29	W-15	7395	57			
W-17	5853	35	61	W-18	1917	8	21	W-19	3200	26			
W-20	5486	33	56	W-21	2785	15	24	W-22	7462	55			
W-23	3113	19	33	W-24	3585	18	29	W-25	3140	26			

TABLE XI

TOTAL CORTISONE & VEHICLE ADMINISTRATION - mg.

Group I			Group II			Group III			Group IV		
Animal No	Vehicle	Expm. Day	Animal No.	Cortisone	Expm. Day	Animal No.	Cortisone	Expm. Day	Animal No.	Vehicle	Expm. Day
V-52	218	55	V-53	313	56	V-54	249	56	V-55	313	56
V-56	303	61	V-57	157	29	V-58	178	24	V-59	335	61
V-60	303	61	V-61	199	25	V-62	234	40	V-63	303	61
V-67	308	55	V-68	304	55	V-69	246	55	V-70	313	56
V-97	213	45	V-98	189	45	V-99	222	45	V-100	263	45
W-1	197	49	W-2	259	49	W-3	197	49	W-4	231	49
W-5	194	46	W-6	186	46	W-7	186	46	W-8	198	46
W-9	254	54	W-1o	210	54	W-11	202	54	W-12	239	54
W-29	335	61	W-30	278	60	W-31	326	60	W-32	335	61
V-64	317	57	V-65	267	57	V-66	317	57			
V-71	209	44	V-72	230	44	V-73	189	41			
V-74	266	61	V-75	145	24	V-76	294	29			
V-77	331	60	V-79	246	60	V-80	246	60			
V-81	335	61	V-82	245	41	V-83	293	39			
V-84	308	55	V-85	249	55	V-87	291	55			
V-88	317	57	V-89	219	57	V-90	123	13			
V-94	261	61	V-95	188	24	V-96	-	-			
W-13	285	57	W-14	157	29	W-15	233	57			
W-17	285	61	W-18	151	21	W-19	154	26			
W-20	243	56	W-21	145	24	W-22	213	55			
W-23	161	33	W-24	157	29	W-25	151	26			

TABLE XII - BODY WEIGHTS.- gm.

Group	Animal No.	Experimental Day -1	6	13	20	27	34	41	48	Final Wt.	% Wt.change of initial Wt.	Carcass Wt.
I.	V-52	2159	2087	2135	2270	2500	2483	2465	2225	1779	-17.6	1550
II.	V-53	2852	2830	2593	2615	2720	2630	2675	2735	2627	- 7.9	1940
III.	V-54	2640	2720	2250	2143	1975	2000	1880	2110	2279	-13.7	1590
IV.	V-55	2780	2785	2880	2915	2975	3050	3183	3170	3240	+16.5	2605
I.	V-56	2450	2375	2500	2570	2750	2730	2805	2908	2857	+16.6	2265
II.	V-57	2382	2300	2160	2220	2485				2090	-12.3	1525
III.	V-58	2600	2600	2400	2400					2050	-21.2	1740
IV	V-59	3114	3010	3035	3145	3125	3265	3428	3395	3345	+13.8	2803
I.	V-60	2518	2455	2620	2750	2770	2770	2930	2938	2956	+17.4	2439
II.	V-61	2895	2728	2500	2450					2075	-28.3	1608
III.	V-62	2897	2790	2620	2623	2440	2330			2445	-15.6	1589
IV.	V-63	2458	2420	2595	2605	2680	2670	2930	2710	2981	+21.3	2322
I.	V-64	3368	3288	3395	3375	3440	3448	3585	3583	3720	+10.5	2785
II.	V-65	2690	2565	2385	2533	2445	2488	2497	2455	2248	-16.4	1296
III.	V-66	2932	2812	2735	2650	2585	2645	2800	2865	2290	-21.9	1668
I.	V-67	2968	2884	2967	3030	3140	3165	3235	3245	2975	+ 0.2	2295
II.	V-68	2810	2808	2550	2543	2485	2599	2760	2770	2690	- 4.3	1881
III.	V-69	2585	2585	2405	2380	2362	1915	2230	2265	2004	-22.5	1521
IV.	V-70	2655	2674	2725	2790	2860	2910	2965	2990	3329	+25.4	2530
I.	V-71	2293	2315	2425	2525	2625	2655	2845		2717	+18.5	2121
II.	V-72	2810	2760	2526	2485	1960	2157	2343		2345	-16.5	1492
III.	V-73	2360	2560	2290	1765	1775	1770			1425	-39.6	1085
I.	V-74	2335	2120	2315	2390	2485	2620	2700	2763	2882	+23.4	2299
II.	V-75	2440	2295	2150	2285					2245	- 8.0	1360
III.	V-76	2605	2515	2357	2380	2385	2515	2685	2610	2497	- 4.1	1749
I.	V-77	2600	2570	2625	2770	2715	2675	2875	2895	2955	+13.7	2274
II.	V-78	2315	2298	2170	2170	2100	2275	2390	2510	2540	+ 9.7	1852
III.	V-80	2400	2470	2350	2370	2280	2440	2400	2520	2576	+ 7.3	1700
I.	V-81	2787	2740	2875	2950	3040	3104	3075	3205	3349	+20.2	2639
II.	V-82	3225	3055	2860	2770	2785	2765			2740	-15.0	1966
III.	V-83	3195	3068	2940	2845	2830	2925			2835	-11.3	2010
I.	V-84	2935	2823	2835	2960	3080	3055	3110	3180	3191	+ 8.7	2518
II.	V-85	2545	2469	2340	2443	2405	2460	2570	2520	2400	- 5.7	1754
III.	V-87	2900	2760	2490	2635	2635	2670	2770	2885	2962	+ 2.1	2080
I.	V-88	2860	2863	2925	3040	3025	3110	3267	3310	3440	+20.3	2762
II.	V-89	2450	2455	2303	2355	2345	2210	2325	2210	1985	-19.0	1341
III.	V-90	2835	2850	2675						2570	- 9.3	1730

TABLE XII - BODY WEIGHTS. (Cont'd)- gm.

Group	Animal No	Experimental Day -1	6	13	20	27	34	41	48	Final WT.	% Wt.change of initial Wt.	Carcass Wt.
I.	V-94	2455	1882	2210	2340	2470	2470	2591	2765	2949	+20.1	2254
II.	V-95	2760	2550	2389	2600					2525	-8.5	1715
III.	V-96	2605								--	--	--
I.	V-97	2167	2330	2440	2595	2635	2670	2860		2798	+29.1	2145
II	V-98	2445	2460	2265	2383	2370	2415	2522		2720	+11.2	1975
III	V-99	2620	2693	2455	2435	2405	2430	2675		2690	+ 2.7	1755
IV	V-loo	2575	2596	2690	2765	2750	2855	2975		3010	+16.9	2410
I.	W-1	1884	1985	2180	2260	2255	2418	2475	2617	2572	+36.5	1935
II.	W-2	2751	2750	2470	2560	2535	2480	2730	2835	2475	-10.0	1983
III.	W-3	2165	2125	2065	2150	2135	2280	2480	2505	2485	+14.8	1855
IV.	W-4	2205	2372	2460	2640	2700	2720	2945	2930	2890	+31.1	2236
I.	W-5	2042	1960	2095	2215	2330	2473	2560		2595	+27.1	1788
II.	W-6	2205	2170	2009	2100	2235	2295	2425		2460	+11.6	1649
III.	W-7	1990	1970	1825	1910	1970	1944	2065		2145	+ 7.8	1155
IV.	W-8	2020	2120	2225	2315	2375	2525	2755		2735	+35.4	2058
I.	W-9	2310	2359	2415	2670	2695	2805	2930	2880	3035	+31.4	2337
II.	W-lo	2420	2377	2235	1990	2055	2077	2310	2330	2482	+ 2.6	1493
III.	W-11	1643	1620	1450	1435	1360	1320	1500	1670	1571	- 4.4	1081
IV.	W-12	2188	2260	2345	2495	2535	2753	2745	2805	2910	+33.0	2290
I.	W-13	2440	2378	2510	2645	2705	2830	2850	2903	2885	+18.2	2309
II.	W-14	2025	2140	1965	2115	2030				2032	+ 0.3	1350
III.	W-15	2327	2277	2180	2205	2260	2353	2420	2535	2330	+ 0.1	1520
I.	W-17	2375	2263	2410	2540	2580	2655	2840	2835	2952	+24.3	2322
II.	W-18	2038	2180	1966	1850					1591	-21.9	1227
III.	W-19	2393	2345	2350	2365					2253	- 5.9	1636
I.	W-20	2230	2185	2255	2340	2440	2505	2695	2683	2747	+23.2	2072
II.	W-21	2285	2290	2125	2180					2142	- 6.3	1500
III.	W-22	2032	2060	1970	2040	2040	2148	2325	2330	2452	+20.7	1785
I.	W-23	1984	2040	2160	2275	2400				2390	+20.5	1620
II.	W-24	2293	2242	2140	2145	2150				2200	- 4.1	1471
III.	W-25	2418	2465	2330	2385					2249	- 7.0	1480
I.	W-29	3650	3628	3475	3695	3200	3250	3380	3370	3455	- 5.3	2630
II.	W-30	2888	2817	2598	2340	1860	2003	2190	2320	2435	-15.7	1818
III.	W-31	2845	2700	2683	2625	2525	2388	2710	2695	2853	+ 0.3	1995
IV.	W-32	2685	2753	2805	2850	2880	2935	3020	2960	3040	+13.2	2509

TABLE XIII-a

BLOOD SUGAR - mg%.

Group	Animal No.	Experimental Day -10	-3	4	5	11	12	18	19	25	26	32	33	39	40	46	47	53	54	57	60
I	V-52	124	139		146	157			121	108	108		138	144			119	136		X	
II	V-53	122	190			155			193		222		133	116			150	115		143	X
III	V-54	113	103	138		113			164		123		110		99		115	139		164	X
IV	V-55	179		99		121			107	140			114	104			130	116		92	X
I	V-56	97	91	120		127			102		91		127	118			122	106			118
II	V-57	115	109	153		171			108		203	X									
III	V-58	96	98	157		134			126	X											
IV	V-59	77	101	107		103			100		85		103	103			129	125			110
I	V-60	106	93		111	102			104	93	93		124	98			102	115			104
II	V-61	86	108	252		187			143		X										
III	V-62	117	153	208		151			127		114		115	102		X					
IV	V-63	100	106		96		106	110			83	103			97	107			98		98
I	V-64	104	115	100		89			110		103		106	104			118	110		95	X
II	V-65	104	110	160		113			132		128		122	91			104	107		96	X
III	V-66	114	113	177		197			135	90	96		113	89			123	190			X
I	V-67	137	121	113		114			108		116		144	121			126	134		X	
II	V-68	107	104	244		143			113	143			133	121			186	106		X	
III	V-69	120	107	164		136			150	131			115	118			159	398		X	
IV	V-70	132	108	99		111			130		118		115	101			143	121		92	X
I	V-71	120	83		95		101	91			100	107			94	X					
II	V-72	124	114		166		170	145			92	91			105	X					
III	V-73	126	102		157		126	137			73	99			47	X					
I	V-74	113	91		93		102	93			102	107			86	96			108		114
II	V-75	107	101		123		149	142		X											
III	V-76	120	106		178		127		185		118	114			113	133			194		152
I	V-77	111	98		140		107	84			90	99			100	110			121		107
II	V-79	128	92		160		151	192			140	164			106	156			170		266
III	V-80	112	93		145		98	131			84	104			121	148			119		160
Dose Cortisone-mg/kilo Body Wt.				3						1		1/2		1		1 1/2					

TABLE XIII-b

BLOOD SUGAR - mg%.

Group.	Animal No.	Experimental Day																		
		-10	-3	4	5	11	12	18	19	25	26	32	33	39	40	46	47	53	54	57	60
I.	V-81	117	118		98		105	116			111	120			111	117			140		116
II.	V-82	127	107		149		203	193			127	136			127	X					
III.	V-83	131	110		214		184	195			121	119			X						
I.	V-84	117	104		91		88	84			91	105			102	104			119	X	
II.	V-85	114	123		140		156	189			123	104			107	144			238	X	
III.	V-87	116	110		189		132				95	109			137	168			244	X	
I.	V-88	133	120		114		115	106			114	100			104	124			125	115	X
II.	V-89	113	120		100		175	160			119	90			115	120			116	83	X
III.	V-90	122	126		213		203	X													
Dose Cortisone - mg/kilo Body Wt.				3						1		1/2		1		1 1/2					

TABLE XIII-c

BLOOD SUGAR - mg%.

Group.	Animal No.	Experimental Day -9	-2	4	5	11	12	18	19	25	26	32	33	39	40	46	47	49	53	54	57	60
I.	V-94	115	180		87		116	128			118	141			110	121				114		120
II.	V-95	152	117		166		175	139		X												
III.	V-96	153	236		X																	
I.	V-97	113	104	125		113			115				114	128		116	(45 days - X)					
II.	V-98	111	148	162		135			120	118			164	126		239	(45 " - X)					
III.	V-99	130		147		131			101		79		131	124		X						
IV.	V-100	113		92		99			111	124	99		128	102		99	(45 days - X)					
I.	W-1	97	113	114		126			103		119		115	110			133	104	X			
II.	W-2	104	115	156		107			102		87		104	91			138	111	X			
III.	W-3	112	139		170	183			142	129			172	107			208		X			
IV.	W-4	121			100		121	114			96	128			103	142		93	X			
I.	W-5	139	185	130		109			106	110			133	121		100	X					
II.	W-6	123	105	166		145			142	127			141	121			X					
III.	W-7	98	136	114		149			120		82		123	77		148	X					
IV.	W-8	143	123	112		108			118		107		144	113		118	X					
I.	W-9	108	109	120		107			104		107		113	110			113		113		X	
II.	W-10	124	122	189		173			97		76		101	91			127		157		X	
III.	W-11	111	106	153		177			136	106	128		123	114			142		132		X	
IV.	W-12	104	172				111	121			51	113			124	121				116	X	
Dose Cortisone mg/kilo Body Wt.				3						1		1/2		1		1 1/2						

TABLE XIII-d

BLOOD SUGAR - mg%.

Group.	Animal No.	Experimental Day																				
		-9	-2	4	5	11	12	18	19	25	26	32	33	39	40	46	47	49	53	54	57	60
I.	W-13	112	129		104		111	111			104	120			114	123				115	113	X
II.	W-14	97	103		151		169	136			165	X										
III.	W-15	118	123		157		163	175			96	113			100	113				144		X
I.	W-17	111	113		94		99	106			102	113			104	115				109		121
II.	W-18	119	113		101		118	104														
III.	W-19	117	108		106		142	137				X										
I.	W-20	111	103		96		125	101			119	121			109	115				107	110	
II.	W-21	120	112		246		255	219														
III.	W-22	104	118		127		150	172			109	115			137	197				216		
I.	W-23	103	114	124		103			110	100												
II.	W-24	125	134	136		172			200	140												
III.	W-25	109	154		98		183	129														
I.	W-29		99	101			112		96	161			92	96			104		91			104
II.	W-30	123	124	157		113			136		145		124	104			145		102			188
III.	W-31	113		178		180			122		127		135	108					125			131
IV.	W-32	136	120		116		127	112			107	123			119	118				133		117
Dose Cortisone mg/kilo Body Wt.				3						1		1/2		1		1 1/2						

TABLE XIV

BLOOD SUGAR - MEAN VALUES - mg.%

Group	Experimental Day										
	-9,-10	-2,-3	4,5	11,12	18,19	25,26	32,33	39,40	46,47	49,53,54	57,60
I	114.4	115.3	110.3	110.9	104.8	107.3	117.0	109.5	114.6	115.7	111.4
II	116.4	117.7	163.9	158.8	147.9	134.7	123.6	109.3	146.3	135.8	155.2
III	116.8	123.1	159.1	153.0	143.6	105.6	119.4	106.2	150.4	190.1	151.8
IV	122.8	121.7	102.6	111.9	113.7	101.0	119.0	107.3	123.0	114.6	101.8

TABLE XV

RATIOS OF THE MEAN VALUES OF THE SERUM LIPIDS

Ratio	Experim. Group	Experimental Day							
		-10	4	11	18	25	30-32	38-39	45-46
$\frac{T}{E}$ *	I	$\frac{1.3}{1}$	$\frac{1.3}{1}$	$\frac{1.3}{1}$	$\frac{1.4}{1}$	$\frac{1.3}{1}$	$\frac{1.3}{1}$	$\frac{1.4}{1}$	$\frac{1.3}{1}$
	II	$\frac{1.2}{1}$	$\frac{1.3}{1}$	$\frac{1.4}{1}$	$\frac{1.5}{1}$	$\frac{1.6}{1}$	$\frac{1.8}{1}$	$\frac{1.5}{1}$	$\frac{1.6}{1}$
	III	$\frac{1.2}{1}$	$\frac{1.3}{1}$	$\frac{1.8}{1}$	$\frac{1.9}{1}$	$\frac{2.1}{1}$	$\frac{1.9}{1}$	$\frac{1.8}{1}$	$\frac{1.8}{1}$
	IV	$\frac{1.4}{1}$	$\frac{1.2}{1}$	$\frac{1.4}{1}$	$\frac{1.5}{1}$	$\frac{1.1}{1}$	-	$\frac{1.4}{1}$	$\frac{1.3}{1}$

* T = Serum Cholesterol-Total
E = Serum Cholesterol-Ester

TABLE XVI

RATIOS OF THE MEAN VALUES OF THE SERUM LIPIDS

Ratio	Experim. Group	Experimental Day							
		-10	4	11	18	25	30-32	38-39	45-46
$\frac{F}{E}$ *	I	$\frac{0.30}{1}$	$\frac{0.32}{1}$	$\frac{0.31}{1}$	$\frac{0.36}{1}$	$\frac{0.32}{1}$	$\frac{0.33}{1}$	$\frac{0.42}{1}$	$\frac{0.35}{1}$
	II	$\frac{0.22}{1}$	$\frac{0.27}{1}$	$\frac{0.38}{1}$	$\frac{0.53}{1}$	$\frac{0.60}{1}$	$\frac{0.90}{1}$	$\frac{0.52}{1}$	$\frac{0.64}{1}$
	III	$\frac{0.28}{1}$	$\frac{0.30}{1}$	$\frac{0.82}{1}$	$\frac{0.88}{1}$	$\frac{1.15}{1}$	$\frac{0.87}{1}$	$\frac{0.76}{1}$	$\frac{0.80}{1}$
	IV	$\frac{0.37}{1}$	$\frac{0.16}{1}$	$\frac{0.37}{1}$	$\frac{0.17}{1}$	$\frac{0.35}{1}$	-	$\frac{0.36}{1}$	$\frac{0.32}{1}$

* F = Serum Cholesterol - Free.
E = Serum Cholesterol - Ester

TABLE XVII

RATIOS OF THE MEAN VALUES OF THE SERUM LIPIDS

Ratio	Experim. Group	Experimental Day							
		-10	4	11	18	25	30-32	38-39	45-46
$\frac{T^*}{P}$	I.	$\frac{12.4}{1}$	$\frac{25.6}{1}$	$\frac{39.9}{1}$	$\frac{36.6}{1}$	$\frac{45.9}{1}$	$\frac{37.0}{1}$	$\frac{29.7}{1}$	$\frac{35.0}{1}$
	II	$\frac{10.4}{1}$	$\frac{16.4}{1}$	$\frac{23.3}{1}$	$\frac{19.2}{1}$	$\frac{17.7}{1}$	$\frac{11.1}{1}$	$\frac{16.0}{1}$	$\frac{24.5}{1}$
	III	$\frac{10.0}{1}$	$\frac{8.1}{1}$	$\frac{8.0}{1}$	$\frac{10.1}{1}$	$\frac{10.1}{1}$	$\frac{11.1}{1}$	$\frac{12.4}{1}$	$\frac{12.5}{1}$
	IV	$\frac{8.4}{1}$	$\frac{9.9}{1}$	$\frac{8.5}{1}$	$\frac{13.3}{1}$	$\frac{10.9}{1}$	-	$\frac{13.9}{1}$	$\frac{(21.4)}{(1)}$?

