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THE PATHOGENESIS OF LESIONS PRODUCED IN RABBITS BY ADMINISTRATION OF FOREIGN SERUM PROTEINS

by

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Ph.D. Thesis. Pathology.

April 1951.

ACKNOWLEDGMENTS.

The experimental work reported in this thesis was done during the tenure of a Post-doctoral Fellowship of the Life Insurance Medical Research Fund during the years 1949-1951. The work was supported by grants to Dr. R. H. More by the National Research Council of Canada and the Defence Research Board of Canada. The ACTH used in these experiments was supplied free of charge by the Medical Division of Armour and Co., Chicago, Ill., U. S. A., and the Cortisone by grants and allocations from the Committee on Cortisone and ACTH of the National Research Council of Canada.

The direct supervision of the experimental work was by Professor Robert H. More and Dean G. Lyman Duff to whose perspicacity any virtues of this work must be attributed. They were particularly helpful in understanding the many difficulties encountered in the experiments and were responsible for many lessons learned during the past years. The supervision of the experiments cannot be held accountable for the deficiencies in the performance of the experiments for these belong exclusively to the writer. I owe much to Dr. Douglas Waugh who preceded me in this work and who initiated me into the practical aspects of experimental work.

I am more than grateful for the conscientiousness with which Miss Jacqueline Chausse and Mrs. Angelica Sungaila carried out the many technical procedures that fell to their lot during the experiments and for the many hours spent doing extra work during the crucial periods of

the experiments. Special mention should be made of the excellent draughtsmanship displayed by Miss Chausse and of the hard work involved in preparing many of the charts included in the text. Mr. Harold Coletta deserves my thanks for the excellent photographic work that may be seen in the photomicrographs and in the reproduction of the charts that do so much to clarify and enhance the appearance of the text. I also wish to acknowledge the help of Mr. C. Dementlin who worked with me during the summer of 1949, and of Miss Eva Bernstein's help during the summer of 1950. I am also most appreciative of the help of my brother Eli Kobernick and Dr. M. D. Charendoff who mounted all the photographs in the text. My sister Mrs. Hilda Greenspoon I thank for helping me with the preparation of the index to the authors and the table of contents and Mrs. Margaret Paasuke for typing and checking the bibliography. It would be an error not to acknowledge the patient forbearance of my wife during the past three years of neglect to my family and her help in the final preparation of this thesis.

I wish to make special mention to Miss Lilian Brown through whose superb skill, patience and perseverance the major portion of this thesis was typed.

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INTRODUCTION

Ever since it was demonstrated that pathological alterations of the hearts [1-13], arteries[2-18], and kidneys[14,19-22] can result from administration of foreign serum proteins in animals, interest in these lesions has resulted from their apparent resemblance to certain diseases in man whose cause is still unknown. The lesions of the heart have been likened by most investigators to the lesions occurring in the hearts of human cases of rheumatic fever[2,3,4,6,7,8,9,10,11,12,21]; the lesions of the arteries to the lesions occurring in human periarteritis nodosa[2-18,21] and those of the kidney to human glomerulonephritis[14,19-22]. Whereas the opinion regarding the comparison of the lesions of the arteries and kidneys to the human diseases has been almost unanimous, the idea has been expressed that the lesions of the heart cannot be justifiably compared to the lesions of human rheumatic fever[13,23-25]. Since our interest in the experimental lesions stems from interest in their relationship to the lesions of the human diseases, it is pertinent to begin with a consideration of the validity of the comparison of the experimentally-induced with the spontaneously occurring diseases.

COMPARISON OF THE MORPHOLOGICAL ASPECTS OF THE LESIONS OF EXPERIMENTAL ANIMALS AND MAN.

Heart Lesions:

The lesions of the heart occurring in human rheumatic fever were first brought into prominence by Aschoff in 1904[26] however, he credited Romberg with having first described them a year previously. Subsequent to this there was some doubt raised as to the specificity of the cardiac lesions described for rheumatic fever[27], but numerous well-documented studies eventually established them as lesions occurring only in rheumatic fever (28-32,38).

The earliest students of the human material emphasized the cellular granuloma of the myocardium located in the interstitial septa of the myocardium as minute submiliary nodules. These were composed of a central zone of amorphous material surrounded by and mixed with large mononuclear cells having abundant cytoplasm with ragged edges (Fig.1), and one to ten centrally placed vesicular nuclei. The nuclei had an oval or round shape and the chromatin was condensed in the centre giving the appearance of a longitudinal bar in the sagitally-cut and of a central dot in the transversely-cut nuclei. The emphasis must be layed on the specificity of the cytoplasm of the cells, for it is the abundant, basophilic cytoplasm with ragged edges that is specific to the Aschoff cells

and to rheumatic fever[31]. The structure of the nuclei is not specific, since Anitschkow cells found normally in the hearts of man and animals[33] and under a variety of pathological circumstances have nuclei that are identical, but which differ from the Aschoff cells in that the cytoplasm of the former is scarcely visible as a faintly acidophilic cap situated