* T = Serum Cholesterol - Total

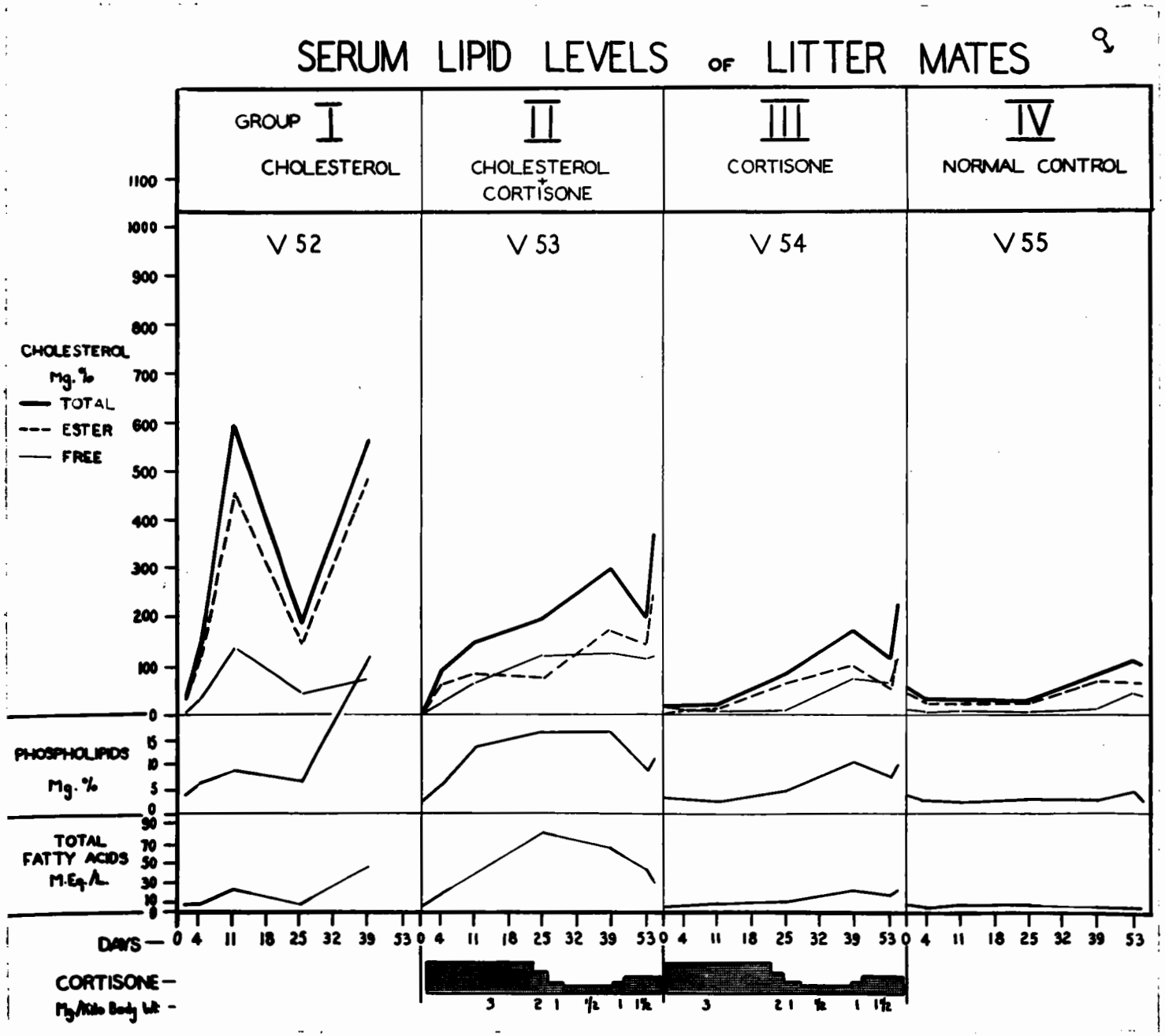
P = Serum Lipid Phosphorus.

TABLE XVIII

RATIOS OF THE MEAN VALUES OF THE SERUM LIPIDS

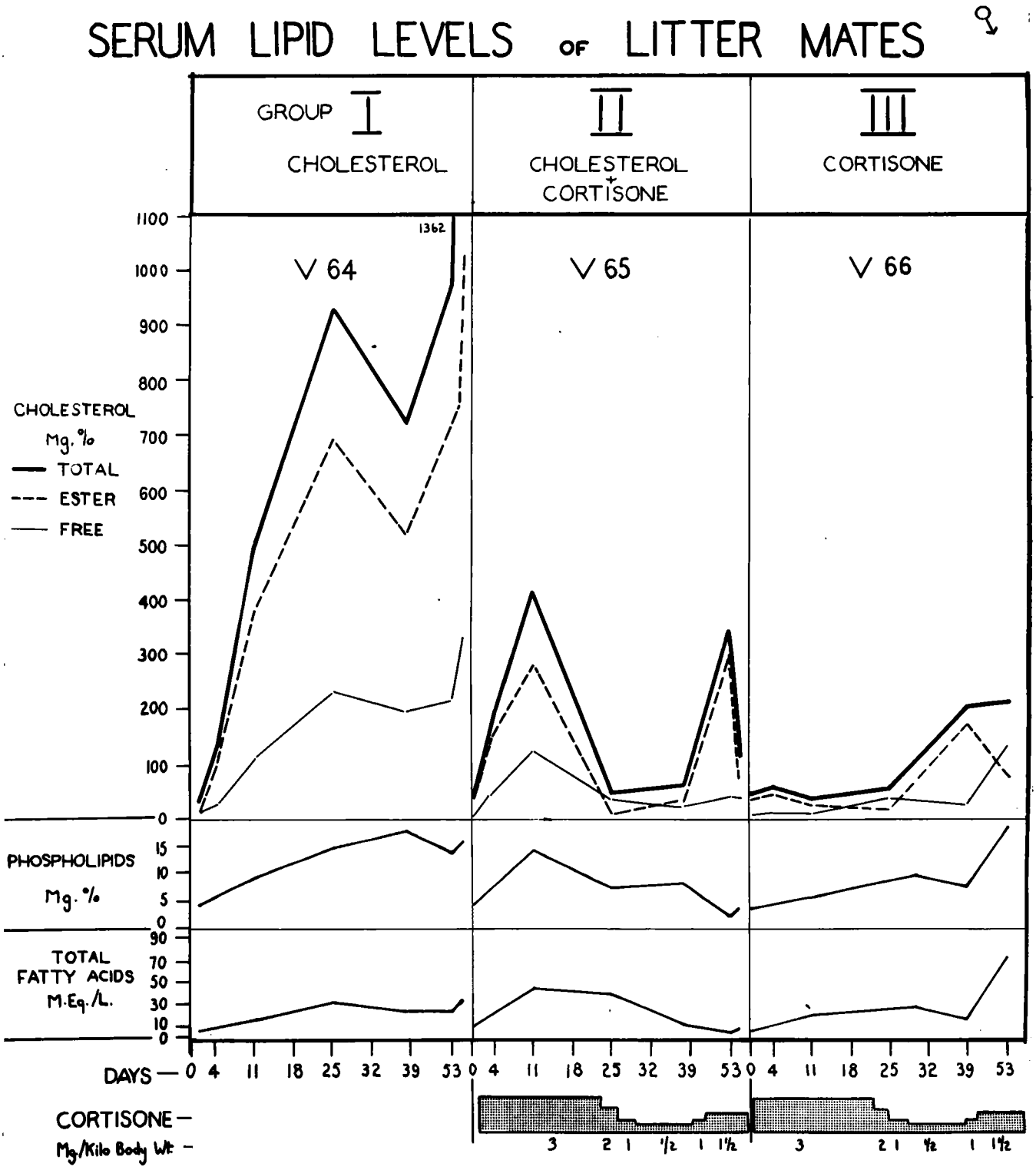
Ratio	Experim. Group	Experimental Day							
		-10	4	11	18	25	30-32	38-39	45-46
F.A.*/E	I	0.20/1	0.07/1	0.05/1	0.06/1	0.05/1	0.05/1	0.07/1	0.05/1
	II	0.21/1	0.16/1	0.20/1	0.30/1	0.44/1	0.46/1	0.23/1	0.17/1
	III	0.20/1	0.36/1	0.96/1	0.80/1	1.12/1	0.54/1	0.38/1	0.66/1
	IV	0.26/1	0.17/1	0.26/1	0.15/1	0.16/1	-	0.14/1	0.10/1

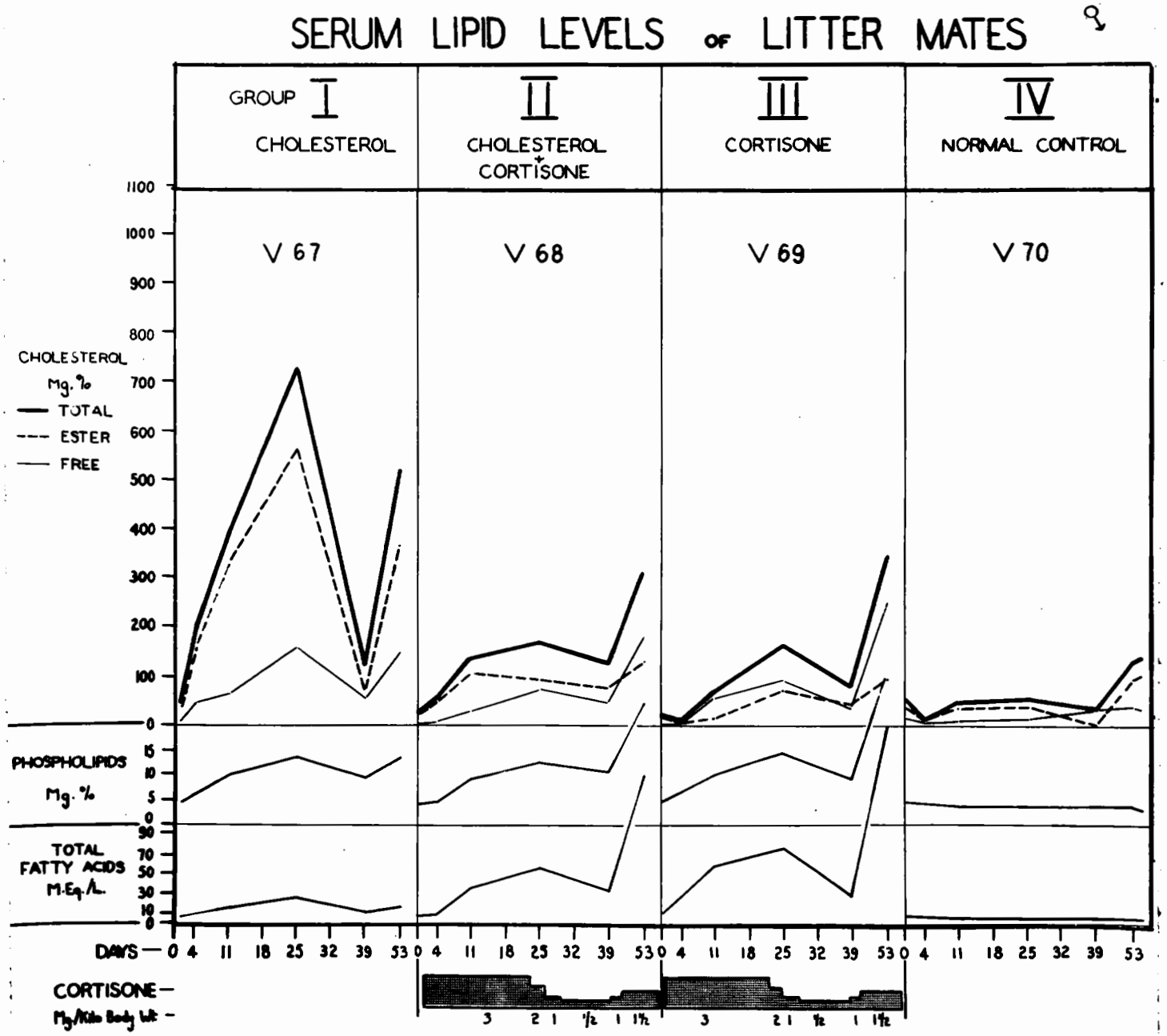
* F.A. = Serum Total Fatty Acid
E. = Serum Cholesterol-Ester.


SERUM LIPID LEVELS OF LITTER MATES
GROUP I
CHOLESTEROL
II
CHOLESTEROL + CORTISONE
III
CORTISONE
IV
NORMAL CONTROL
V 52
V 53
V 54
V 55
CHOLESTEROL Mg.%
TOTAL
ESTER
FREE
1100
1000
900
800
700
600
500
400
300
200
100
0
PHOSPHOLIPIDS Mg.%
15
10
5
0
TOTAL FATTY ACIDS M.Eq./L.
90
70
50
30
10
0
DAYS — 0 4 11 18 25 32 39 53 0 4 11 18 25 32 39 53 0 4 11 18 25 32 39 53 0 4 11 18 25 32 39 53
CORTISONE —
Mg/Kilo Body Wt. —
3 2 1 ½ 1 1½ 3 2 1 ½ 1 1½

GRAPH 1.

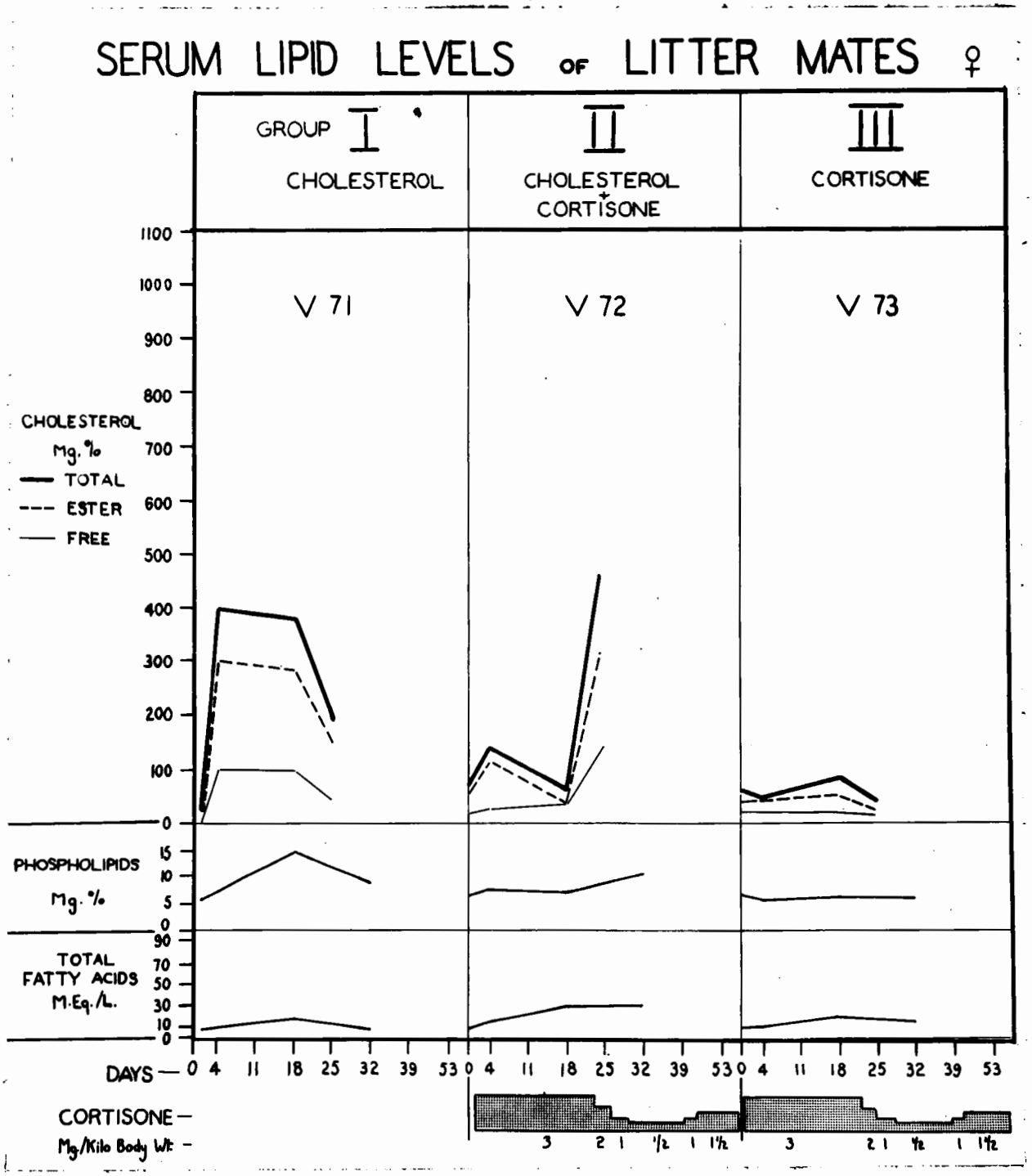
GRAPH 2


SERUM LIPID LEVELS OF LITTER MATES
GROUP I
CHOLESTEROL
II
CHOLESTEROL + CORTISONE
III
CORTISONE
V 64
V 65
V 66
1362
CHOLESTEROL Mg.%
TOTAL
ESTER
FREE
1100
1000
900
800
700
600
500
400
300
200
100
0
PHOSPHOLIPIDS Mg.%
15
10
5
0
TOTAL FATTY ACIDS M.Eq./L.
90
70
50
30
10
0
DAYS — 0 4 11 18 25 32 39 53 0 4 11 18 25 32 39 53 0 4 11 18 25 32 39 53
CORTISONE — Mg/Kilo Body Wt: —
3 2 1 1/2 1 1 1/2
3 2 1 1/2 1 1 1/2