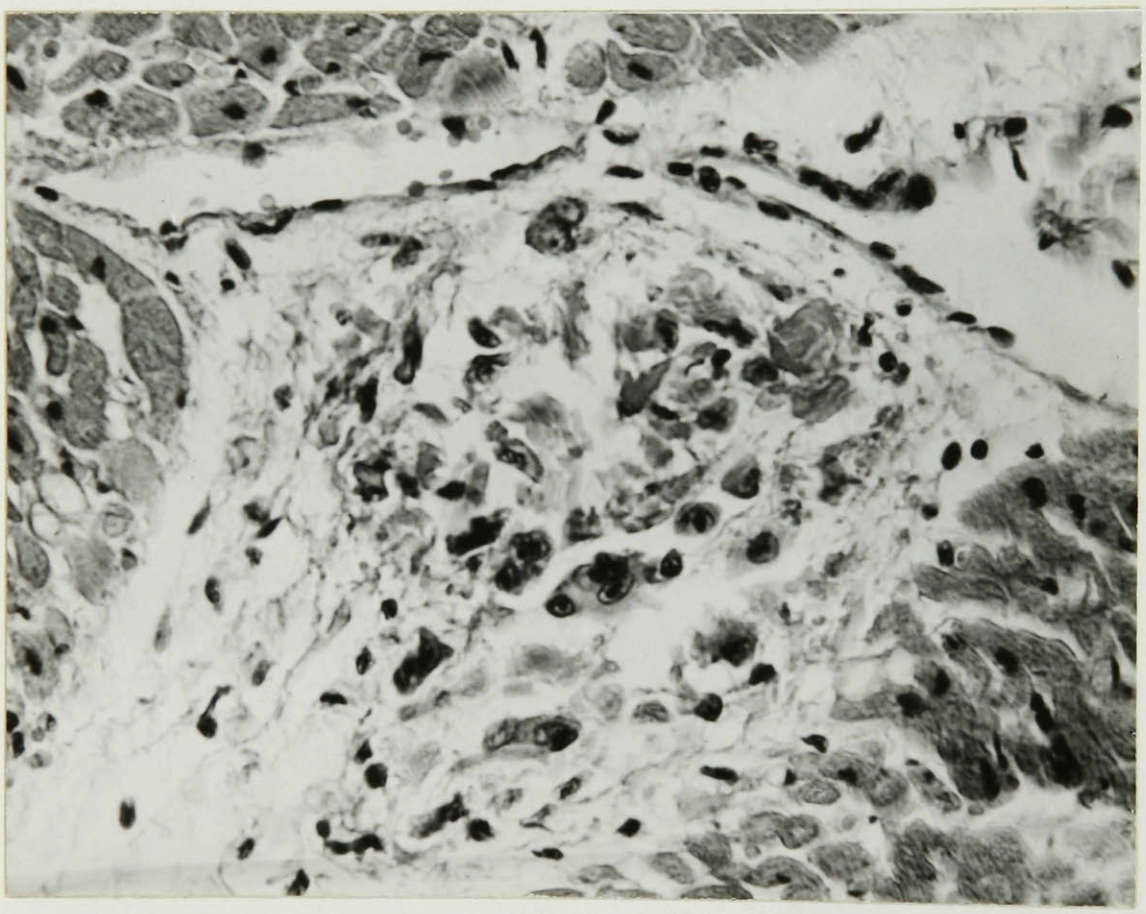


Fig.1. Aschoff body of the myocardium of a case of human rheumatic fever showing the fragmented collagen in the centre, and numerous Aschoff cells.

at either end of the nuclei. So inconspicuous is the cytoplasm of the Anitschkow cells that the nuclei appear to be bare of cytoplasm except in careful observations of good preparations[34]. A few lymphocytes and polymorphonuclear leukocytes are occasionally seen mixed with the Aschoff cels.

It was later shown that the material at the centre of the nodules stained positively for fibrin[35]. However, it was not until the studies of Klinge that it was emphasized that the origin of this "fibrinoid" material was from, or in, association with the collagen fibres[35]. He observed the fibrinoid material developing from the clumping of an increased amount of interstitial ground substance. He did not believe that the collagen became fragmented, but this was disputed by Gross and his co-workers[31]. The

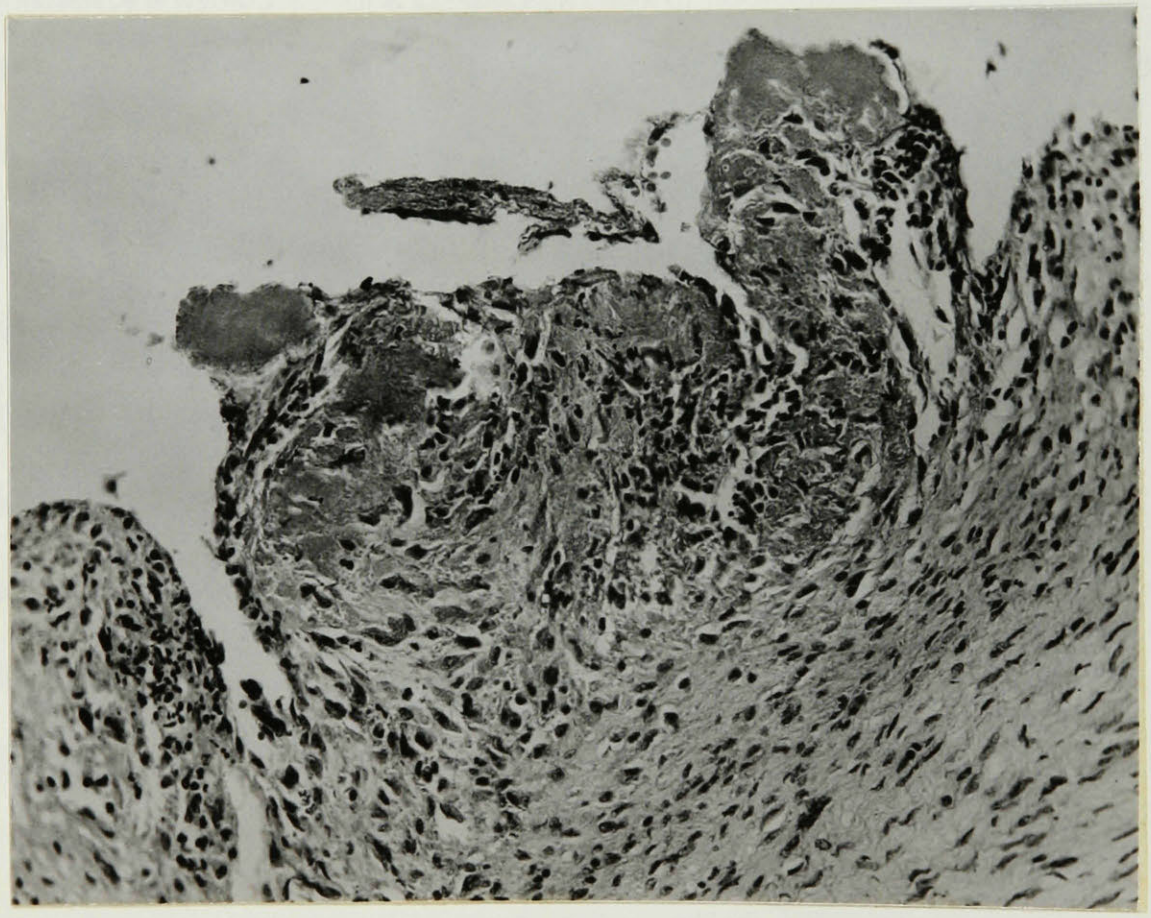


Fig. 2. Verrucosa vegetation of a valve in human rheumatic fever showing fibrinoid necrosis of collagen breaking through the endothelium. Aschoff cells are visible at the base of the vegetation.

latter, in a series of studies[32], pointed out that the Aschoff nodule did not have a single morphological structure but a cycle of development in which different stages could be recognized. These stages correspond

approximately with the clinical history of the patients.

In addition to the Aschoff bodules in the myocardium, lesions with similar basic components, fibrinoid necrosis of collagen and Aschoff cells were usually found in the valves (Fig.2), where the breaking of the necrotic collagen through the endothelium created the nidus for the deposition of the verrucose vegetations of rheumatic endocarditis[35]. Similar lesions were seen in the endocardium of the left atrium localized to an area from two to five centimeters in diameter just above the auricular cusp of the mitral valve[36,37]. This zone is popularly known as "MacCallum's patch". Rheumatic subcutaneous nodules[61], arteritis[56-59,62], aortitis[64], pericarditis[64] and pneumonitis[65] have also been described. However, the specificity of the pneumonitis[65,66] and the arteritis[46] have been questioned. In view of the widespread focal distribution of the lesions in the body and their relation to fibrinoid degeneration of the connective tissue, this disease has recently been grouped with lupus erythematosus, dermatomyositis, rheumatoid arthritis and periarteritis nodosa under the heading of "diffuse collagen diseases"[67-72].

Since the recognition of the specificity of the heart lesions of rheumatic fever, attempts have been made to induce the lesions in animals in an effort to uncover the etiological factors and pathogenetical mechanisms concerned in their production. Among these, streptococci of non-haemolytic[39,40,42,44] and haemolytic varieties[41,43], both alive and killed, were injected into animals, and these failed to produce lesions of the heart comparable to the lesions of human rheumatic heart disease.

However, for many years controversy raged over the lesions produced in experimental animals, and it was not until the work of Gross and his co-workers[45], who applied the criteria for the diagnosis of rheumatic fever rigidly and repeated the previous work, that the myth was finally exploded.

It was within this background that interest was raised by the demonstration that lesions of the heart could be produced by rendering rabbits hypersensitive to horse serum by means of large intravenous doses[1-13]. Many authors again drew attention to the similarity between the lesions of the hearts of experimental animals so-produced to the lesions of human rheumatic fever. In expressing their opinions regarding the lesions, most of the investigators were cautious enough to state that the lesions had a "similar reaction pattern" to Aschoff nodules or were "Aschoff-like nodules"[2,4,7,8,10], although they were also referred to as Aschoff nodules[9].

It thus became important first to evaluate the lesions produced in the hearts of animals by foreign serum proteins. It should not be inferred from the remarks in the foregoing paragraph that opinion was unanimous in accepting the similarity between the experimental serum lesions of the heart and the lesions of human rheumatic fever. On the contrary, no less an authority than Aschoff[24] was not convinced of the similarity between the lesions. This opinion he expressed after seeing some of the work of Klinge[2]. However, many who followed Klinge with similar investigations were of the opinion that they were dealing with

lesions that were related morphologically to the lesions of human rheumatic fever. In this laboratory study of the hearts of animals sensitized to massive doses of bovine gamma globulin did not uphold the similarity of the experimental valvular lesions to those of human rheumatic fever[13]. Nevertheless, it was decided to attempt a quantitative or qualitative alteration of the lesions of the heart by modifying the mode of administration of foreign serum proteins[46,47]. Detailed analysis of the lesions of the heart in these studies revealed several types of lesions of the myocardium.

The first lesion was a spontaneous lesion of the myocardium consisting of focal collections of lymphocytes and larger mononuclear cells, often clustered about an indistinct giant cell(Fig.3). These

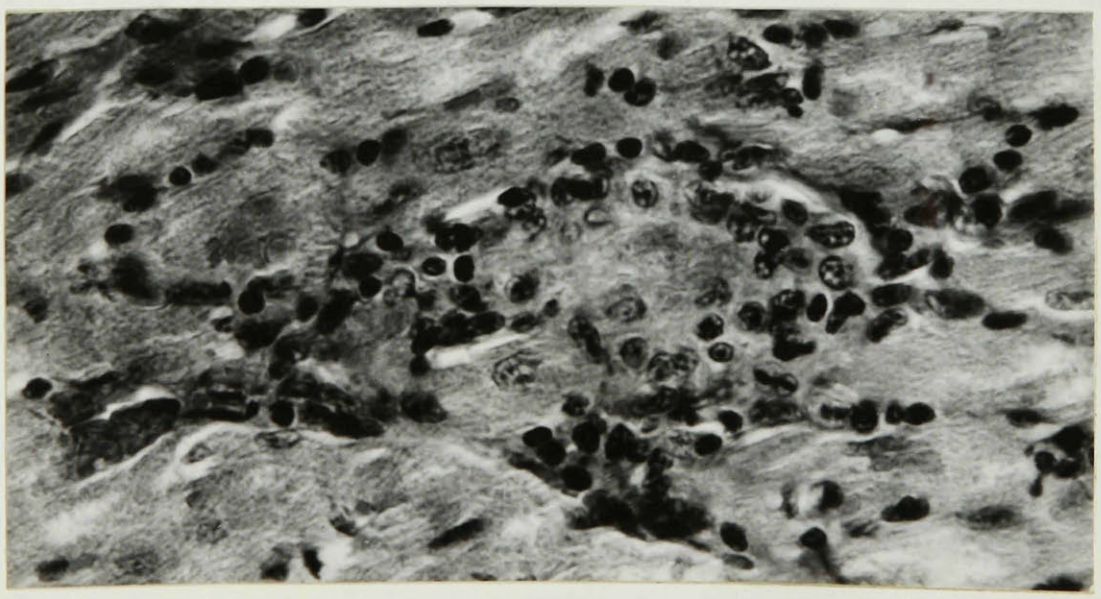


Fig. 3. Spontaneous focal lesion of the myocardium in normal, untreated rabbit. Note the indistinct giant cell in the centre.

lesions occurred spontaneously, even in untreated rabbits, although they have been attributed by some[8,9] to the treatment employed and compared

to human Aschoff bodies. The recognition of these as spontaneous lesions is not new, having been recognized by Miller in 1924[48]. Numerous investigators have since recognized that these lesions may be confused with experimentally-induced ones[5,10,13,49], but more have not distinguished them.