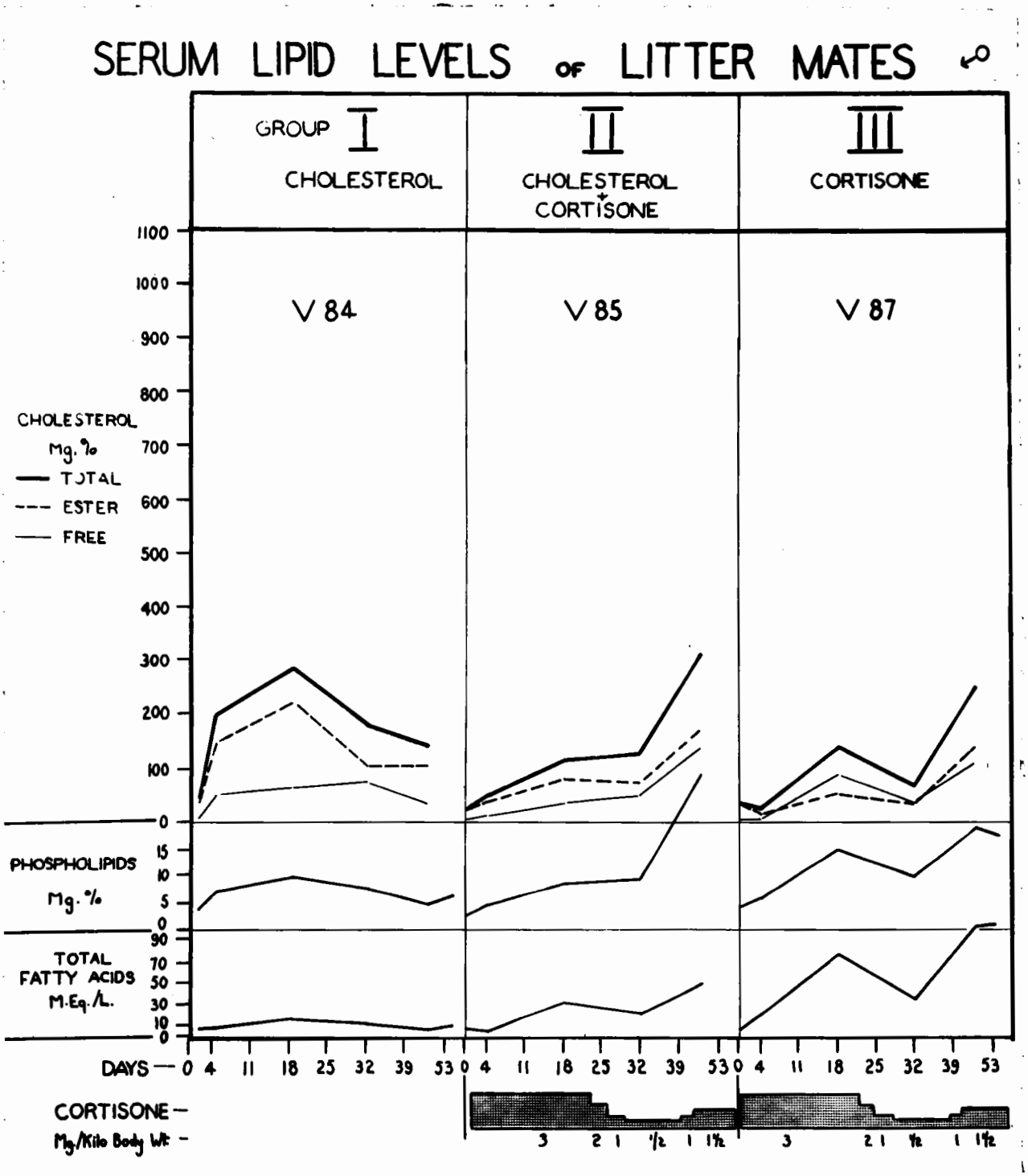

SERUM LIPID LEVELS of LITTER MATES
GROUP I
CHOLESTEROL
II
CHOLESTEROL + CORTISONE
III
CORTISONE
IV
NORMAL CONTROL
V 67
V 68
V 69
V 70
CHOLESTEROL Mg.%
TOTAL
ESTER
FREE
1100
1000
900
800
700
600
500
400
300
200
100
0
PHOSPHOLIPIDS Mg.%
15
10
5
0
TOTAL FATTY ACIDS M.Eq./L.
90
70
50
30
10
0
DAYS — 0 4 11 18 25 32 39 53
CORTISONE — Mg./Kilo Body Wt. —
3 2 1 ½ 1 1½

GRAPH 3.

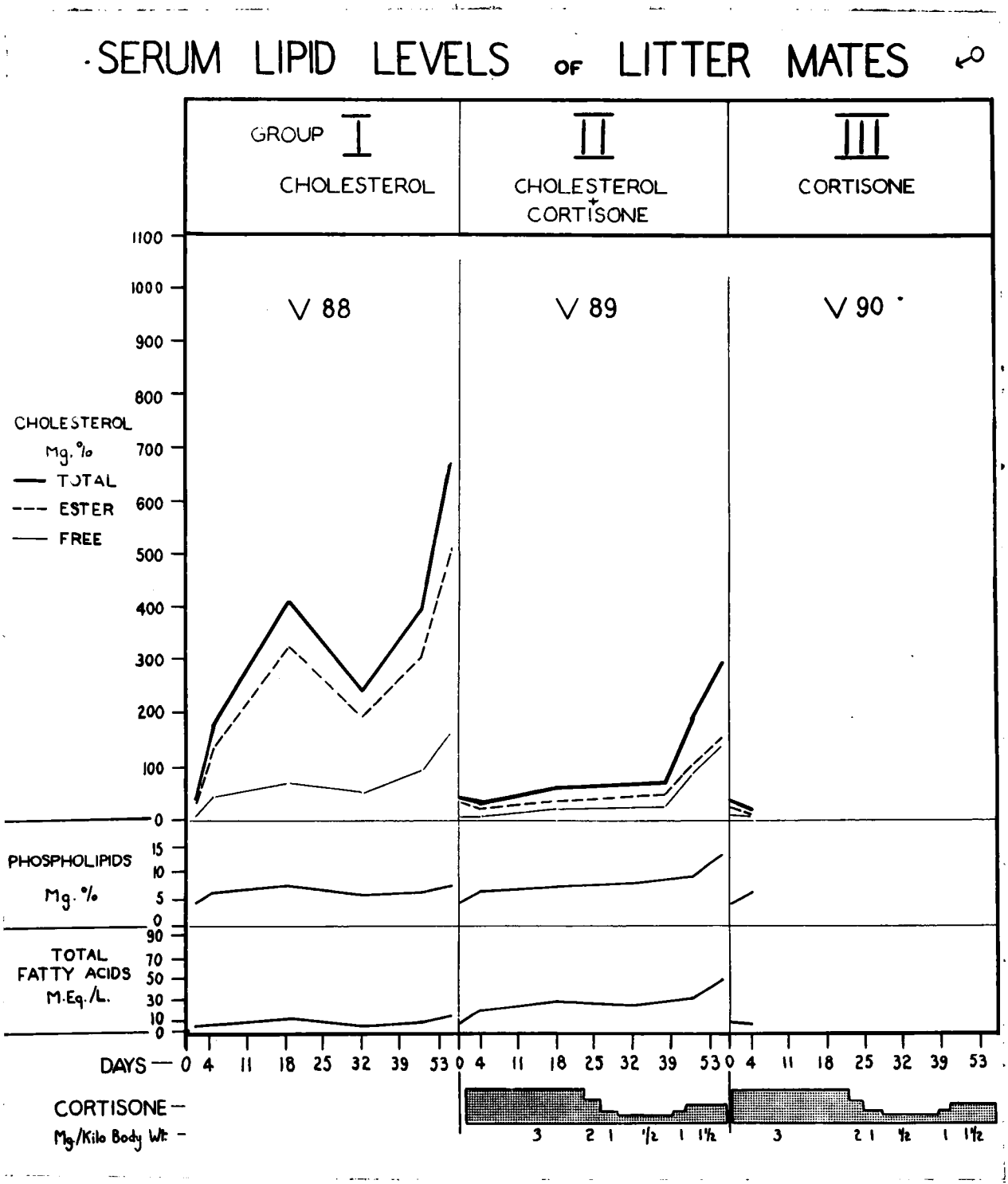
GRAPH 4


SERUM LIPID LEVELS OF LITTER MATES ♀
GROUP I
CHOLESTEROL
II
CHOLESTEROL + CORTISONE
III
CORTISONE
V 71
V 72
V 73
CHOLESTEROL Mg.%
TOTAL
ESTER
FREE
1100
1000
900
800
700
600
500
400
300
200
100
0
PHOSPHOLIPIDS Mg.%
15
10
5
0
TOTAL FATTY ACIDS M.Eq./L.
90
70
50
30
10
0
DAYS — 0 4 11 18 25 32 39 53 0 4 11 18 25 32 39 53 0 4 11 18 25 32 39 53
CORTISONE —
Mg/Kilo Body Wt —
3 2 1 ½ 1 1½ 3 2 1 ½ 1 1½

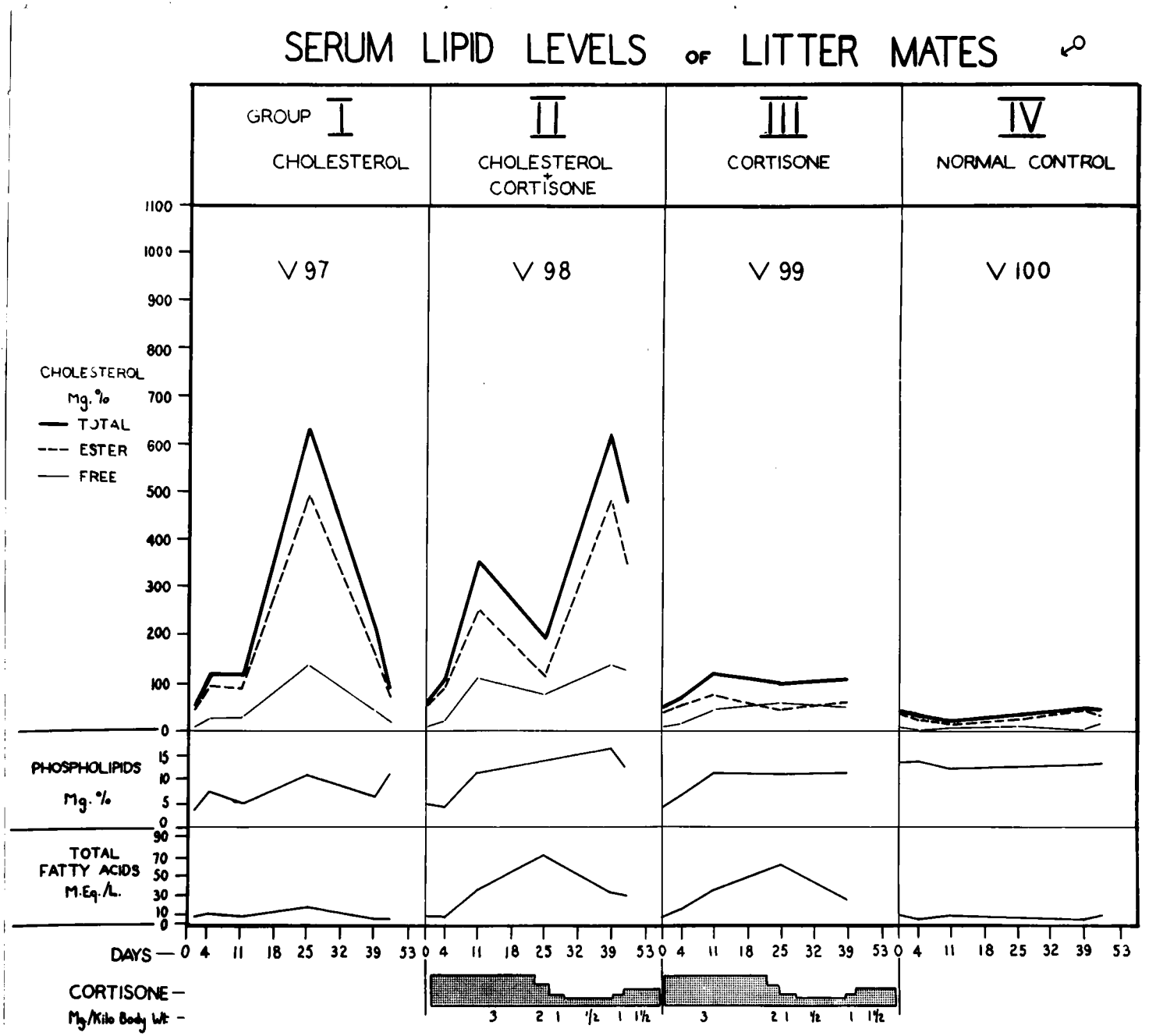
GRAPH 5


SERUM LIPID LEVELS OF LITTER MATES
GROUP I
CHOLESTEROL
II
CHOLESTEROL + CORTISONE
III
CORTISONE
V 84
V 85
V 87
CHOLESTEROL Mg. %
TOTAL
ESTER
FREE
1100
1000
900
800
700
600
500
400
300
200
100
0
PHOSPHOLIPIDS Mg. %
15
10
5
0
TOTAL FATTY ACIDS M.Eq./L.
90
70
50
30
10
0
DAYS — 0 4 11 18 25 32 39 53 0 4 11 18 25 32 39 53 0 4 11 18 25 32 39 53
CORTISONE — Mg/Kilo Body Wt. —
3 2 1 ½ 1 1½
3 2 1 ½ 1 1½

GRAPH 6

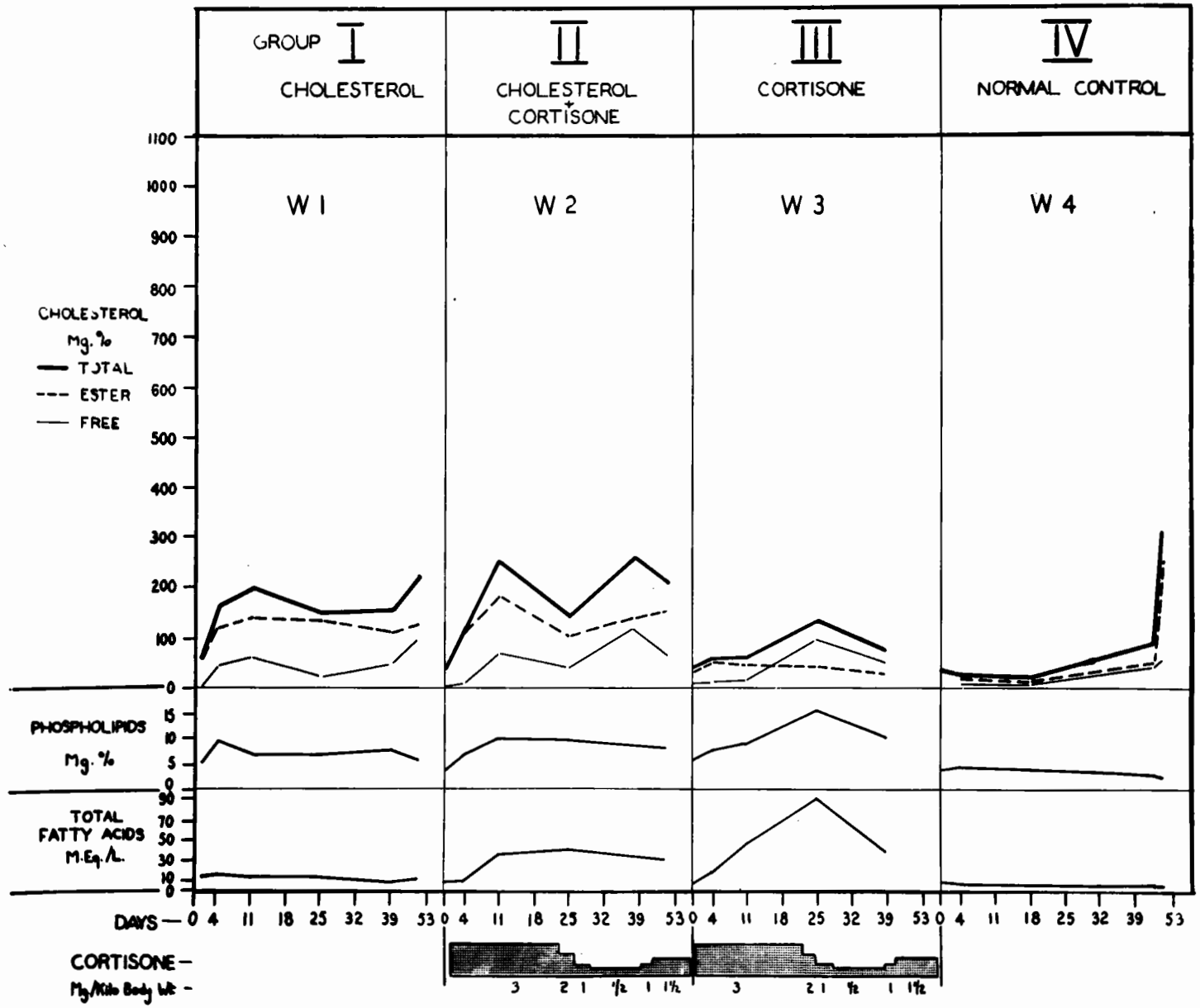

SERUM LIPID LEVELS OF LITTER MATES
GROUP I
CHOLESTEROL
II
CHOLESTEROL + CORTISONE
III
CORTISONE
V 88
V 89
V 90
CHOLESTEROL Mg. %
TOTAL
ESTER
FREE
PHOSPHOLIPIDS Mg. %
TOTAL FATTY ACIDS M.Eq./L.
DAYS
CORTISONE Mg/Kilo Body Wt.

Errata: The serum phospholipid tracing of V-100 - Group IV, corrected, should follow along a base line - 3.4, 3.5, 3.7, 2.2, 3.2, 3.4 mg%.

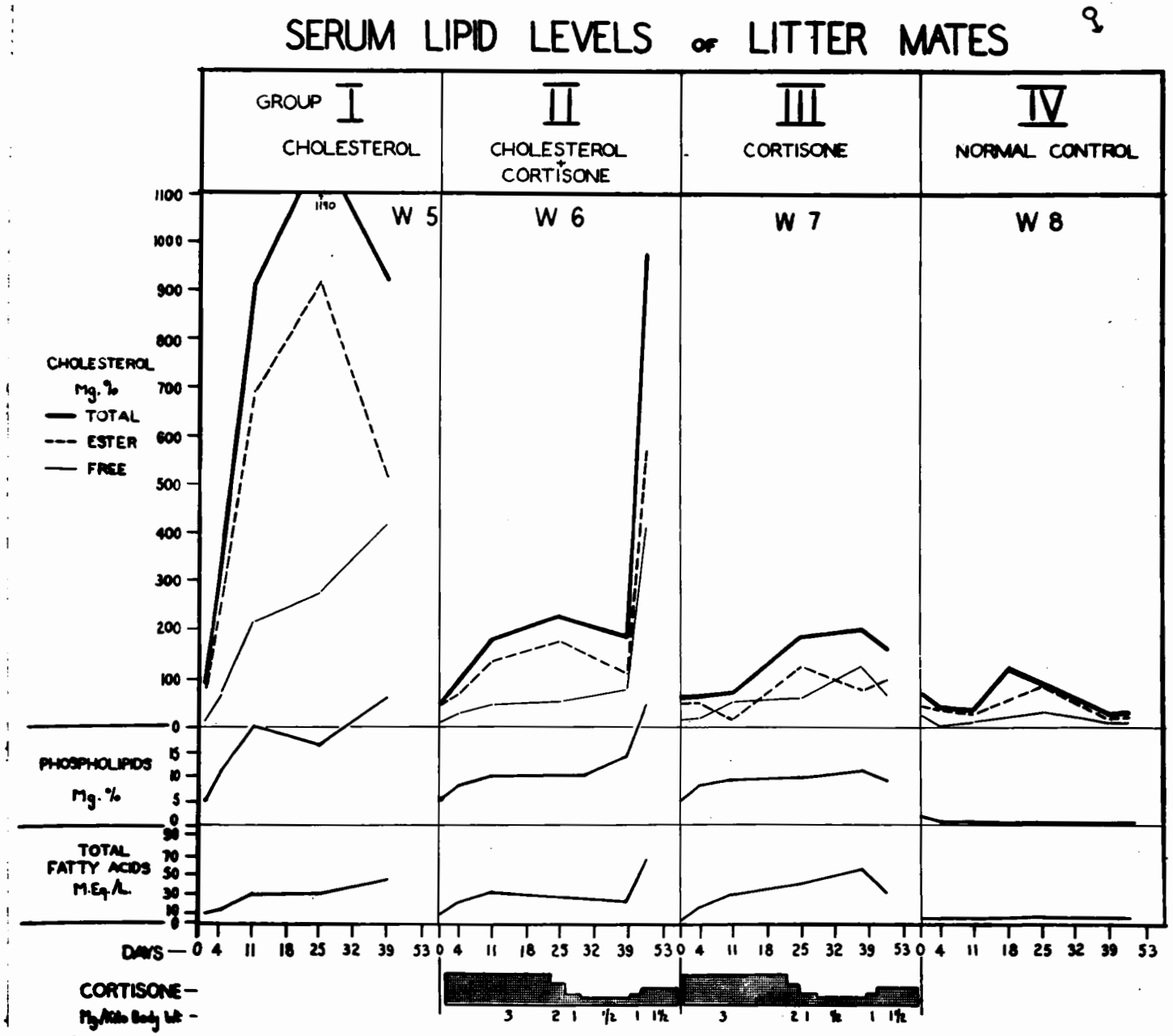


GRAPH 7.

SERUM LIPID LEVELS of LITTER MATES ♀

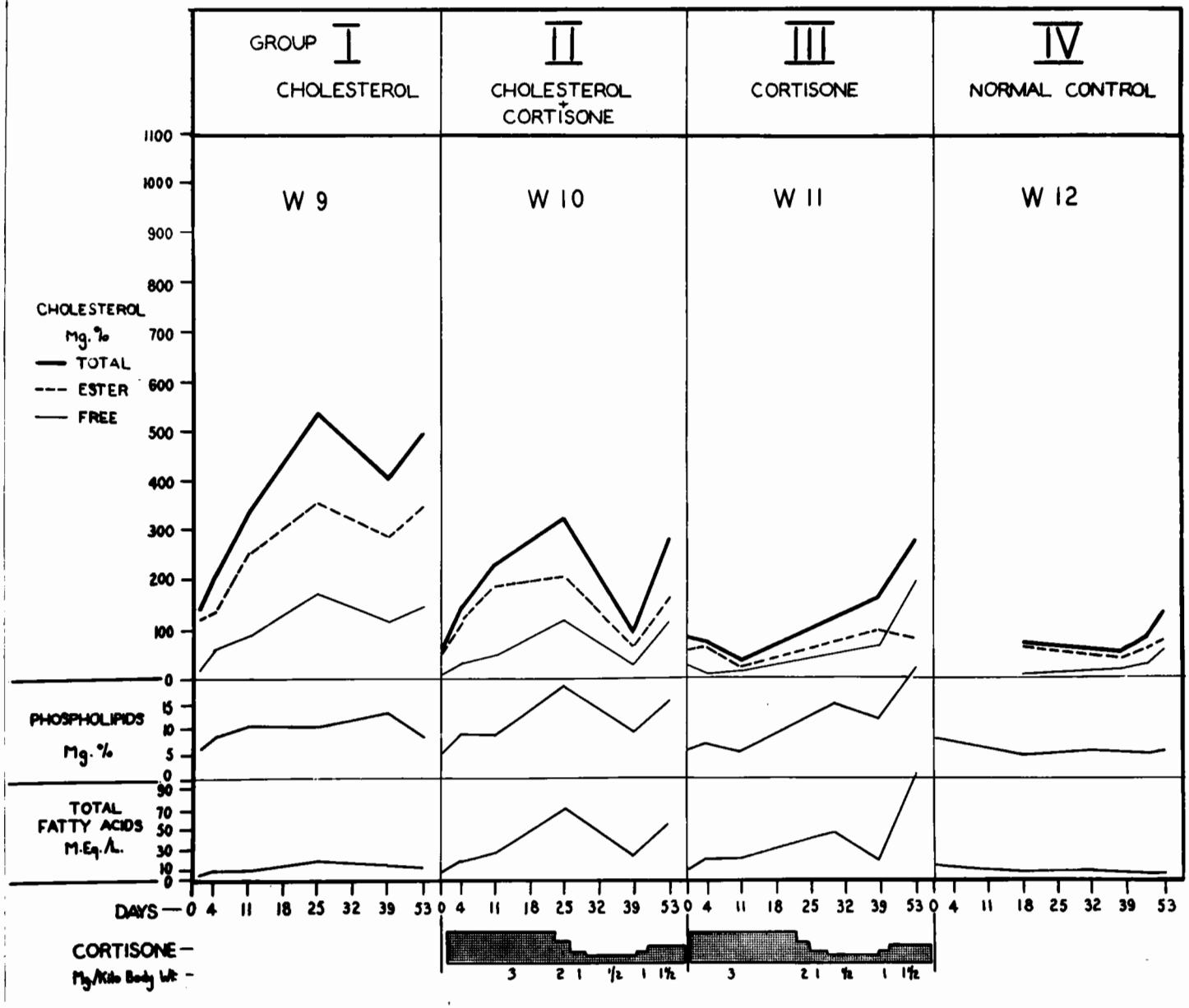


GRAPH 8

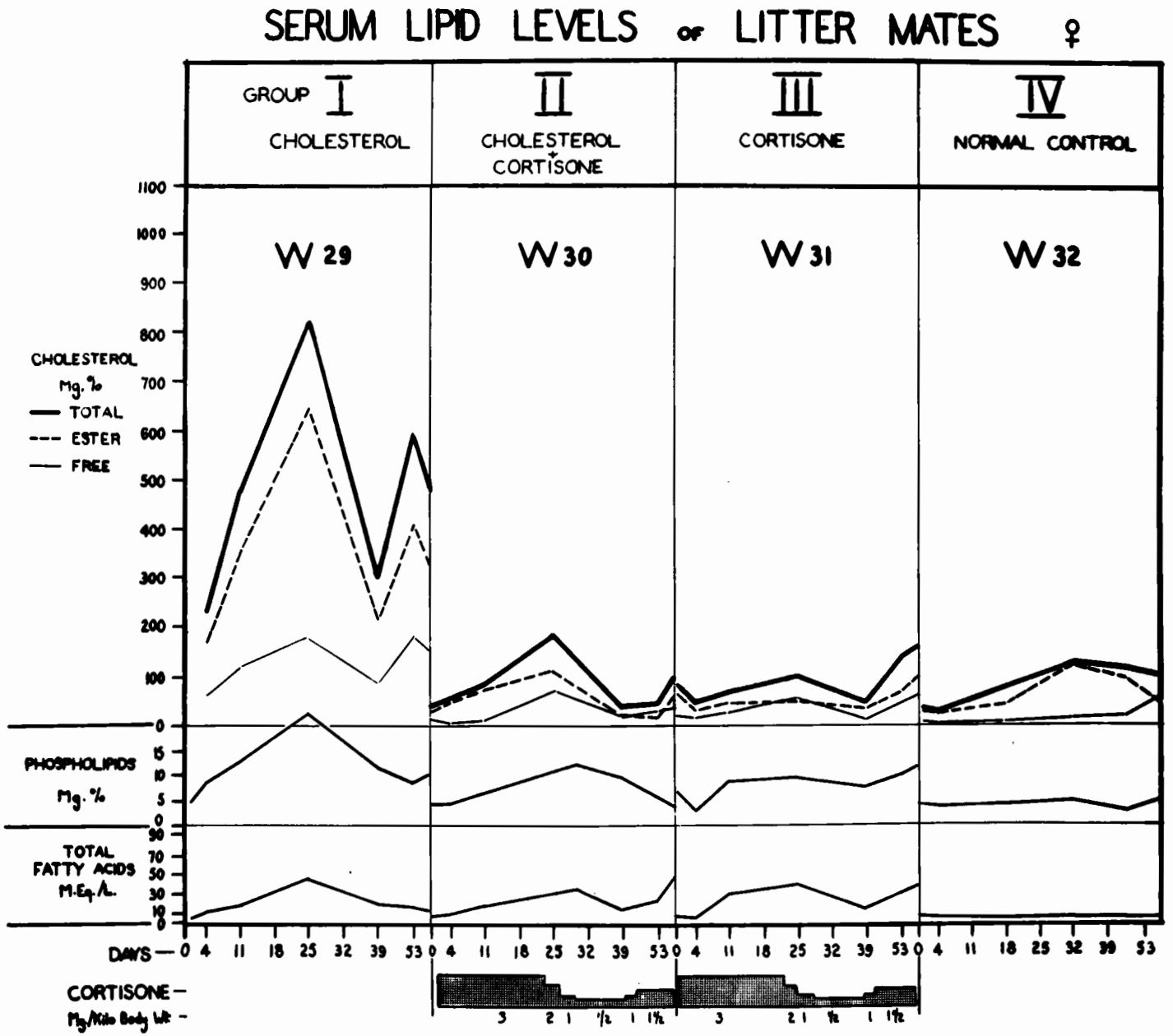

SERUM LIPID LEVELS of LITTER MATES
GROUP I
CHOLESTEROL
II
CHOLESTEROL + CORTISONE
III
CORTISONE
IV
NORMAL CONTROL
W 5
W 6
W 7
W 8
1190
CHOLESTEROL Mg.%
TOTAL
ESTER
FREE
PHOSPHOLIPIDS Mg.%
TOTAL FATTY ACIDS M.Eq./L.
DAYS
0 4 11 18 25 32 39 53
CORTISONE
Mg/Kilo Body Wt
3 2 1 ½ 1 1½
3 2 1 ½ 1 1½

GRAPH 9

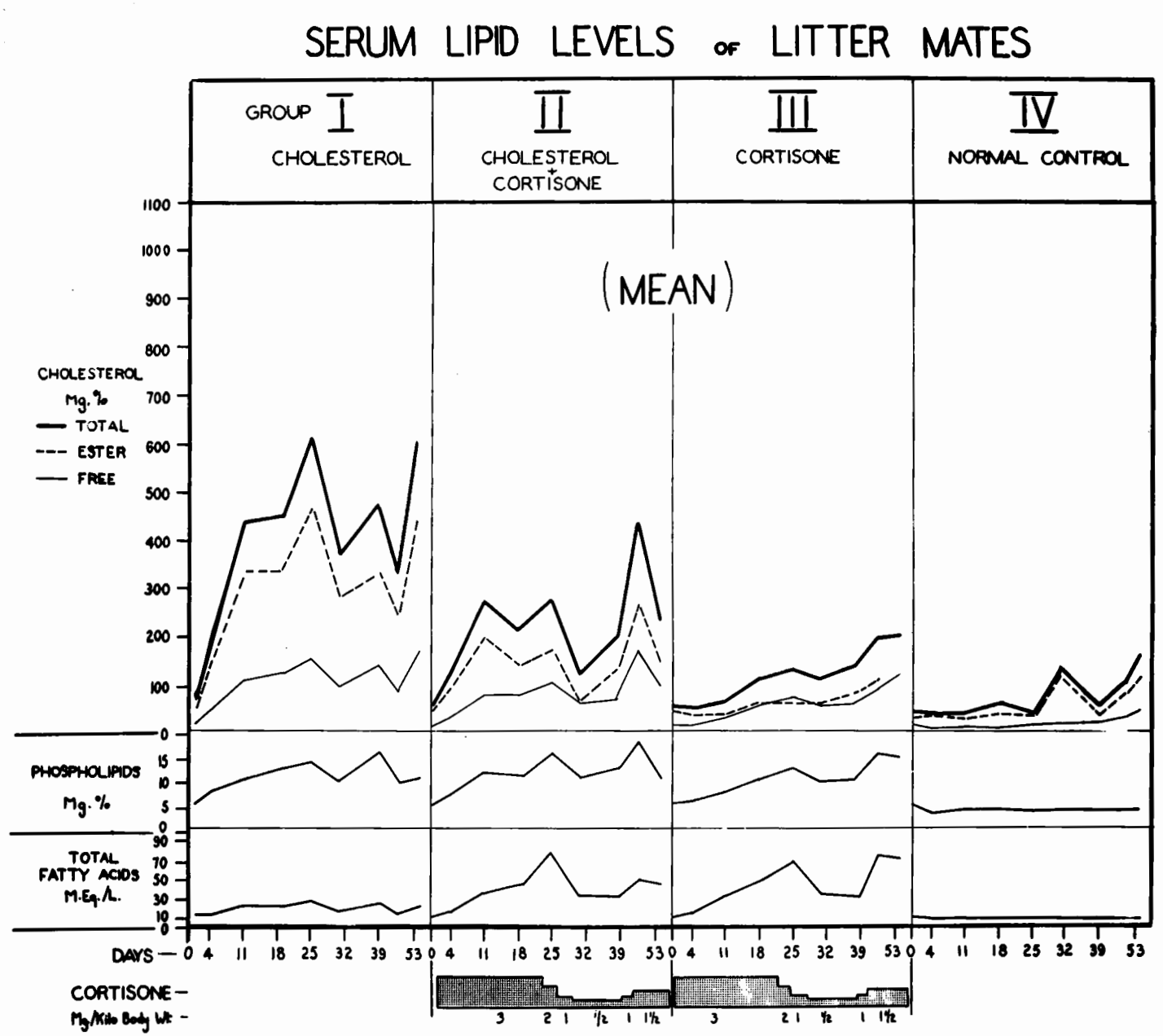
SERUM LIPID LEVELS of LITTER MATES ♀



GRAPH 10

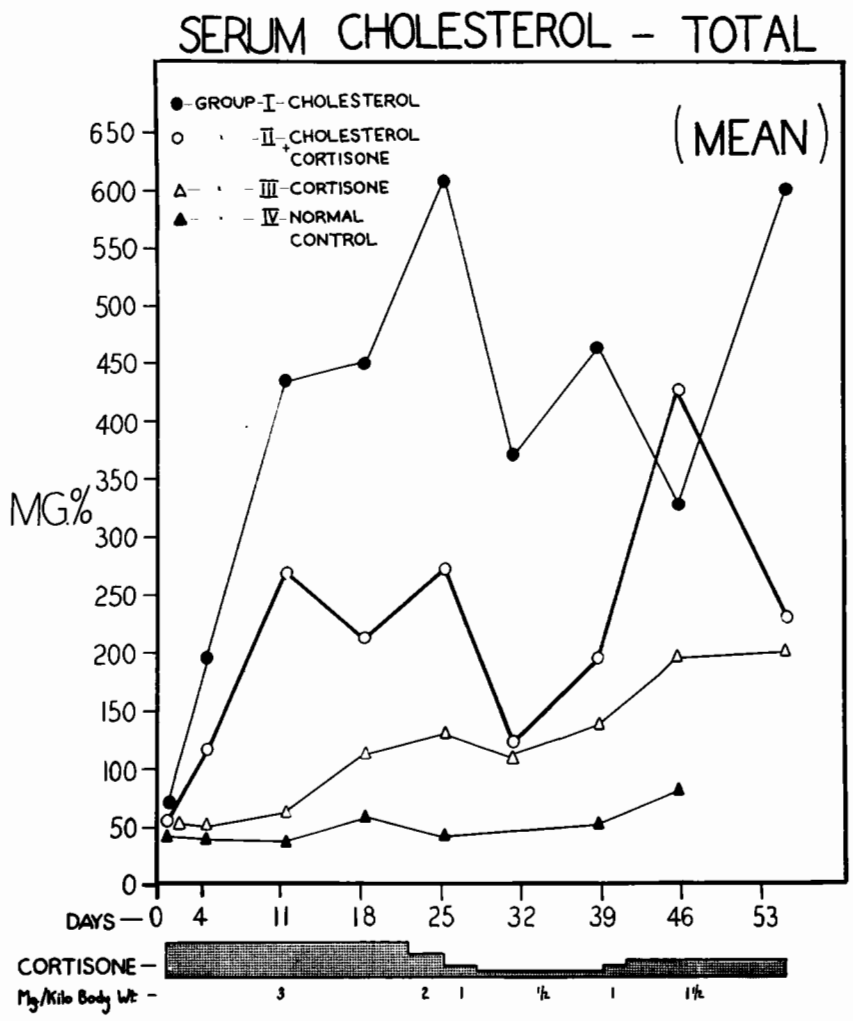

SERUM LIPID LEVELS of LITTER MATES ♀
GROUP I
CHOLESTEROL
II
CHOLESTEROL
CORTISONE
III
CORTISONE
IV
NORMAL CONTROL
W 29
W 30
W 31
W 32
CHOLESTEROL
Mg. %
TOTAL
ESTER
FREE
PHOSPHOLIPIDS
Mg. %
TOTAL
FATTY ACIDS
M.Eq./L.
DAYS
CORTISONE
Mg/Kilo Body Wt

GRAPH 11

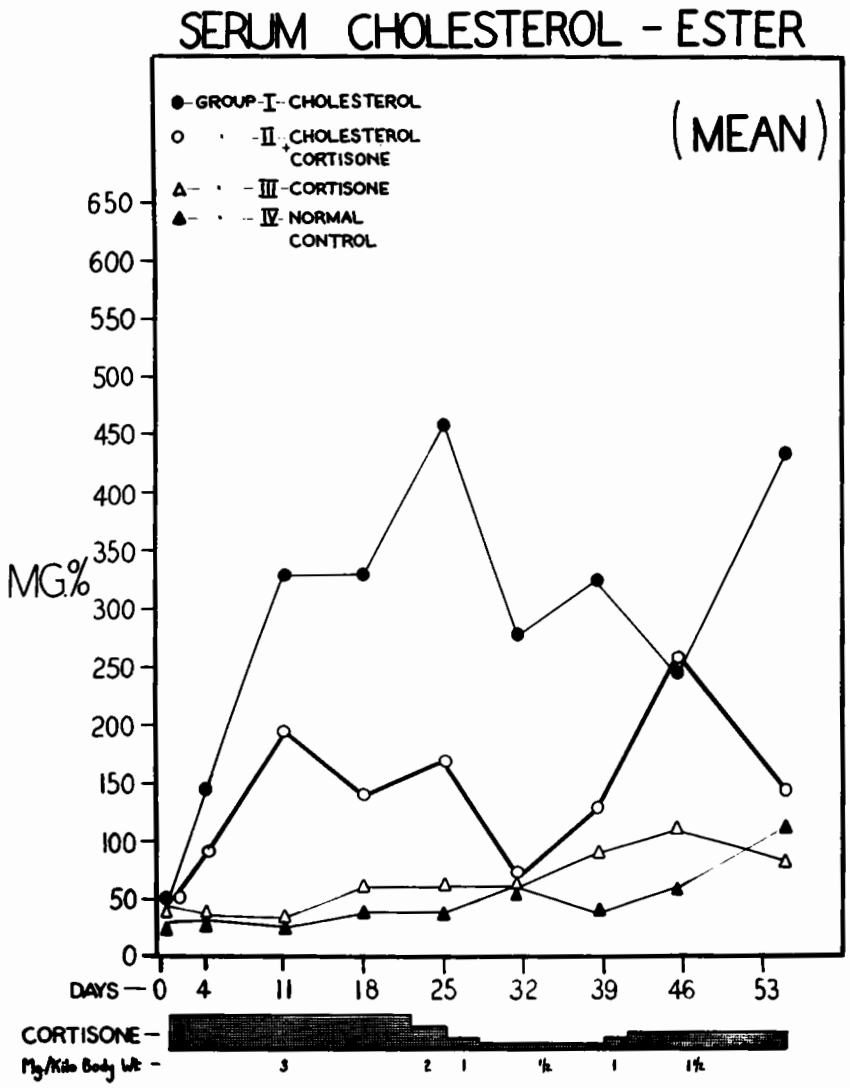

SERUM LIPID LEVELS OF LITTER MATES
GROUP I
CHOLESTEROL
II
CHOLESTEROL + CORTISONE
III
CORTISONE
IV
NORMAL CONTROL
(MEAN)
CHOLESTEROL Mg.%
TOTAL
ESTER
FREE
PHOSPHOLIPIDS Mg.%
TOTAL FATTY ACIDS M.Eq./L.
DAYS
CORTISONE
Mg./Kilo Body Wt