The second type of myocardial lesion that was distinguished in experimental animals was a diffuse myocarditis occurring most often in the right ventricular muscle. This consisted of a rather generalized destruction of muscle fibres with infiltration of large mononuclear cells and lymphocytes. This lesion is pictured in its acute phase in Fig.4. Later on, this type of lesion may give rise to a diffuse fibrosis of the myocardium with focal calcification, and giant cell formation. Focal areas of this type of lesion have been illustrated in published material as resembling Aschoff bodies[9], but on the other hand have been recognized as an entity by most other observers[5,10]. Far from being similar to the lesions of the myocardium of human rheumatic fever (see Fig.1), these lesions of the heart are very similar to those seen in the hearts of cases dying after sensitization to sulphonamides. The similarity of the lesions in these two conditions may be seen by comparing Figs.4 and 5.

The third type of lesion seen in the myocardium of animals sensitized to foreign serum proteins was a peri- and paravascular accumulation of large and small mononuclear cells, sometimes associated with fibrinoid necrosis of the collagen. However, these lesions always occurred in hearts that were the seat of necrotizing arteritis. Some of

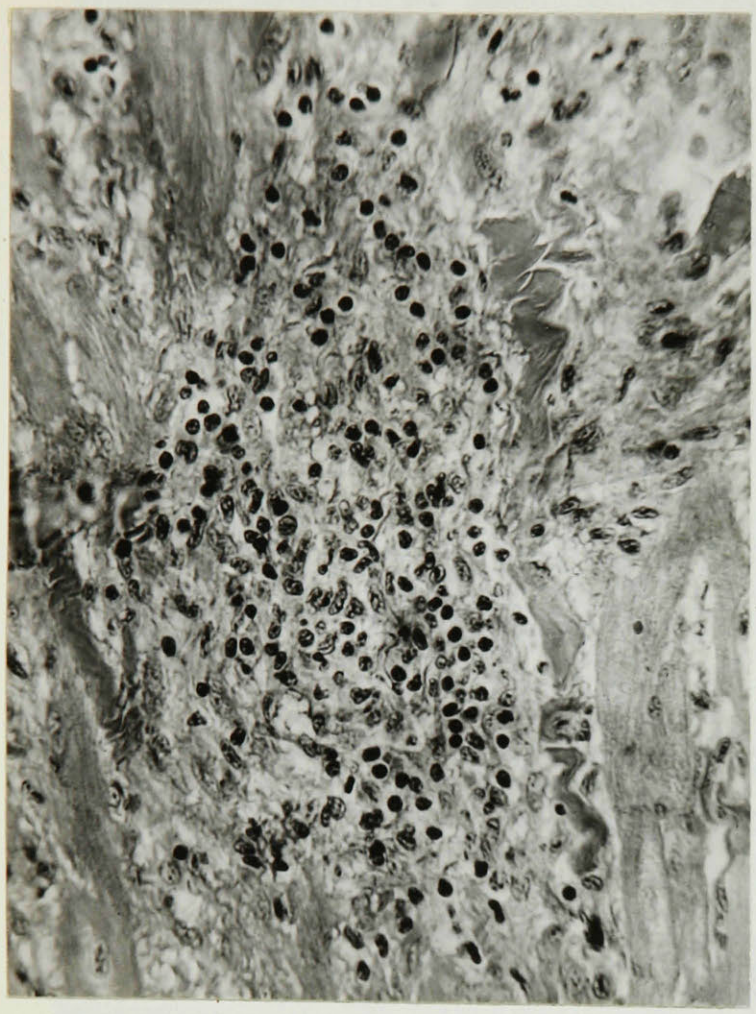


Fig.4. Diffuse myocarditis of the right ventricle of a rabbit treated with globulin and streptococci. X 125.



Fig.5. Heart from a fatal case of "sulphonamide allergy"(5).

these infiltrations were associated with definite areas of necrosis of the arterial wall (Fig.6), but others were near arteries which, in the areas observed, were free of necrosis (Fig.7). It was pointed out, however, that since the arteritis is a segmental lesion, it is highly probable that the infiltrations were associated with actual necrosis of the arterial walls lying just outside the planes of section(46,47). The similarity of this type of lesion with the lesions seen in fatal cases of human serum sickness(51) may be seen by comparing Figs.7 and 8.