GRAPH 12

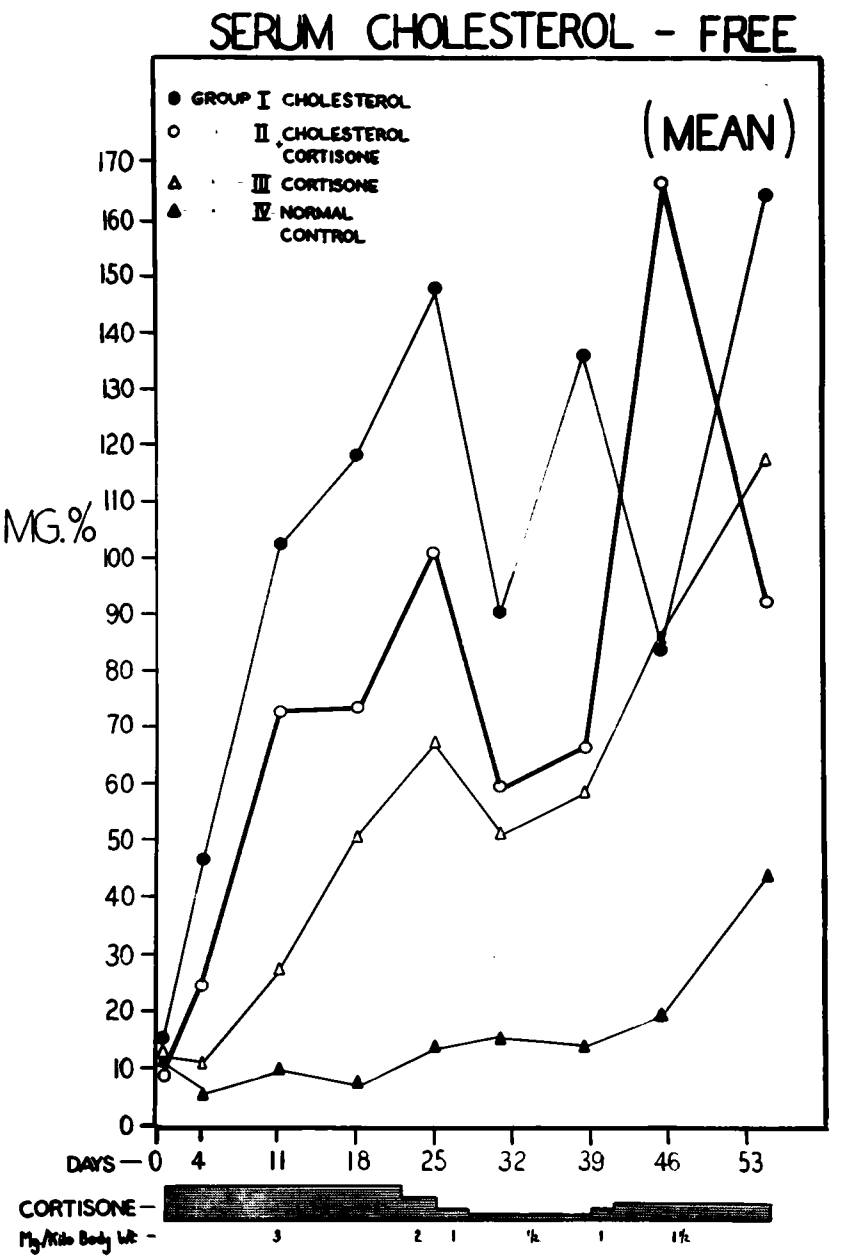
GRAPH 13


SERUM CHOLESTEROL - TOTAL
(MEAN)
GROUP I - CHOLESTEROL
II - CHOLESTEROL + CORTISONE
III - CORTISONE
IV - NORMAL CONTROL
MG%
650
600
550
500
450
400
350
300
250
200
150
100
50
0
DAYS — 0 4 11 18 25 32 39 46 53
CORTISONE —
Mg/Kilo Body Wt - 3 2 1 ½ 1 1½

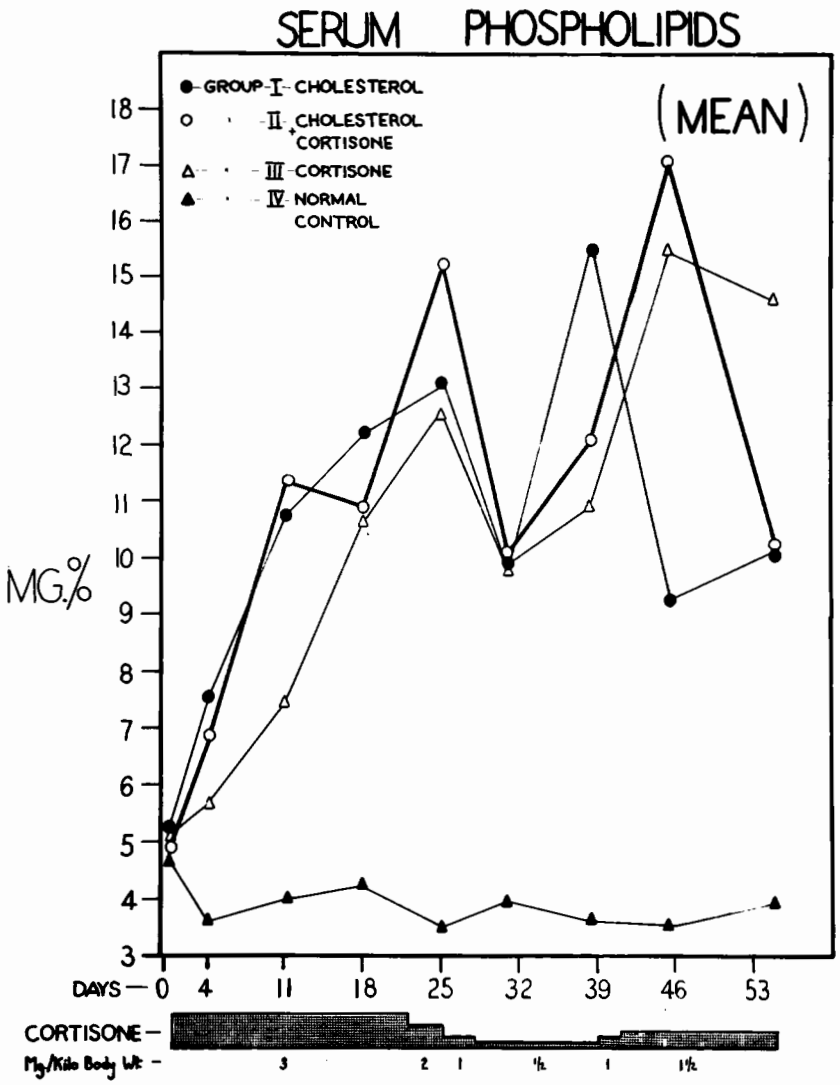
GRAPH 14


SERUM CHOLESTEROL - ESTER
(MEAN)
GROUP I - CHOLESTEROL
II + CHOLESTEROL CORTISONE
III - CORTISONE
IV - NORMAL CONTROL
MG%
650
600
550
500
450
400
350
300
250
200
150
100
50
0
DAYS — 0 4 11 18 25 32 39 46 53
CORTISONE —
Mg/Kilo Body Wt —
3
2
1
½
1
1½

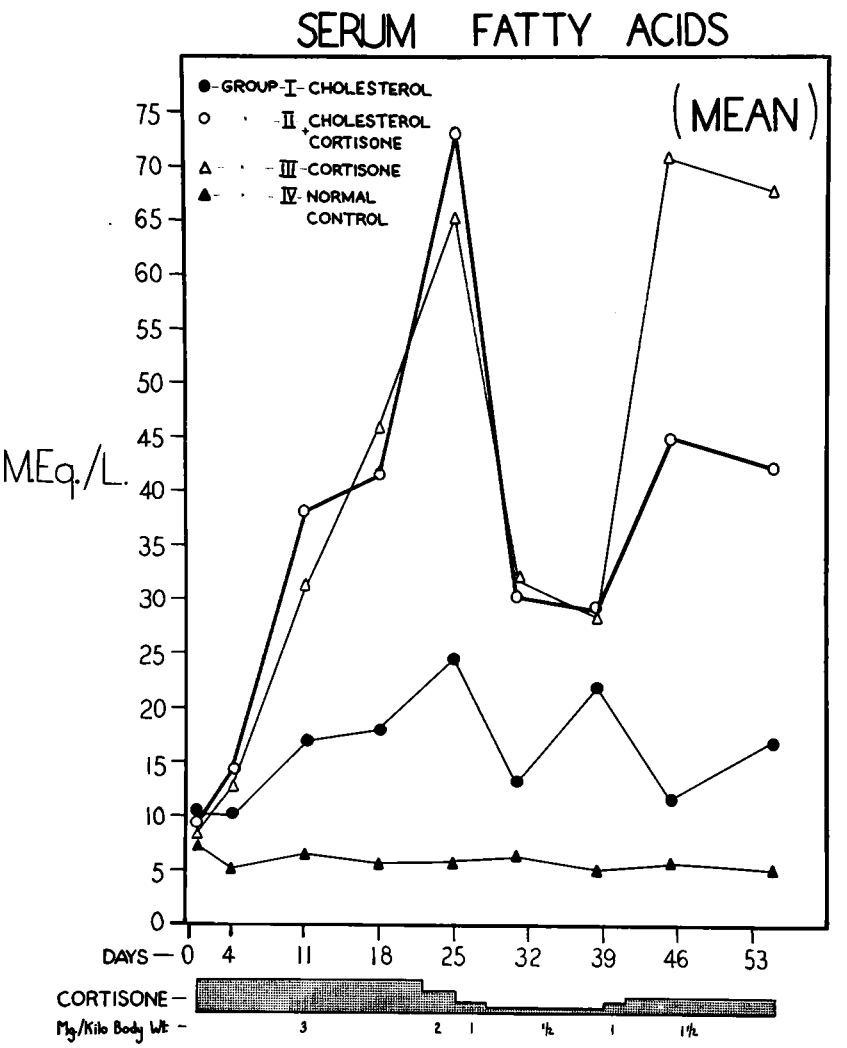
GRAPH 15


SERUM CHOLESTEROL - FREE
(MEAN)
GROUP I CHOLESTEROL
II CHOLESTEROL CORTISONE
III CORTISONE
IV NORMAL CONTROL
MG.%
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
DAYS — 0 4 11 18 25 32 39 46 53
CORTISONE —
Mg/Kilo Body Wt: - 3 2 1 ½ 1 1½

GRAPH 16

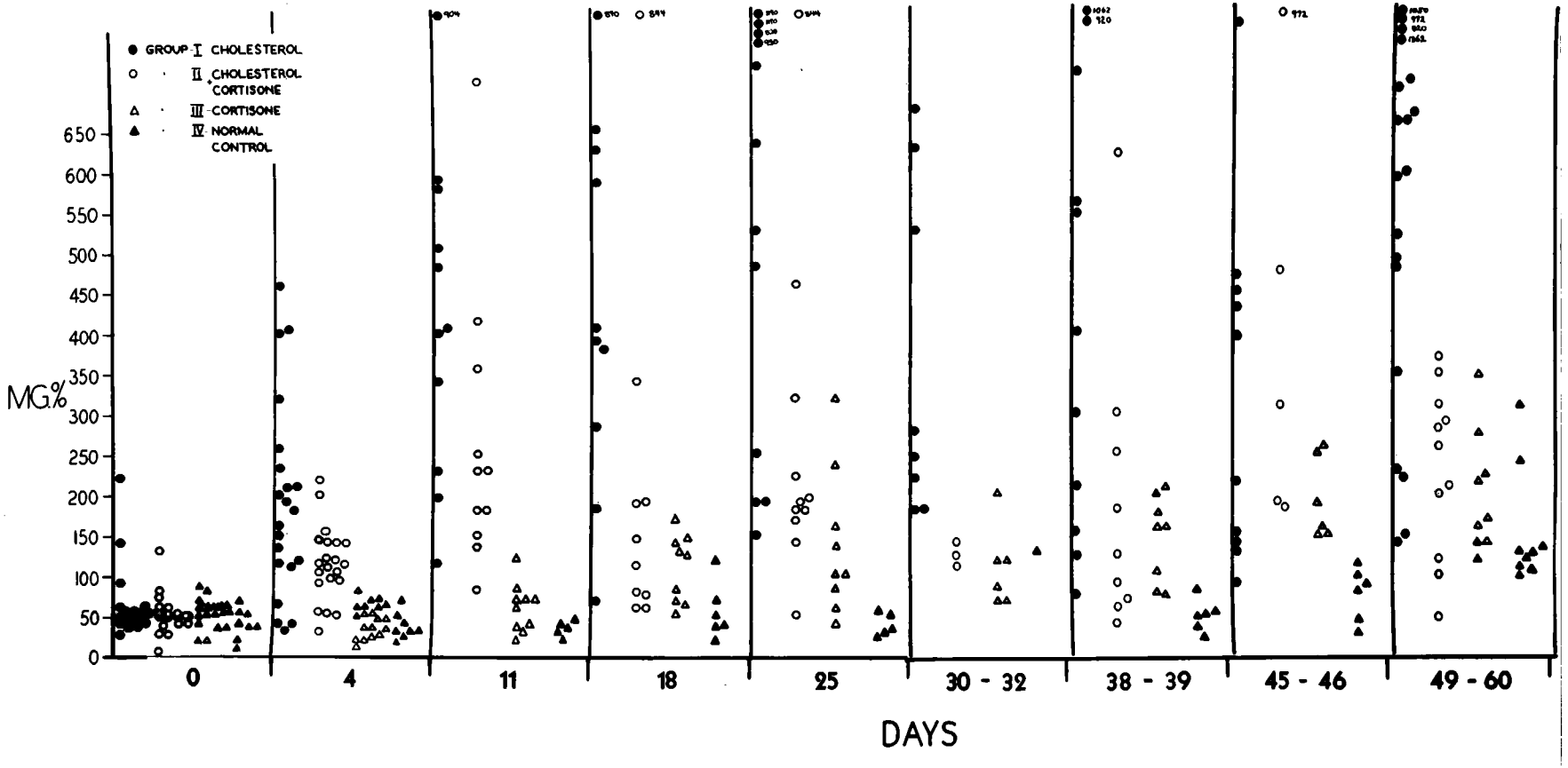

SERUM PHOSPHOLIPIDS
(MEAN)
GROUP I CHOLESTEROL
II CHOLESTEROL + CORTISONE
III CORTISONE
IV NORMAL CONTROL
MG.%
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
DAYS — 0 4 11 18 25 32 39 46 53
CORTISONE —
Mg./Kilo Body Wt. — 3 2 1 ½ 1 1½

GRAPH 17


SERUM FATTY ACIDS
(MEAN)
●-GROUP-I-CHOLESTEROL
○ · -II-CHOLESTEROL + CORTISONE
△ · -III-CORTISONE
▲ · -IV-NORMAL CONTROL
MEq./L.
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
DAYS — 0 4 11 18 25 32 39 46 53
CORTISONE —
Mg/Kilo Body Wt — 3 2 1 ½ 1 1½

G R A P H 18

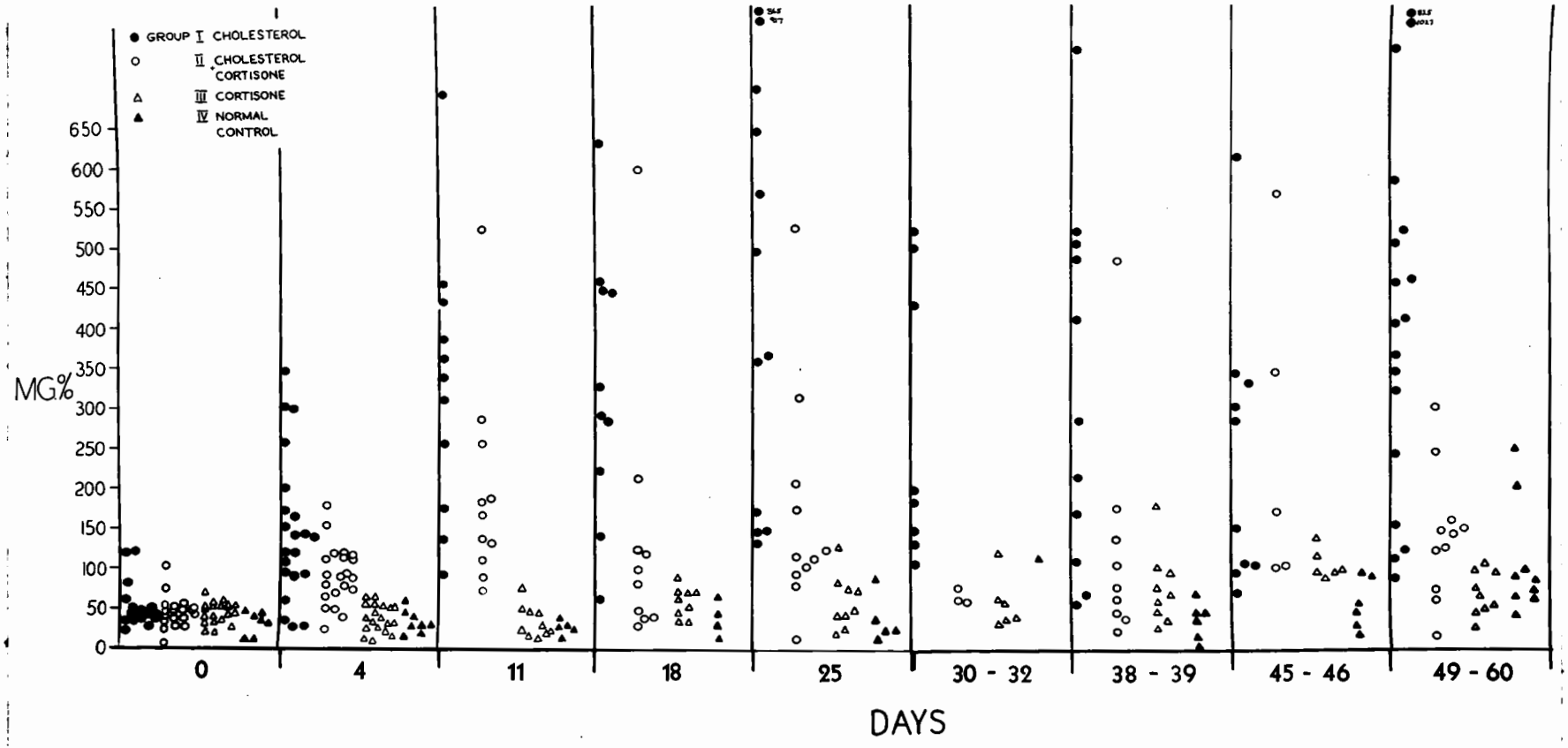
Scatter graph of the Serum Total Cholesterol levels in the animals of comparable experimental and control groups on respective bleeding days.