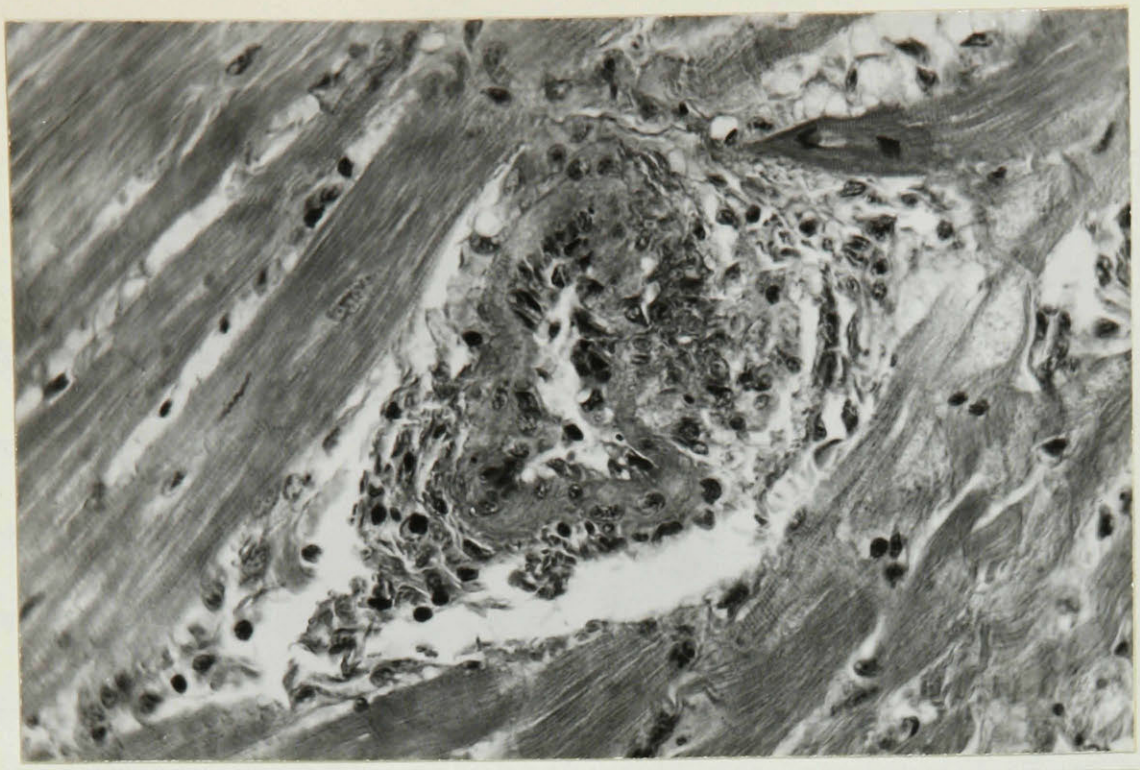


Fig.6. Artery from rabbit showing necrotizing arteritis and paravascular infiltration with fibrinoid necrosis.

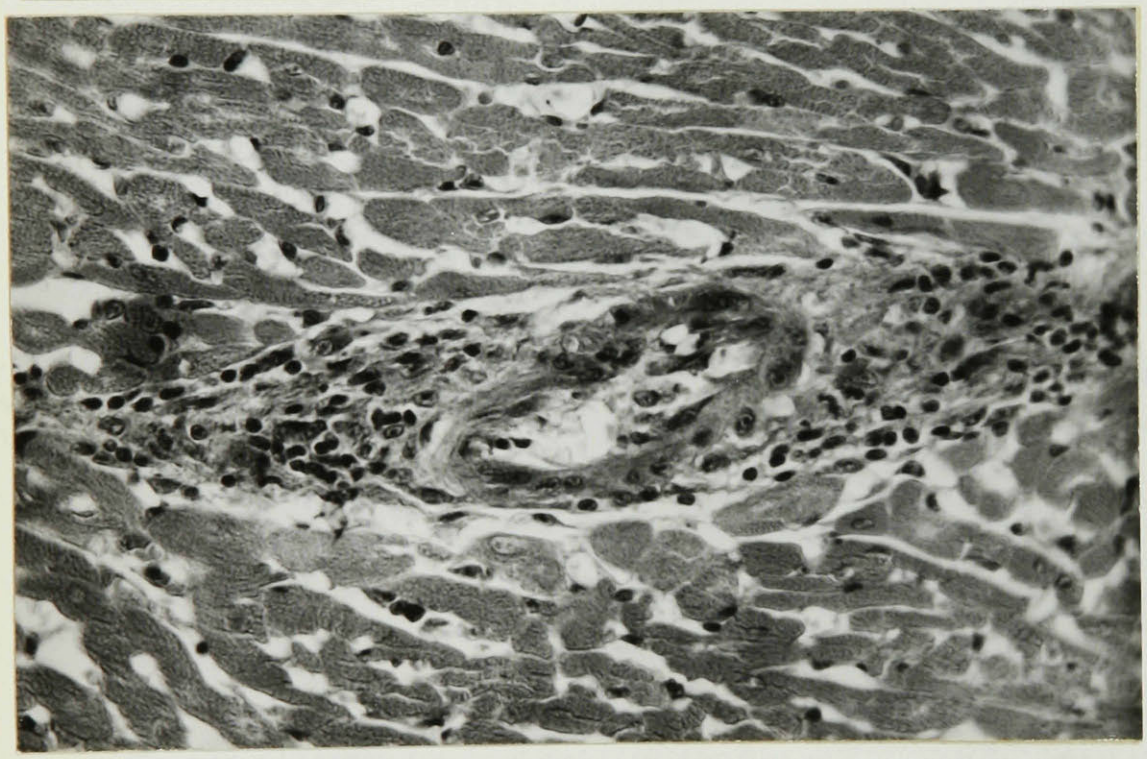


Fig.7. Paravascular infiltration about relatively normal artery of rabbit myocardium.

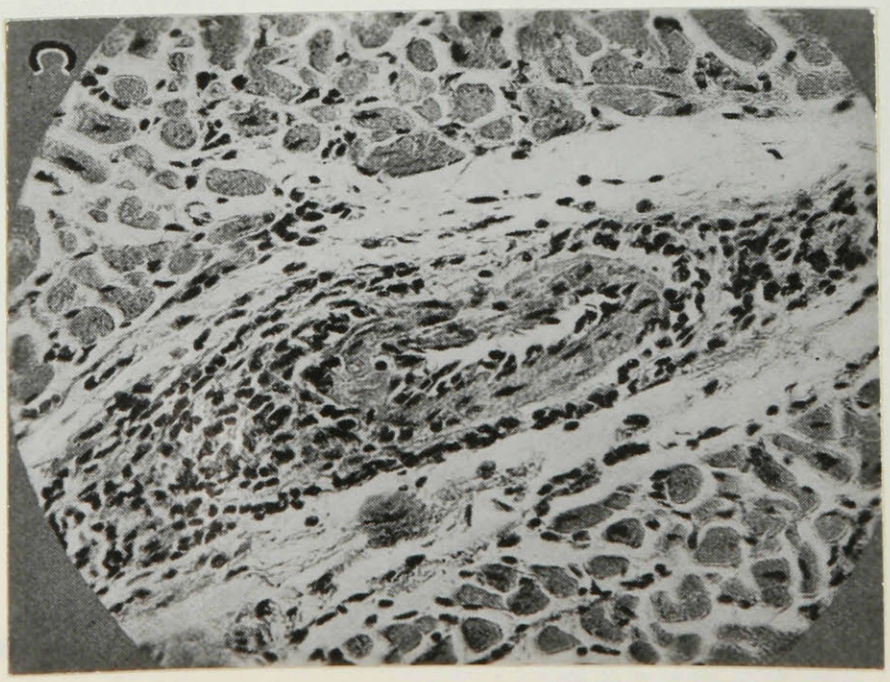


Fig.8. Lesion of human case of serum sickness from (51). Note similarity to Fig.7.

The valve lesions observed in sensitized rabbits consist mainly of a proliferation of endothelial and large mononuclear cells near the surface of the valves and not, as in rheumatic fever, of a necrotizing process of the connective tissue. Fibrinoid necrosis, so prominent in rheumatic valvulitis, is almost uniformly absent in the valves of sensitized rabbits. The similarity between the lesions of rabbits and those of human serum sickness may be seen from Figs. 9 and 10.

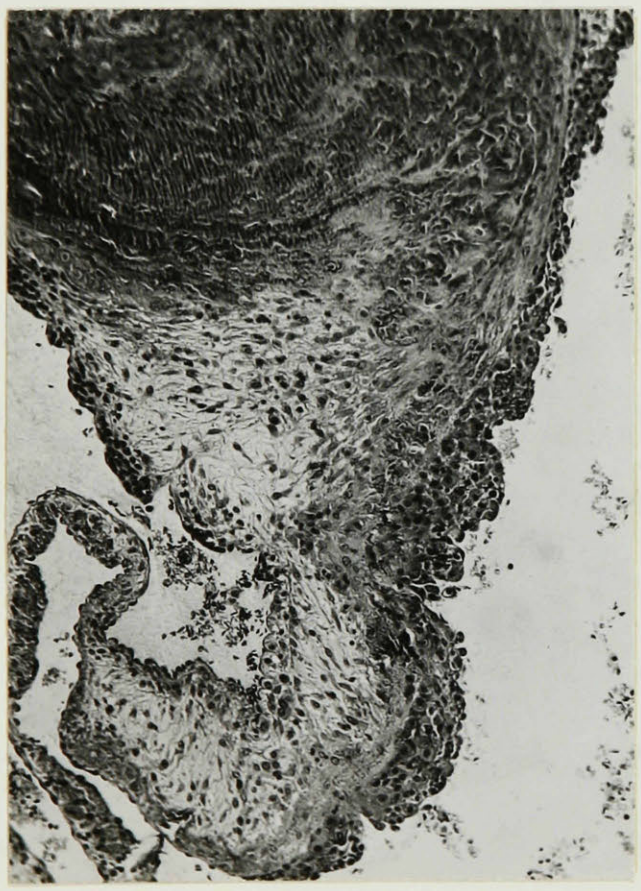


Fig.9. Valve lesion from sensitized rabbit showing surface cellular proliferation.

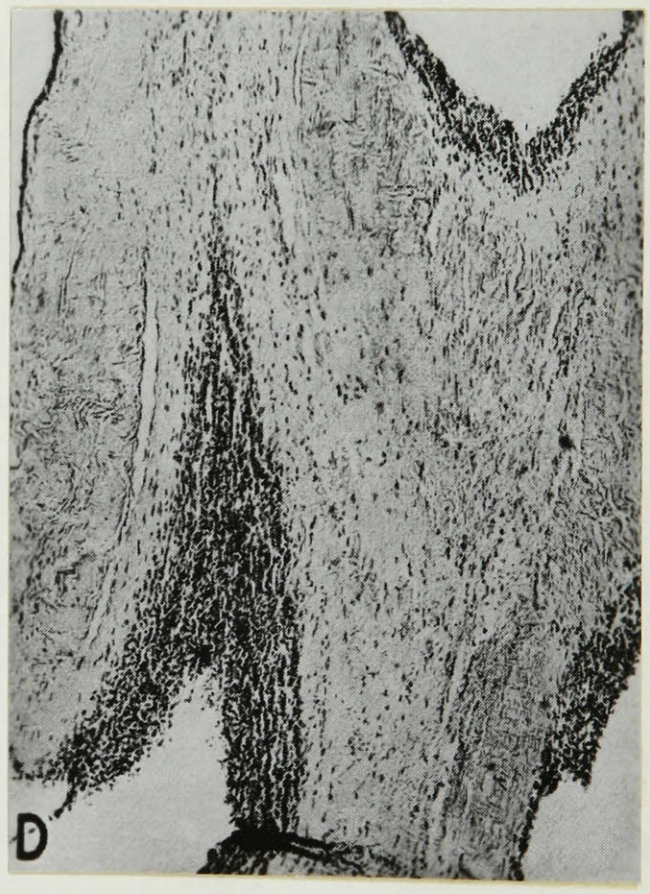


Fig.10. Valve lesion from case of human serum sickness (51) showing lesion similar to Fig.9.

Thus, it is apparent that the lesions of the myocardium and of the valves of sensitized rabbits do not resemble the lesions of human

rheumatic fever, but rather those seen in the hearts of human cases of fatal sulphonamide allergy[50] or of serum sickness[51]. It should also be noted that a further similarity exists between the disease induced in rabbits by foreign serum sensitization and that of sulphonamide allergy and serum sickness; that is, the occurrence of necrotizing arteritis[50-52].

Although the rigid interpretation of the lesions of human rheumatic fever and the insistence on a closer identity of the experimental with the human lesions has been regarded as "purist"[55], justification for a strict attitude has appeared from several sources. Firstly, from a theoretical point of view it is impossible to fairly compare two entities in which the only criteria of similarity are morphological, if the morphological pictures are different. This apparently obvious logical fallacy was perpetrated on the ground of "species differences" wherewith it was supposed that the rabbit could not react in the same manner as the human organism. This was an unproven assumption. Secondly, lesions have been produced in rabbits by repeated intradermal infections with different strains of haemolytic streptococci which possess all the characteristics of Aschoff nodules in the human[54,55]. It is to be hoped that the lesions induced in the hearts of rabbits by rendering them hypersensitive to foreign serum proteins will no longer be compared to the cardiac lesions of human rheumatic fever.