GROUP I CHOLESTEROL
II CHOLESTEROL CORTISONE
III CORTISONE
IV NORMAL CONTROL
MG%
650
600
550
500
450
400
350
300
250
200
150
100
50
0
0
4
11
18
25
30 - 32
38 - 39
45 - 46
49 - 60
DAYS

GRAPH 18

G R A P H 19

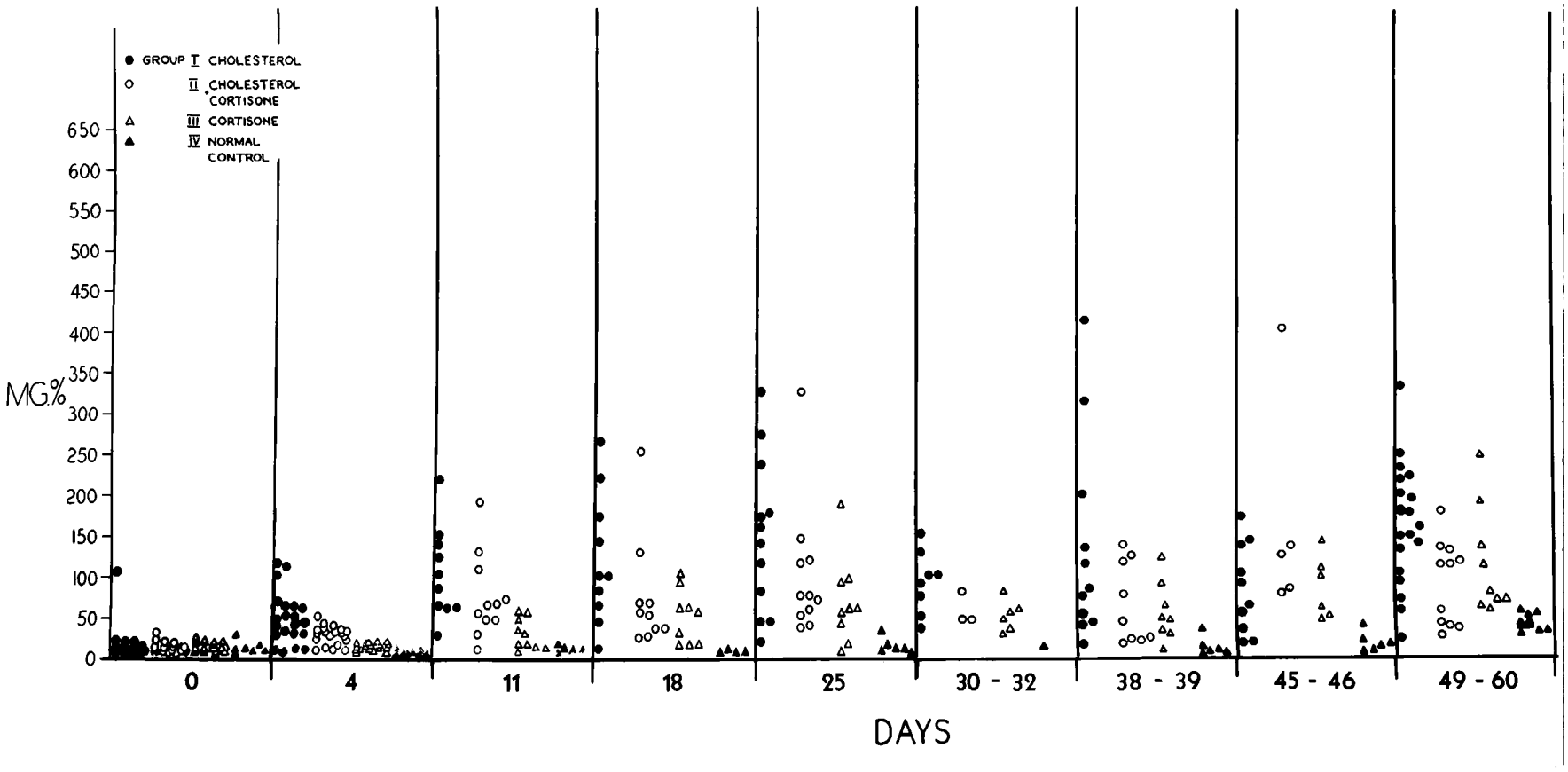
Scatter graph of the Serum Ester Cholesterol levels in the animals of comparable experimental and control groups on respective bleeding days.


GROUP I CHOLESTEROL
II CHOLESTEROL CORTISONE
III CORTISONE
IV NORMAL CONTROL
MG%
650
600
550
500
450
400
350
300
250
200
150
100
50
0
0
4
11
18
25
30 - 32
38 - 39
45 - 46
49 - 60
DAYS

GRAPH 19

G R A P H 20

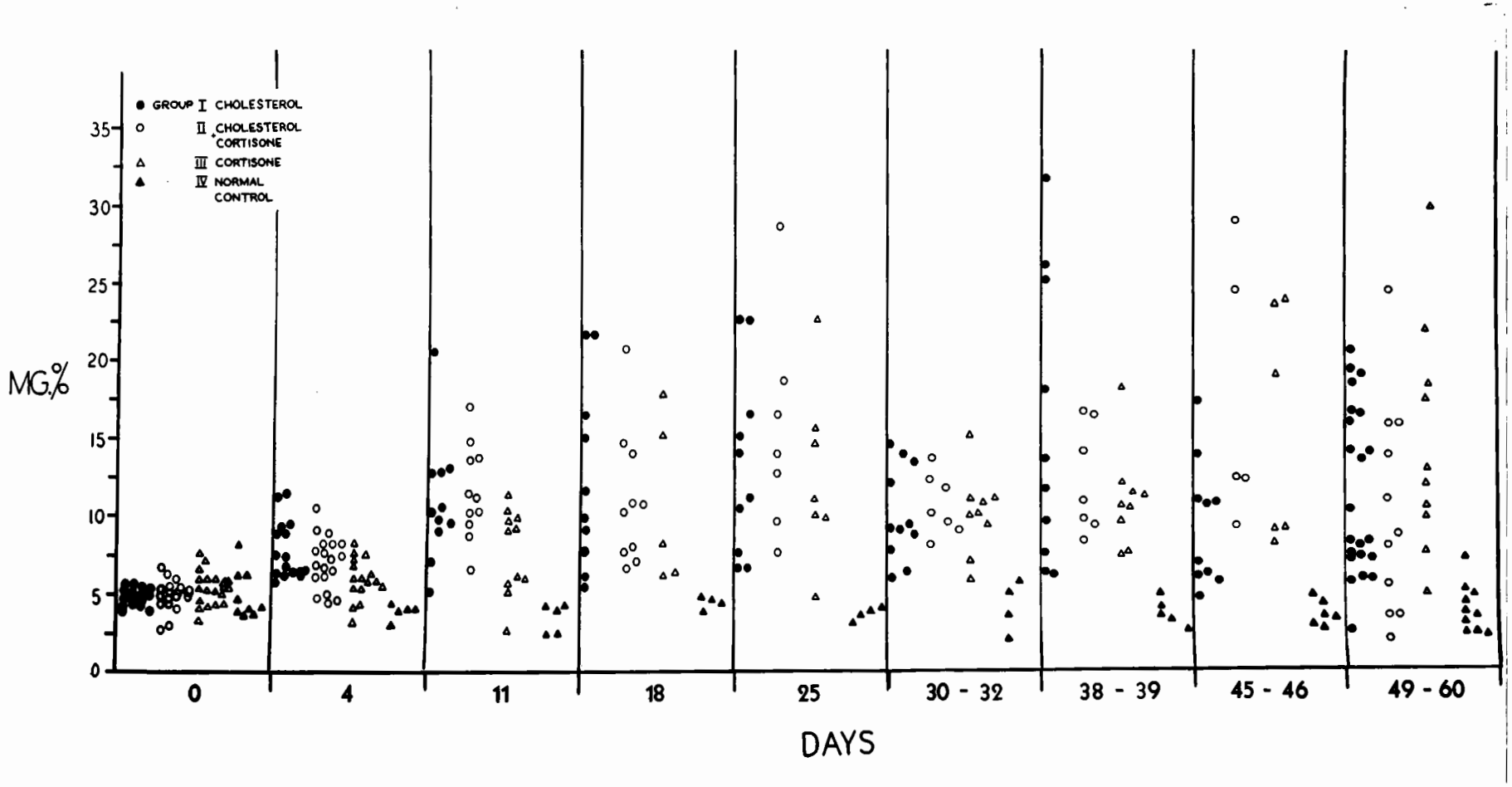
Scatter graph of the Serum Free Cholesterol levels in the animals of comparable experimental and control groups on respective bleeding days.


GROUP I CHOLESTEROL
II CHOLESTEROL CORTISONE
III CORTISONE
IV NORMAL CONTROL
MG.%
650
600
550
500
450
400
350
300
250
200
150
100
50
0
0
4
11
18
25
30 - 32
38 - 39
45 - 46
49 - 60
DAYS

GRAPH 20

G R A P H 21

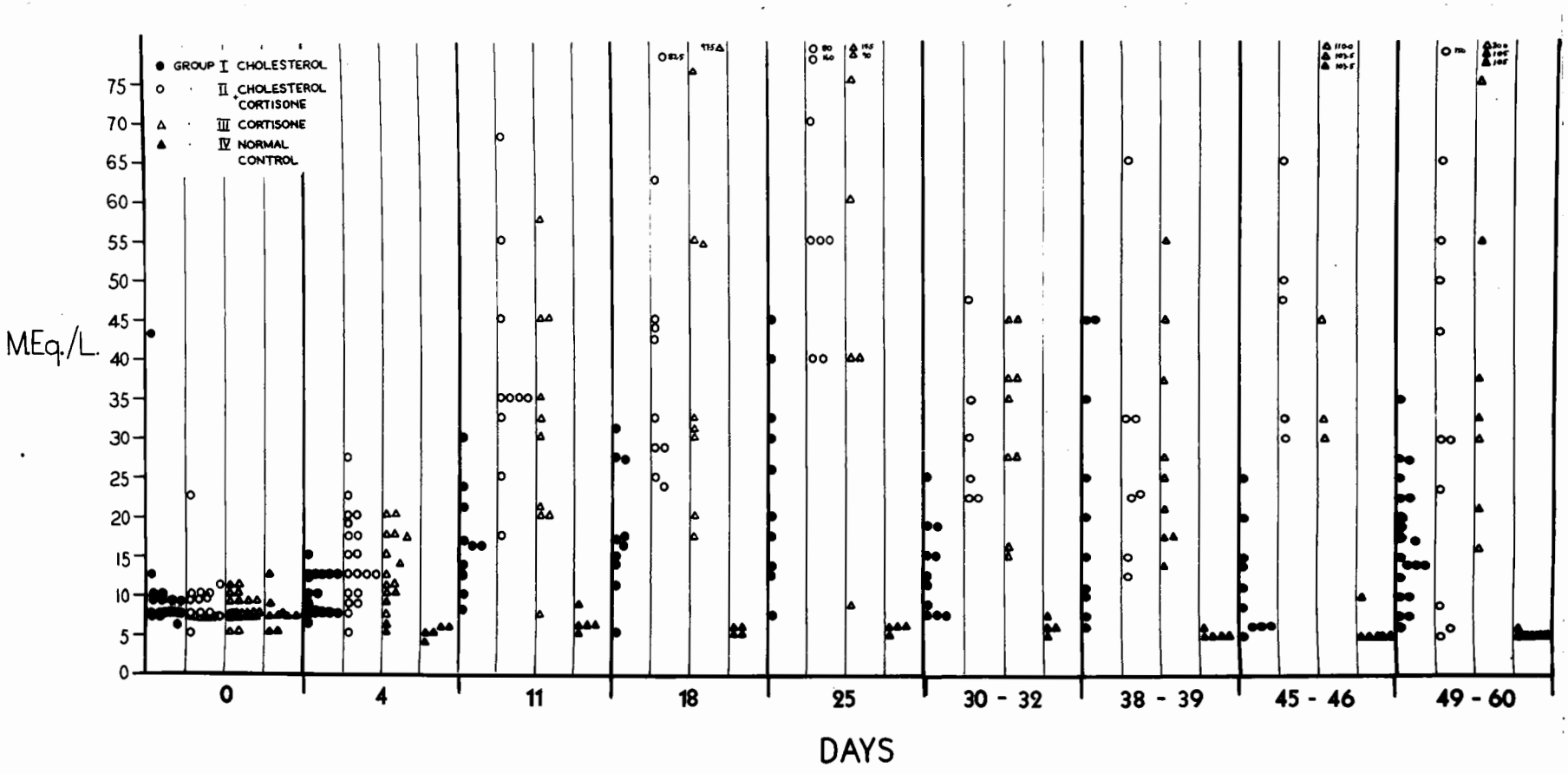
Scatter graph of the Serum Phospholipid levels in the animals of comparable experimental and control groups on respective bleeding days.


GROUP I CHOLESTEROL
II CHOLESTEROL CORTISONE
III CORTISONE
IV NORMAL CONTROL
MG.%
35
30
25
20
15
10
5
0
0
4
11
18
25
30 - 32
38 - 39
45 - 46
49 - 60
DAYS

GRAPH 21

G R A P H 22

Scatter graph of the Serum Total Fatty Acids in the animals of comparable experimental and control groups on respective bleeding days.


GROUP I CHOLESTEROL
II CHOLESTEROL CORTISONE
III CORTISONE
IV NORMAL CONTROL
MEq./L.
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
0
4
11
18
25
30 - 32
38 - 39
45 - 46
49 - 60
DAYS

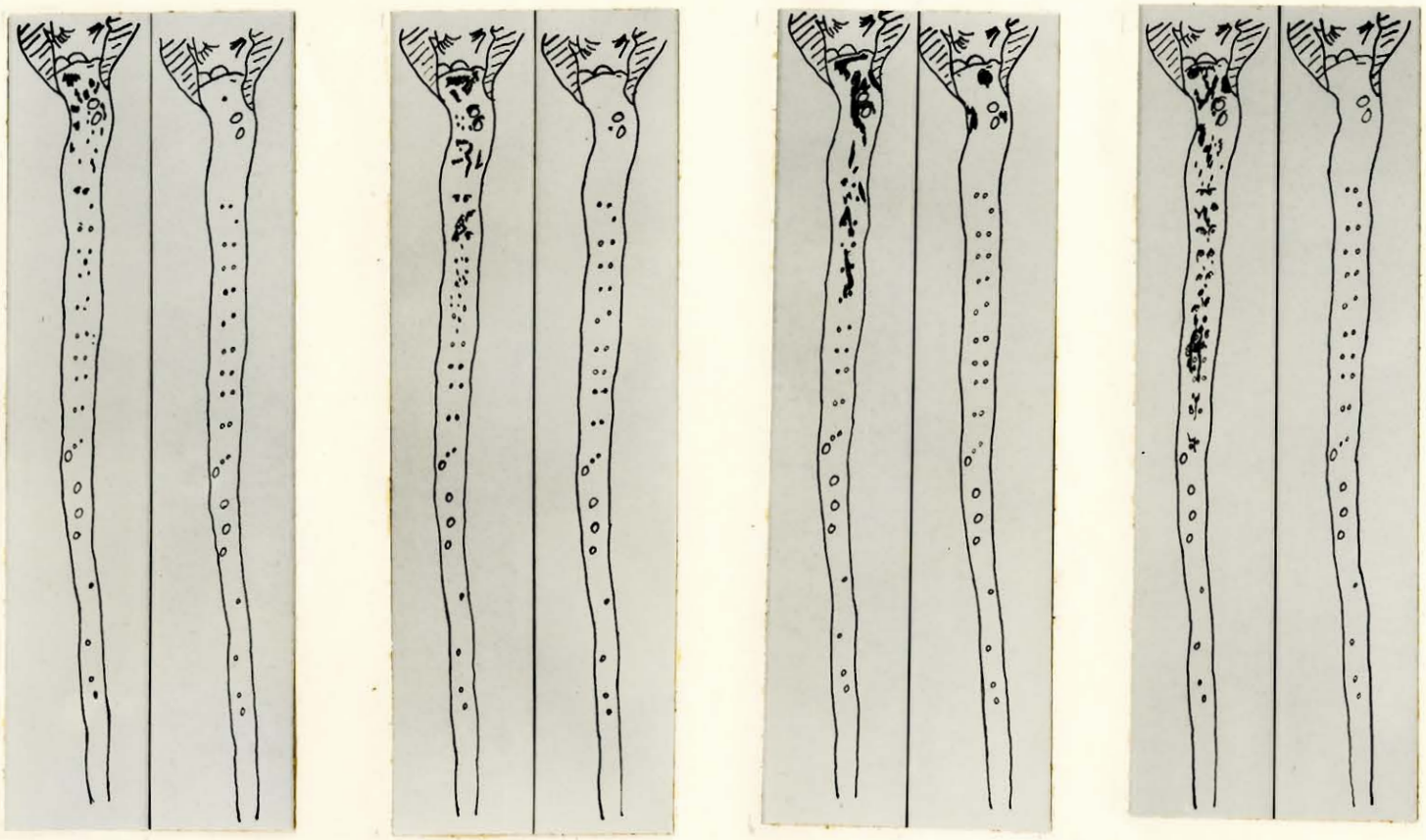
GRAPH 22

D I A G R A M S

Diagrams 1, 2 and 3 which follow show the degree of atherosclerosis in the aortae of the Cortisone cholesterol-fed rabbits and their cholesterol-fed controls. These are compared according to litter mates.

D I A G R A M 1

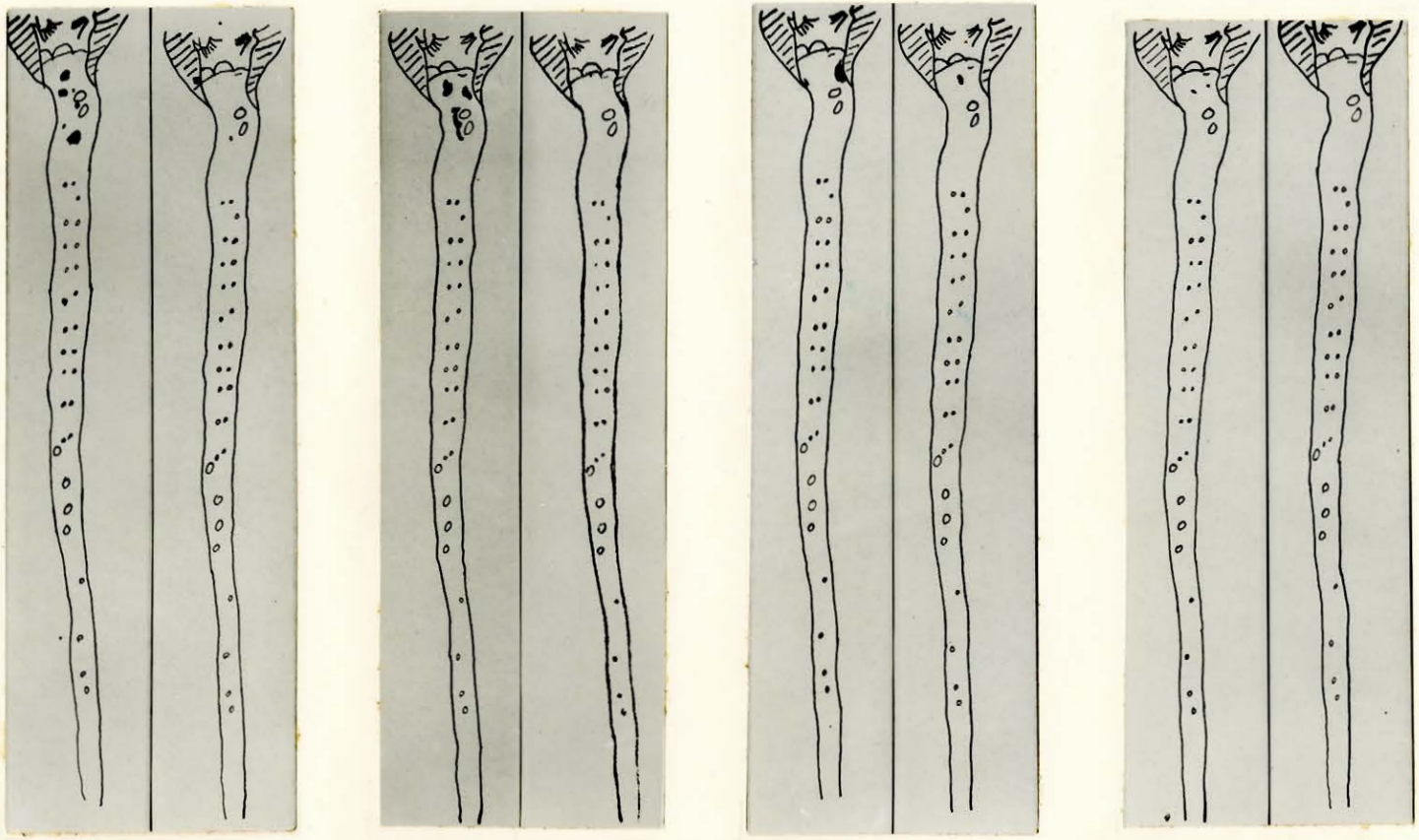
Exptl. Group.	I	II	I	II	I	II	I	II
Animal number	V-64	V-65	V-88	V-89	W-5	W-6	W-9	W-10
Sex.	M	M	M	M	M	M	F	F
Aortic atherosclerosis.	+	tr.	++	tr.	++	+	++	0



V-64 V-65 V-88 V-89 W-5 W-6 W-9 W-1o

D I A G R A M 2

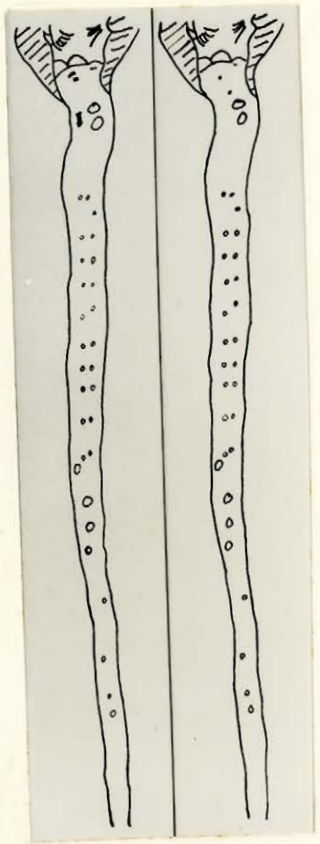
Exptl. Group	I	II	I	II	I	II	I	II
Animal number	V-52	V-53	V-67	V-68	V-71	V-72	V-84	V-85
Sex.	M	M	M	M	F	F	M	M
Aortic atherosclerosis	+	tr.	+	0	+	tr.	tr.	0



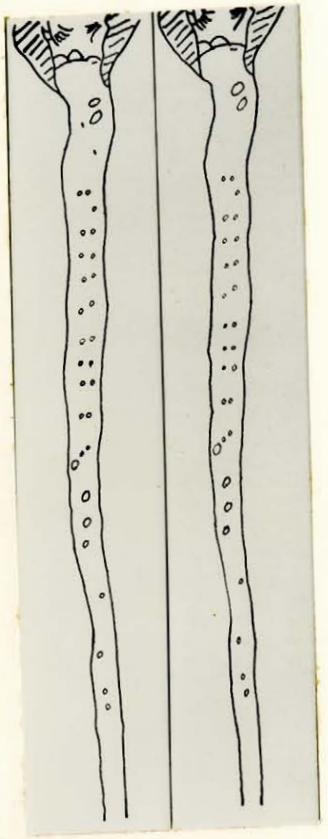
V-52 V-53 V-67 V-68 V-71 V-72 V-84 V-85

D I A G R A M 3

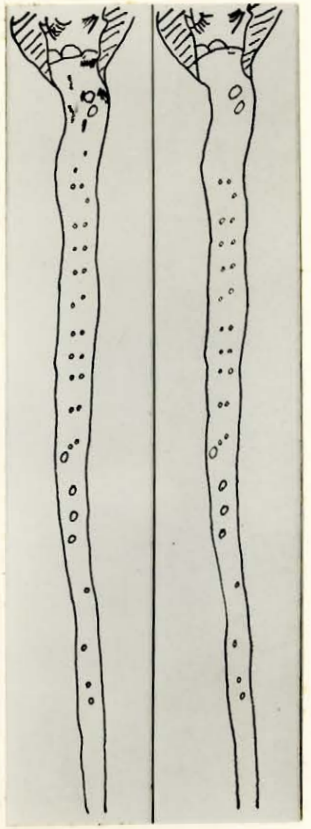
Exptl. Group	I	II	I	II	I	II	I	II
Animal number.	V-97	V-98	W-1	W-2	W-29	W-30	V-77	V-79
Sex	M	M	F	F	F	F	F	F
Aortic atherosclerosis.	+	tr.	tr.	0	+	0	0	0



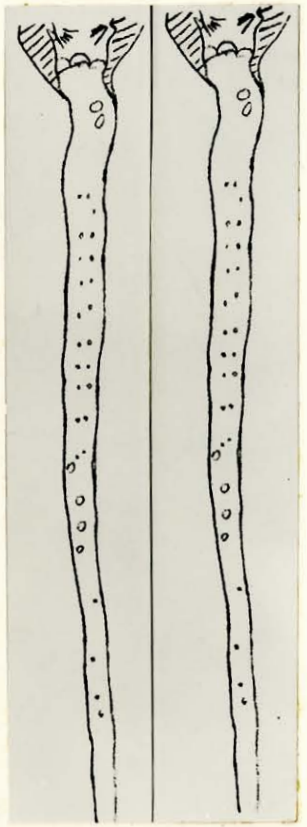
V-97 V-98



W-1 W-2



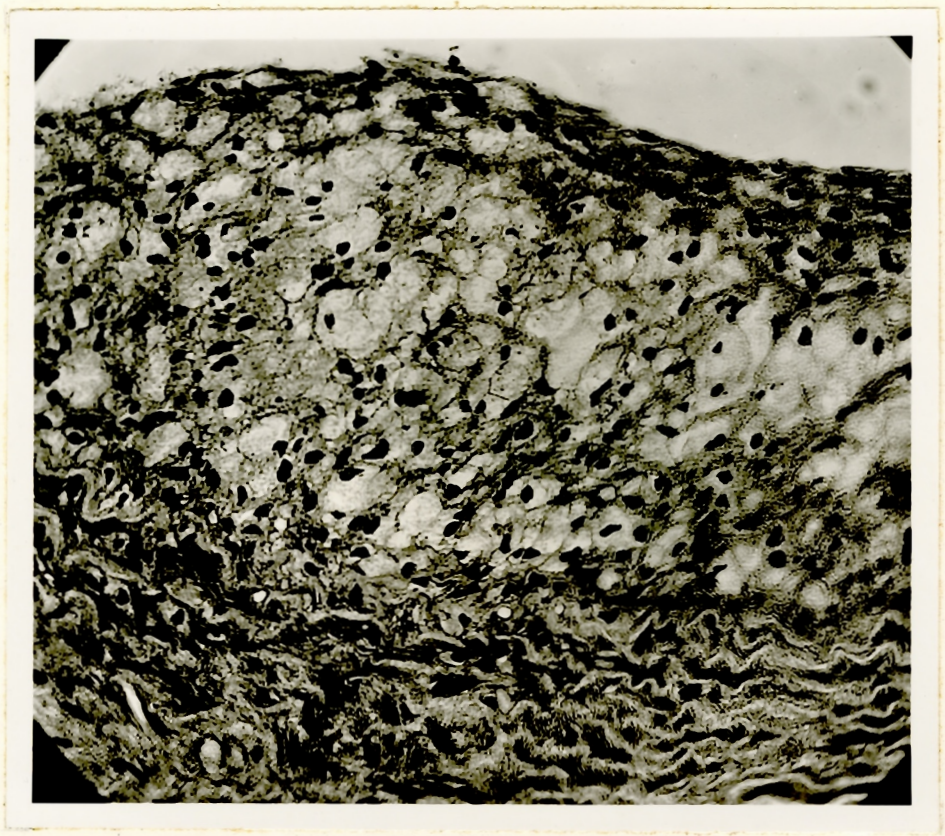
W-29 W-30



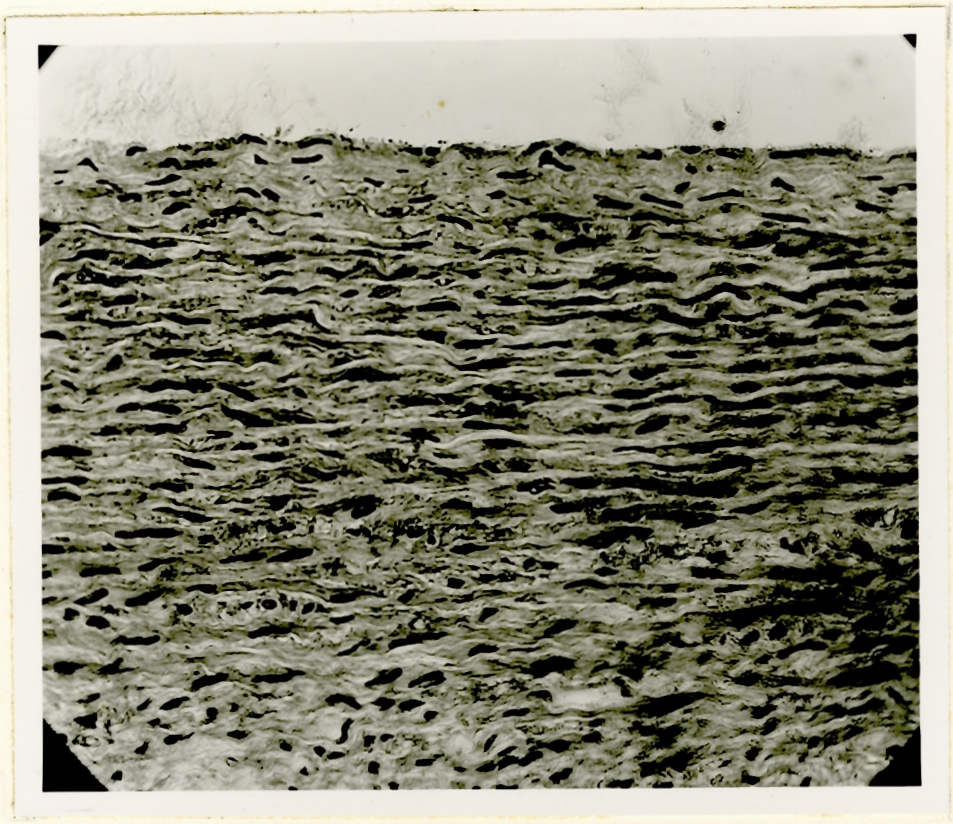
V-77 V-79

Diagrams 4 and 5 which follow are photomicrographs which show the characteristic aortic lesions of experimental cholesterol atherosclerosis in rabbits. The intimal thickenings in the cholesterol-fed control rabbits, W-5 and W-9, contain an abundance of large, pale, spongy, lipid-filled foam cells. W-6 and W-10 are their respective cholesterol-fed Cortisone treated litter mates which do not show these lesions, although there was in each a minimal degree of gross atherosclerosis. In each case the section was taken from the proximal 3 mm. of the aorta immediately below the aortic cusps.

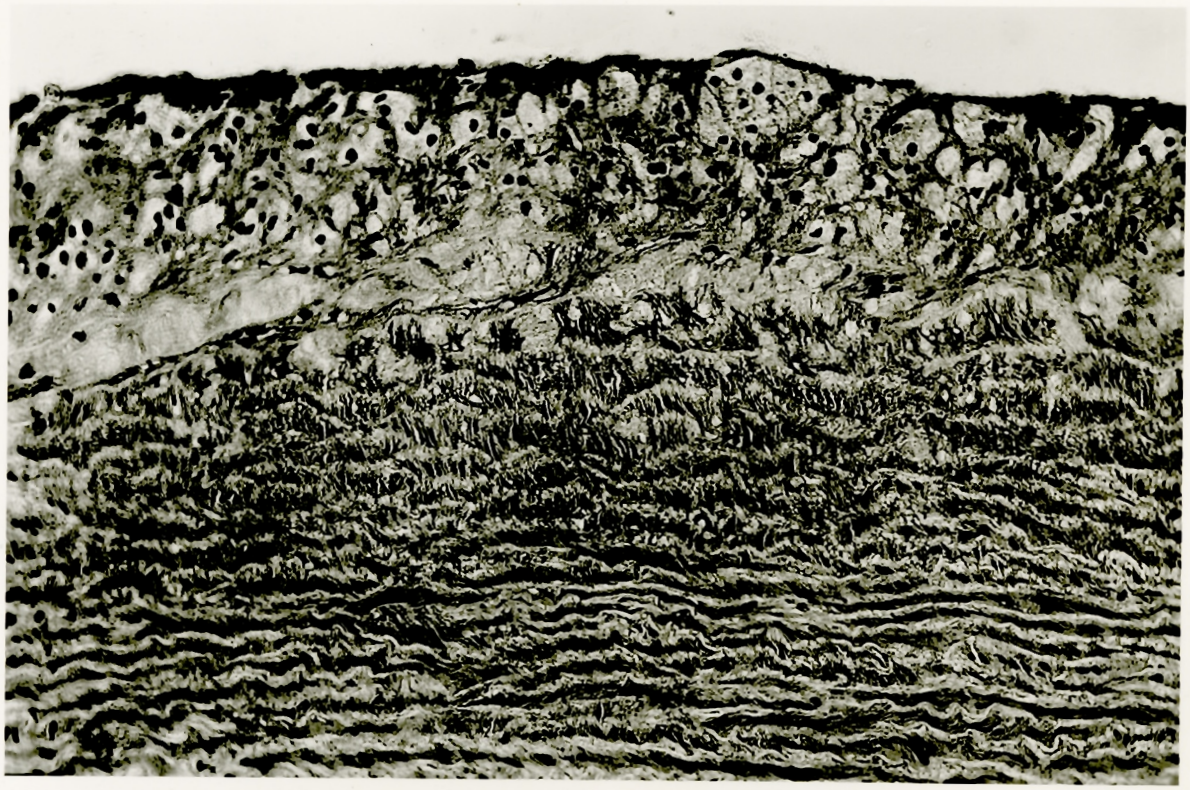
W-5



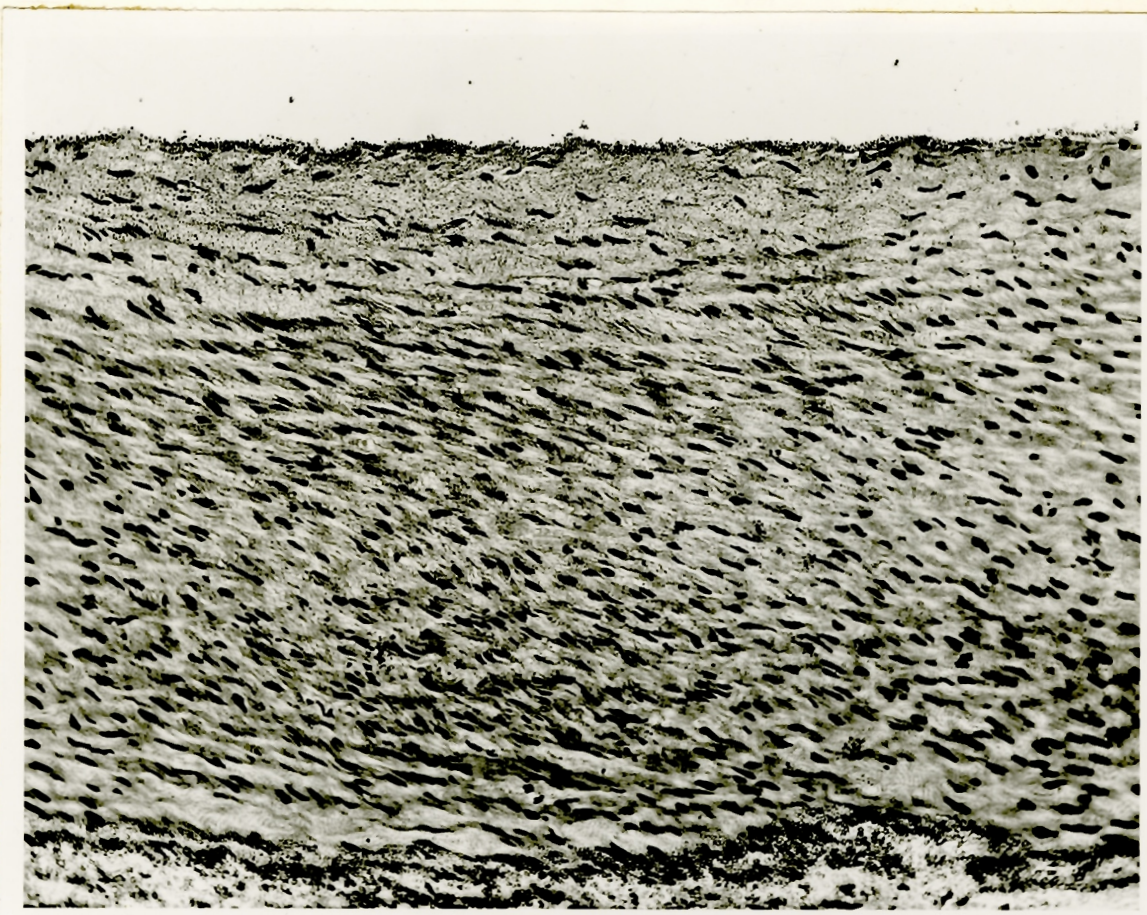
W-6



W-9



W-1o



PROTOCOLS

Group	Group I	Group II	Group III	Group IV
Animal No.	V-52	V-53	V-54	V-55
Progression period-days	55	56	56	56
Normal food intake-gm.	5111	6735	5624	5600
Cholesterol intake-gm.	31	31	-	-
Cortisone mg. Gr.II & III.	-	313	249	-
% wt.change of initial wt.	-17.6	-7.9	-13.7	+16.5
Mode of death	spontaneous	I.V.Nembutal	I.V.Nembutal	I.V.Nembutal
Total body weight-gm.	1779	2627	2279	3240
Carcass wt.-gm.	1550	1940	1590	2605
ORGAN WEIGHTS				
Heart-gm.	5.9	4.4	6.7	7.8
Lungs-gm.	10.4	7.9	11.8	9.6
Spleen-gm.	0.42	0.38	1.25	1.30
Liver-gm.	67.1	126.8	164.5	102.5
Kidneys-gm.	19.5	19.4	20.9	14.2
Adrenals-gm.	0.3506	0.1183	0.0887	0.1993
Pituitary-gm.	0.0156	0.0232	0.0088	0.0260
Brain-gm.	8.16	7.52	7.26	8.65

P R O T O C O L S

Group	Group I	Group II	Group III	Group IV
Animal No.	V-56	V-57	V-58	V-59
Progression period-days	61	29	24	61
Normal food intake-gm.	6095	3397	2746	6100
Cholesterol intake-gm.	38	16	-	-
Cortisone-mg.	-	157	178	-
%wt.change of initial wt.	+16.6	-12.3	-21.2	+13.8
Mode of death	Blow on head	Spon-taneous.	Spon-taneous	Blow on head
Total body weight-gm.	2857	2090	2050	3545
Carcass wt.-gm.	2265	1525	1740	2803
ORGAN WEIGHTS				
Heart-gm.	6.1	6.5		7.8
Lungs-gm.	12.2	19.8	17.6	20.5
Spleen-gm.	1.5	0.8	0.4	1.2
Liver-gm.	126.1	101.9	95.2	98.6
Kidneys-gm.	26.5	21.9	21.4	16.8
Adrenals-gm.	0.5325		0.1260	0.2583
Pituitary-gm.	0.0262		0.0240	0.0337
Brain-gm.	8.10	8.80	8.0	8.90