Necrotizing Arteritis:

In 1865 periarteritis nodosa was first described by Kussmaul and Maier[73]. Since that time sporadic occurrence of cases manifesting

this lesion have been reported and reviewed exhaustively by Lindberg[74], Gruber[75] and Arkin[76]. Since the early reports, many individual cases have been reported[78-88]. In studying the descriptions of the lesions in the various reports, it becomes evident immediately that a great variation in the lesions must exist from case to case. These variations could probably be best explained by considering a process with many stages. The insistence that this or that single criterion must be present before the diagnosis can be made[89,90] cannot be applied with any degree of success to unify the concept. Accordingly, the term polyarteritis nodosa, emphasizing the occurrence of the lesions in a segmental fashion in many muscular arteries of medium size, is a term to be preferred over that of periarteritis nodosa, which emphasizes the periarterial inflammation which may be minimal if many of the cases in the literature are to be accepted as examples of the disease.

The pathogenetic sequence of the development of the lesions has been well described by Duff[71,91]. Here it is pointed out that the fundamental basis of the lesion lies in the necrosis of connective tissue elements of the media and adventitia with the medial muscle cells being preserved in many instances (Fig.11). Accumulation of fibrinoid material beneath an intact intima may occur (Fig.12). Infiltration of inflammatory large and small mononuclear cells is prominent in addition to eosinophile and other polymorphonuclear leukocytes (Figs.11 & 12). Healing of the lesions results in an increase in the amount and density of the adventitial collagen; increased collagen content of the media between preserved muscle cells, and proliferation of the subendothelial connective tissue with resultant narrow-

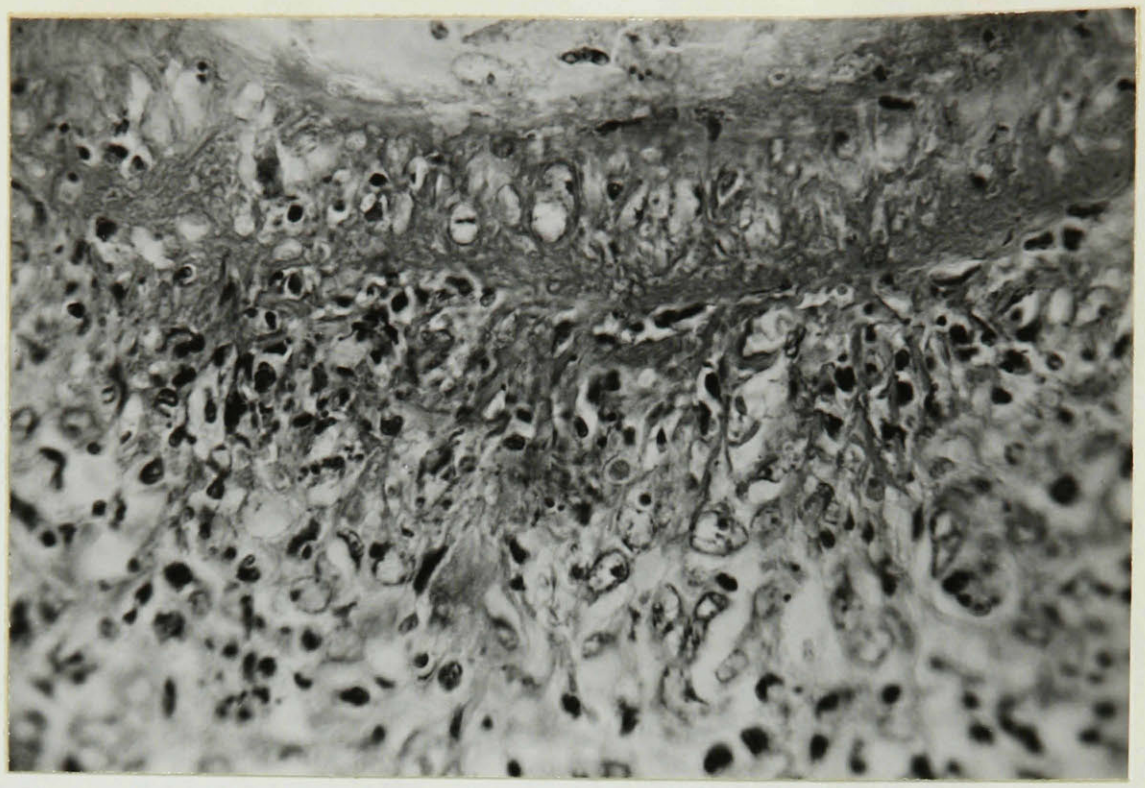


Fig.11. Portion of the arterial wall from a case of periarteritis nodosa(91) showing fibrinoid material in the adventitia and infiltrating between the preserved medial muscle cells. The intima is intact. Perivascular mononuclear infiltration is well shown.