PROTOCOLS

Group	Group I	Group II	Group III	Group IV
Animal No.	V-60	V-61	V-62	V-63
Progression period-days	61	25	40	61
Normal food intake-gm.	6033	2631	4551	6100
Cholesterol intake-gm.	37	14	-	-
Cortisone-mg.	-	199	234	-
% wt.change of initial wt.	+17.4	-28.3	-15.6	+21.3
Mode of death	Blow on head	Spon-taneous	Spon-taneous	Blow on head
Total body weight-gm.	2956	2075	2445	2981
Carcass wt.-gm.	2439	1608	1589	2322
ORGAN WEIGHTS				
Heart-gm.	5.9		8.9	6.3
Lungs-gm.	9.3		22.6	10.2
Spleen-gm.	1.9	1.1	2.0	1.2
Liver-gm.	100.9	140.8	138.3	85.0
Kidneys-gm.	2.7	20.5	19.8	12.3
Adrenals-gm.	0.4113	0.2930	0.1605	0.2042
Pituitary-gm.	0.0374	0.0280	0.0373	0.0407
Brain-gm.	9.7	8.8	9.6	9.1

PROTOCOLS

Group	Group I	Group II	Group III
Animal No.	V-64	V-65	V-66
Progression period-days	57	57	57
Normal food intake-gm.	5612	6684	6662
Cholesterol intake-gm.	35	30	-
Cortisone-mg.	-	267	317
% wt.change of initial wt.	+10.5	-16.4	-21.9
Mode of death	I.V. Nembutal.	I.V. Nembutal	Spontaneous
Total body weight-gm.	3720	2248	2290
Carcass wt.-gm.	2785	1296	1668
ORGAN WEIGHTS			
Heart-gm.	7.8	5.4	5.9
Lungs-gm.	11.9	9.3	9.0
Spleen-gm.	2.0	0.9	0.4
Liver-gm.	106.2	76.8	85.5
Kidneys-gm.	15.7	14.9	19.5
Adrenals-gm.	0.6385	0.0997	0.2308
Pituitary-gm.	0.0360	0.0224	0.0303
Brain-gm.	8.6	6.7	8.2

PROTOCOLS

Group	Group I	Group II	Group III	Group IV
Animal No.	V-67	V-68	V-69	V-70
Progression period-days	55	55	55	56
Normal food intake-gm.	5278	7354	6100	5600
Cholesterol intake-gm.	52	34	-	-
Cortisone-mg.	-	304	246	-
% wt.change of initial wt.	+0.23	-4.3	-22.5	+25.4
Mode of death	I.V. Nembutal	I.V. Nembutal	Spontaneous	I.V. Nembutal
Total body weight-gm.	2975	2690	2004	3329
Carcass wt.-gm.	2295	1881	1521	2530
ORGAN WEIGHTS				
Heart-gm.	5.5	5.8	5.8	7.3
Lungs-gm.	19.0	9.7	9.3	11.7
Spleen-gm.	1.5	0.7	0.3	1.6
Liver-gm.	98.5	147.9	88.2	113.7
Kidneys-gm.	15.5	21.2	21.6	15.1
Adrenals-gm.	0.3105	0.0777	0.1468	0.0630
Pituitary-gm.	0.0247	0.0246	0.0172	0.0265
Brain-gm.	9.2	7.5	7.9	

PROTOCOLS

Group	Group I	Group II	Group III
Animal No.	V-71	V-72	V-73
Progression period-days	44	44	41
Normal food intake-gm.	4365	4670	3211
Cholesterol intake-gm.	27	23	-
Cortisone-mg.	-	230	189
% wt.change of initial wt.	+18.5	-16.5	-39.6
Mode of death	I.V. Nembutal	Spontaneous	Spontaneous
Total body weight-gm.	2717	2345	1425
Carcass wt.-gm.	2121	1492	1085
ORGAN WEIGHTS			
Heart-gm.	5.7	9.5	4.4
Lungs-gm.	9.3	17.3	12.3
Spleen-gm.	2.4	1.1	1.1
Liver-gm.	78.8	93.3	51.1
Kidneys-gm.	14.4	16.7	18.7
Adrenals-gm.	0.3460	0.1220	0.0893
Pituitary-gm.	0;0340	0.0230	0.0301
Brain-gm.	8.4	8.1	9.2

PROTOCOLS

Group	Group I	Group II	Group III
Animal No.	V-74	V-75	V-76
Progression period-days	61	24	29
Normal food intake-gm.	5683	2750	6913
Cholesterol intake-gm.	34	14	-
Cortisone-mg.	-	145	294
% wt.change of initial wt.	+23.4	-8.0	-4.1
Mode of death	Blow on head	Spon-taneous	I.V.Ether
Total body weight-gm.	2882	2245	2497
Carcass wt.-gm.	2299	1360	1749
ORGAN WEIGHTS			
Heart-gm.	6.4		5.6
Lungs-gm.	16.2	16.2	11.2
Spleen-gm.	1.3	0.7	0.8
Liver-gm.	108.2	131.0	136.9
Kidneys-gm.	14.3	23.8	22.6
Adrenals-gm.	0.3790	0.2360	0.1o62
Pituitary-gm.	0.0352	0.0275	0.0239
Brain-gm.	8.7		6.9

P R O T O C O L S

Group &Animal No.	Group I V-77	Group II V-79	Group III V-80
Progression period-days	60	60	60
Normal food intake-gm.	5648	8273	7496
Cholesterol intake-gm.	35	37	-
Cortisone-mg.	-	246	246
% wt.change of initial wt.	+13.7	+9.7	+7.3
Mode of death	I.V. Nembutal	I.V. Nembutal	I.V.Ether
Total body weight-gm.	2955	2540	2576
Carcass wt.-gm.	2274	1852	1700
ORGAN WEIGHTS			
Heart-gm.	5.5	7.8	7.4
Lungs-gm.	13.3	10.02	20.0
Spleen-gm.	1.6	0.6	0.7
Liver-gm.	117.9	194.5	205.5
Kidneys-gm.	15.6	25.1	22.7
Adrenals-gm.	0.3888	0.1648	0.1225
Pituitary-gm.	0.0308	0.0268	0.0
Brain-gm.	7.9	7.4	7.6

PROTOCOLS

Group Animal No.	Group I V-81	Group II V-82	Group III V-83
Progression period-days	61	41	39
Normal food intake-gm.	6008	4862	5150
Cholesterol intake-gm.	36	33	-
Cortisone-mg.	-	245	293
% wt.change of initial wt.	+20.2	-15.0	-11.3
Mode of death	Blow on head	Spon-taneous	Spon-taneous
Total body weight-gm.	3349	2740	2835
Carcass wt.-gm.	2639	1966	2010
ORGAN WEIGHTS			
Heart-gm.	6.9	9.3	9.1
Lungs-gm.	21.3	18.5	18.4
Spleen-gm.	2.4	1.3	1.0
Liver-gm.	145.9	111.5	106.3
Kidneys-gm.	14.3	24.5	19.1
Adrenals-gm.	0.5766	0.1533	0.2285
Pituitary-gm.	0.0361	0.0402	0.0343
Brain-gm.	9.1	9.1	8.5

P R O T O C O L S

Group	Group I	Group II	Group III
Animal No.	V-84	V-85	V-87
Progression period-days	55	55	55
Normal food intake-gm.	5402	6600	7439
Cholesterol intake-gm.	32	32	-
Cortisone-mg.	-	249	291
% wt.change of initial wt.	+8.7	-5.7	+2.1
Mode of death	I.V. Nembutal	Spontaneous	I.V. Nembutal
Total body weight-gm.	3191	2400	2962
Carcass wt.-gm.	2518	1754	2080
ORGAN WEIGHTS			
Heart-gm.	7.8	7.2	6.9
Lungs-gm.	13.3	19.5	9.1
Spleen-gm.	2.3	0.6	-
Liver-gm.	89.1	110.5	197.1
Kidneys-gm.	16.3	28.2	22.9
Adrenals-gm.	0.1307	0.1298	0.1641
Pituitary-gm.	0.0289	0.0232	0.0325
Brain-gm.	9.8	7.7	7.5

PROTOCOLS

Group	Group I	Group II	Group III
Animal No.	V-88	V-89	V-90
Progression period-days	57	57	13
Normal food intake-gm.	5656	5867	1300
Cholesterol intake-gm.	34	27	-
Cortisone-mg.	-	219	123
% wt.change of initial wt.	+20.3	-19.0	-9.3
Mode of death	I.V. Nembutal	Spontaneous	Spontaneous
Total body weight-gm.	3440	1985	2570
Carcass wt.-gm.	2762	1341	1730
ORGAN WEIGHTS			
Heart-gm.	6.1	4.3	7.2
Lungs-gm.	10.8	7.9	8.9
Spleen-gm.	1.4	0.8	0.8
Liver-gm.	108.7	72.0	100.6
Kidneys-gm.	16.5	16.2	22.2
Adrenals-gm.	0.2874	0.0853	0.1270
Pituitary-gm.	0.0422	0.0307	-
Brain-gm.	7.8	7.4	-

P R O T O C O L S

Group Animal No.	Group I V-94	Group II V-95
Progression period-days	61	24
Normal food intake-gm.	5322	2613
Cholesterol intake-gm.	33	13
Cortisone-mg.	-	188
% wt.change of initial wt.	+20.1	-8.5
Mode of death	Blow on head	Spon-taneous
Total body weight-gm.	2949	2525
Carcass wt.-gm.	2254	1715
ORGAN WEIGHTS		
Heart-gm.	6.9	-
Lungs-gm.	10.1	18.4
Spleen-gm.	1.6	0.6
Liver-gm.	117.1	148.0
Kidneys-gm.	14.4	20.0
Adrenals-gm.	0.6451	0.2150
Pituitary-gm.	0.0568	0.0230
Brain-gm.	9.1	8.8

P R O T O C O L S

Group	Group I	Group II	Group III	Group IV
Animal No.	V-97	V-98	V-99	V-100
Progression period-days	45	45	45	45
Normal food intake-gm.	4488	6170	5650	4600
Cholesterol intake-gm.	28	27	--	--
Cortisone-mg.	-	189	222	--
% wt.change of initial wt.	+29.1	+11.2	+2.7	+16.9
Mode of death	I.V. Nembutal	I.V. Nembutal	Spontaneous	I.V. Nembutal
Total body weight-gm.	2798	2720	2690	3010
Carcass wt.-gm.	2145	1975	1755	2410
ORGAN WEIGHTS				
Heart-gm.	8.9	5.9	9.8	8.4
Lungs-gm.	9.6	8.3	19.5	10.5
Spleen-gm.	1.9	0.8	0.7	0.6
Liver-gm.	111.5	189.0	110.2	99.1
Kidneys-gm.	12.5	20.6	20.0	14.4
Adrenals-gm.	0.2655	0.1420	0.1455	0.1582
Pituitary-gm.	0.0367	0.0162	0.0210	0.0243
Brain-gm.	9.2	8.7	8.2	8.5

PROTOCOLS

Group	Group I	Group II	Group III	Group IV
Animal No.	W-1	W-2	W-3	W-4
Progression period-days	49	49	49	49
Normal food intake-gm.	4835	6650	6408	4900
Cholesterol intake-gm.	30	30	--	--
Cortisone-mg.	-	259	197	--
% wt.change of initial wt.	+36.5	-10.0	+14.8	+31.1
Mode of death	I.V. Nembutal	I.V. Nembutal	Spontaneous	I.V. Nembutal
Total body weight-gm.	2572	2475	2485	2890
Carcass wt.-gm.	1935	1983	1855	2236
ORGAN WEIGHTS				
Heart-gm.	5.2	9.4	8.5	5.5
Lungs-gm.	8.2	9.9	12.9	9.4
Spleen-gm.	0.9	1.1	0.3	1.0
Liver-gm.	82.4	163.3	116.8	93.7
Adrenals-gm.	0.3303	0.1100	0.0800	0.1624
Kidneys-gm.	10.2	18.3	21.3	15.1
Pituitary-gm.	0.0287	0.0277	0.0150	0.0300
Brain-gm.	8.8	7.5	7.5	8.2

PROTOCOLS

Group	Group I	Group II	Group III	Group IV
Animal No.	W-5	W-6	W-7	W-8
Progression period-days	46	46	46	46
Normal food intake-gm.	4511	6200	5787	4600
Cholesterol intake-gm.	27	28	-	-
Cortisone-mg.	-	186	186	-
% wt.change of initial wt.	+27.1	+11.6	+7.8	+35.4
Mode of death	I.V. Nembutal	Spontaneous	I.V. Nembutal	I.V. Nembutal
Total body weight-gm.	2595	2460	2145	3735
Carcass wt.-gm.	1788	1649	1155	2058
ORGAN WEIGHTS				
Heart-gm.	4.8	5.6	7.7	5.0
Lungs-gm.	8.7	11.2	13.6	9.3
Spleen-gm.	1.8	0.3	1.0	1.3
Liver-gm.	102.9	142.7	141.5	106.1
Kidneys-gm.	16.6	23.7	22.8	15.4
Adrenals-gm.	0.4180	0.1602	0.1001	0.2095
Pituitary-gm.	0.0428	0.0294	0.0212	0.0292
Brain-gm.	8.9	8.4	7.5	8.6

PROTOCOLS

Group	Group I	Group II	Group III	Group IV
Animal No.	W-9	W-10	W-11	W-12
Progression period-days	54	54	54	54
Normal food intake-gm.	5394	6436	5705	5400
Cholsterol intake-gm.	33	30	-	-
Cortisone-mg.	-	210	202	-
% wt.change of initial wt.	+31.4	+2.6	-4.4	+33.0
Mode of death	I.V. Nembutal	I.V. Nembutal	Spontaneous	I.V. Nembutal
Total body weight-gm.	3035	2482	1571	2910
Carcass wt.-gm.	2337	1493	1081	2290
ORGAN WEIGHTS				
Heart-gm.	7.5	8.2	6.3	5.1
Lungs-gm.	9.2	13.1	12.0	8.3
Spleen-gm.	1.5	0.9	0.5	0.8
Liver-gm.	120.7	119.4	92.2	104.8
Kidneys-gm.	14.3	19.6	18.1	13.5
Adrenals-gm.	0.3697	0.1746	0.1215	0.1380
Pituitary-gm.	0.0436	0.0298	0.0282	0.0217
Brain-gm.	8.8	8.3	8.6	8.9

PROTOCOLS

Group	Group I	Group II	Group III
Animal No.	W-13	W-14	W-15
Progression period-days	57	29	57
Normal food intake-gm.	5568	3505	7395
Cholesterol intake-gm.	34	17	-
Cortisone-mg.	-	157	233
% wt.change of initial wt.	+18.2	+0.34	+o.12
Mode of death	I.V. Nembutal	Spontaneous	Spontaneous
Total body weight-gm.	2885	2032	2330
Carcass wt.-gm.	2309	1350	1520
ORGAN WEIGHTS			
Heart-gm.	7.1	5.9	6.0
Lungs-gm.	10.0	10.6	15.2
Spleen-gm.	0.9	1.1	0.9
Liver-gm.	79.0	100.1	111.0
Kidneys-gm.	12.0	19.3	16.7
Adrenals-gm.	0.3409	0.1622	0.1567
Pituitary-gm.	0.0350	0.0335	0.0273
Brain-gm.	8.0	7.7	7.8

P R O T O C O L S

Group	Group I	Group II	Group III
Animal No.	W-17	W-18	W-19
Progression period-days	61	21	26
Normal food intake-gm.	5853	1917	3200
Cholesterol intake-gm.	35	8	-
Cortisone-mg.	-	131	154
% wt.change of initial wt.	+24.3	-21.9	-5.9
Mode of death	Blow on head	Spon-taneous	Spon-taneous
Total body weight-gm.	2952	1591	2253
Carcass wt.-gm.	2322	1227	1636
ORGAN WEIGHTS			
Heart-gm.	5.9	-	9.1
Lungs-gm.	9.2	6.4	23.6
Spleen-gm.	1.7	1.5	0.5
Liver-gm.	85.3	108.2	177.8
Kidneys-gm.	12.9	19.8	18.3
Adrenals-gm.	0.4647	---	---
Pituitary-gm.	0.0237	---	---
Brain-gm.	8.2	---	9.9

PROTOCOLS

Group	Group I	Group II	Group III
Animal No.	W-20	W-21	W-22
Progression period-days	56	24	55
Normal food intake-gm.	5486	2785	7462
Cholesterol intake-gm.	34	15	-
Cortisone-mg.	-	145	213
% wt.change of initial wt.	+23.2	-6.3	+20.7
Mode of death	I.V. Nembutal	Spontaneous	Spontaneous
Total body weight-gm.	2747	2142	2452
Carcass wt.-gm.	2072	1500	1785
ORGAN WEIGHTS			
Heart-gm.	5.4	-	7.1
Lungs-gm.	10.0	12.5	16.8
Spleen-gm.	1.5	1.0	1.0
Liver-gm.	99.6	178.0	175.0
Kidneys-gm.	14.6	21.2	24.1
Adrenals-gm.	0.2700	0.1750	0.0879
Pituitary-gm.	0.0317	0.0120	0.0257
Brain-gm.	8.0	8.0	7.6

P R O T O C O L S

Group	Group I	Group II	Group III
Animal No.	W-23	W-24	W-25
Progression period-days	33	29	26
Normal food intake-gm.	3113	3585	3140
Cholesterol intake-gm.	19	18	-
Cortisone-mg.	-	157	151
% wt.change of initial wt.	+20.5	-4.1	-7.0
Mode of death	Spon-taneous	Spon-taneous	Spon-taneous
Total body weight-gm.	2390	2200	2249
Carcass wt.-gm.	1620	1471	1480
ORGAN WEIGHTS			
Heart-gm.	6.7	6.9	12.1
Lungs-gm.	14.5	7.7	14.7
Spleen-gm.	1.1	1.3	1.1
Liver-gm.	96.6	152.9	130.6
Kidneys-gm.	13.5	19.4	18.1
Adrenals-gm.	-	0.1175	0.1070
Pituitary-gm.	-	0.0270	0.0250
Brain-gm.	7.7	7.5	8.2

PROTOCOLS

Group	Group I	Group II	Group III	Group IV
Animal No.	W-29	W-30	W-31	W-32
Progression period-days	61	60	60	61
Normal food intake-gm.	5600	6255	7368	5871
Cholesterol intake-gm.	32	27	-	-
Cortisone-mg.	-	278	326	-
% wt.change of initial wt.	-5.3	-15.7	+o.3	+13.2
Mode of death	Blow on head	I.V. Nembutal	I.V. Nembutal	Blow on head
Total body weight-gm.	3455	2435	2853	3040
Carcass wt.-gm.	2630	1818	1995	2509
ORGAN WEIGHTS				
Heart-gm.	7.6	6.4	7.5	7.1
Lungs-gm.	11.2	9.3	20.1	9.4
Spleen-gm.	1.5	0.9	1.3	1.0
Liver-gm.	112.0	202.3	173.1	85.2
Kidneys-gm.	18.7	19.9	26.6	15.4
Adrenals-gm.	0.7496	0.1399	0.2056	0.2074
Pituitary-gm.	0.0430	0.0258	0.0266	0.0312
Brain-gm.	8.9	7.5	8.8	9.9

A P P E N D I X

TABLES

TABLES

GRAPHS

DIAGRAMS

PROTOCLS

I N D E X .