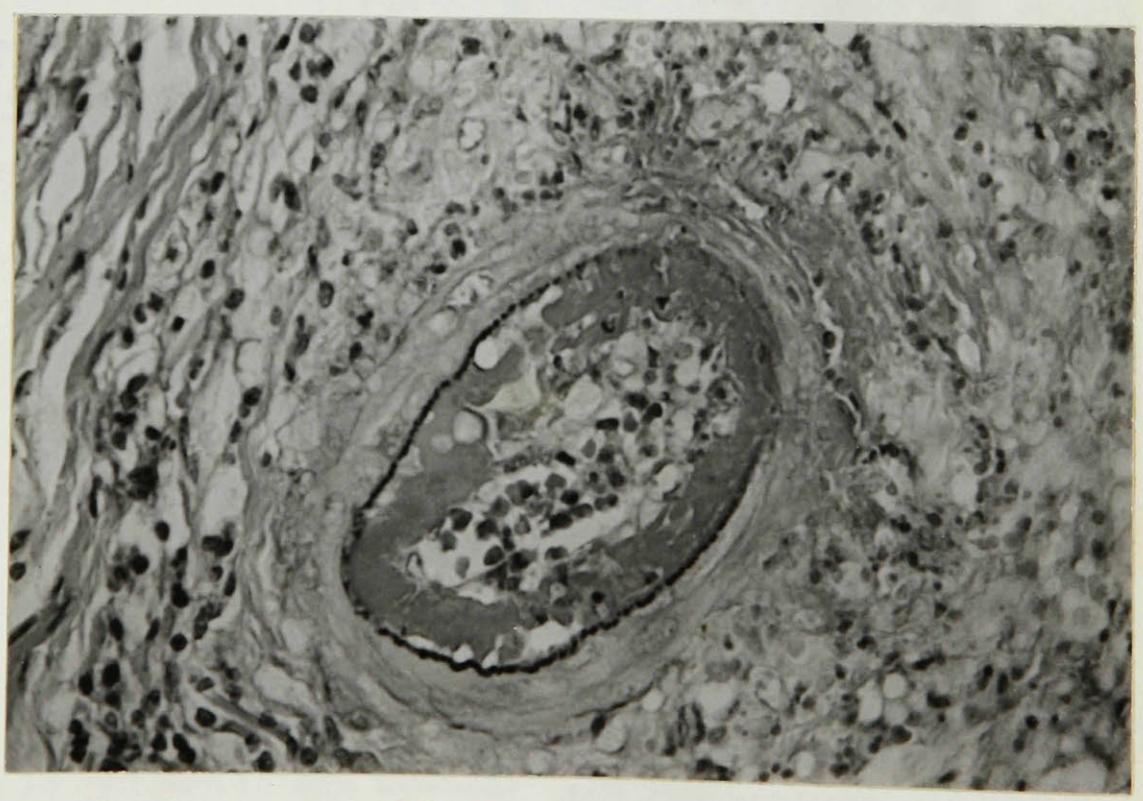


Fig.12. Small artery from case of periarteritis nodosa(91) showing fibrinoid material beneath intact intima and preservation of medial muscle cells, except for small area upper right. Perivascular round cell infiltration is also present.

ing of the lumen of the vessels (Fig.13). In some instances only, is complete destruction of the media evident in the acute stages. When this occurs, it is followed by complete collagenous replacement of the affected segment of the media.

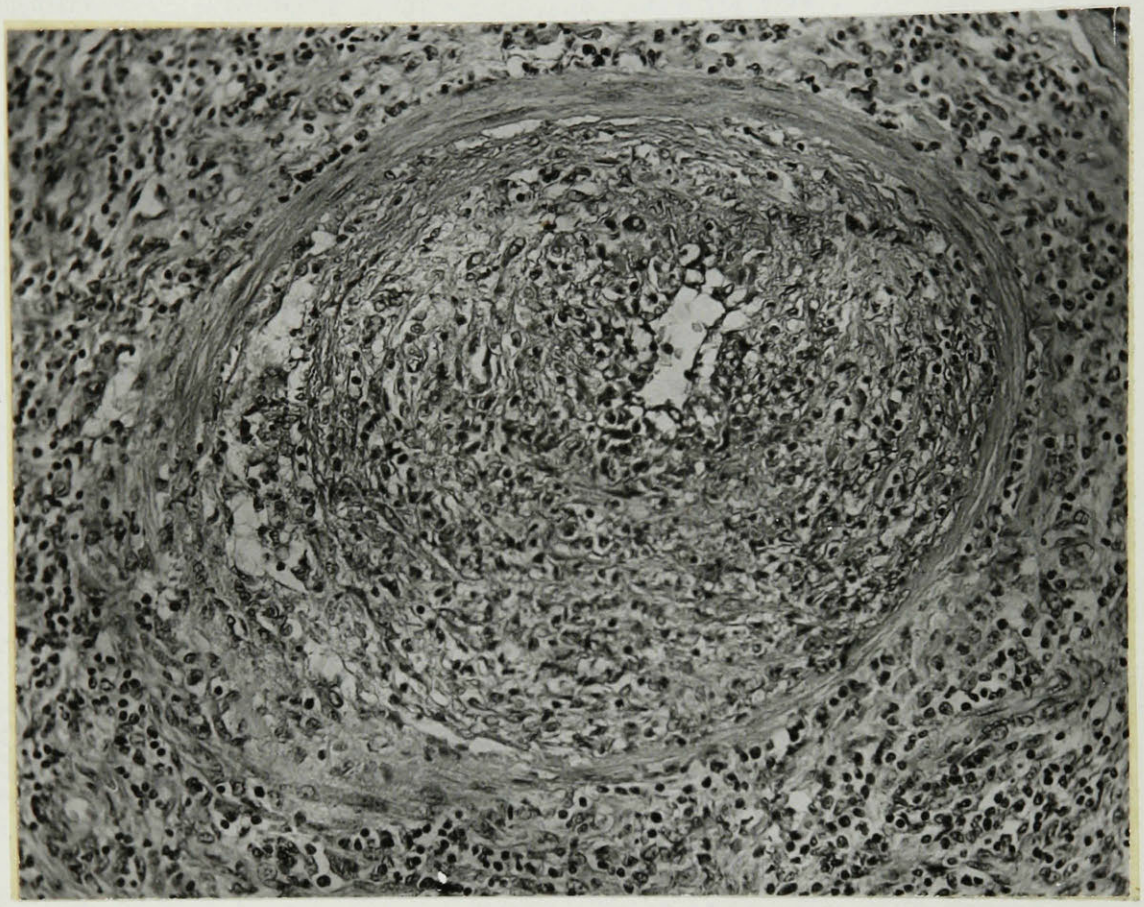


Fig.13. Periarteritis nodosa showing marked subintimal proliferation of connective tissue with narrowing of the lumen. The medial musculature is well-preserved. Perivascular mononuclear infiltration and increased collagen formation is also evident(91).

The process observed in experimental animals made sensitive by massive doses of foreign serum proteins[2-5,9-15,17-19,46,47] is identical to that described in periarteritis nodosa. The complication of aneurysm formation and rupture followed by fatal haemorrhage has even been observed in one of the present writer's rabbits[47]. The experimental lesions have already been adequately described elsewhere, so it has been considered to

suffice for the present purposes to present photomicrographs that illustrate the typical sequence of events in experimental necrotizing arterieis (see Figs.14,15,16,17)

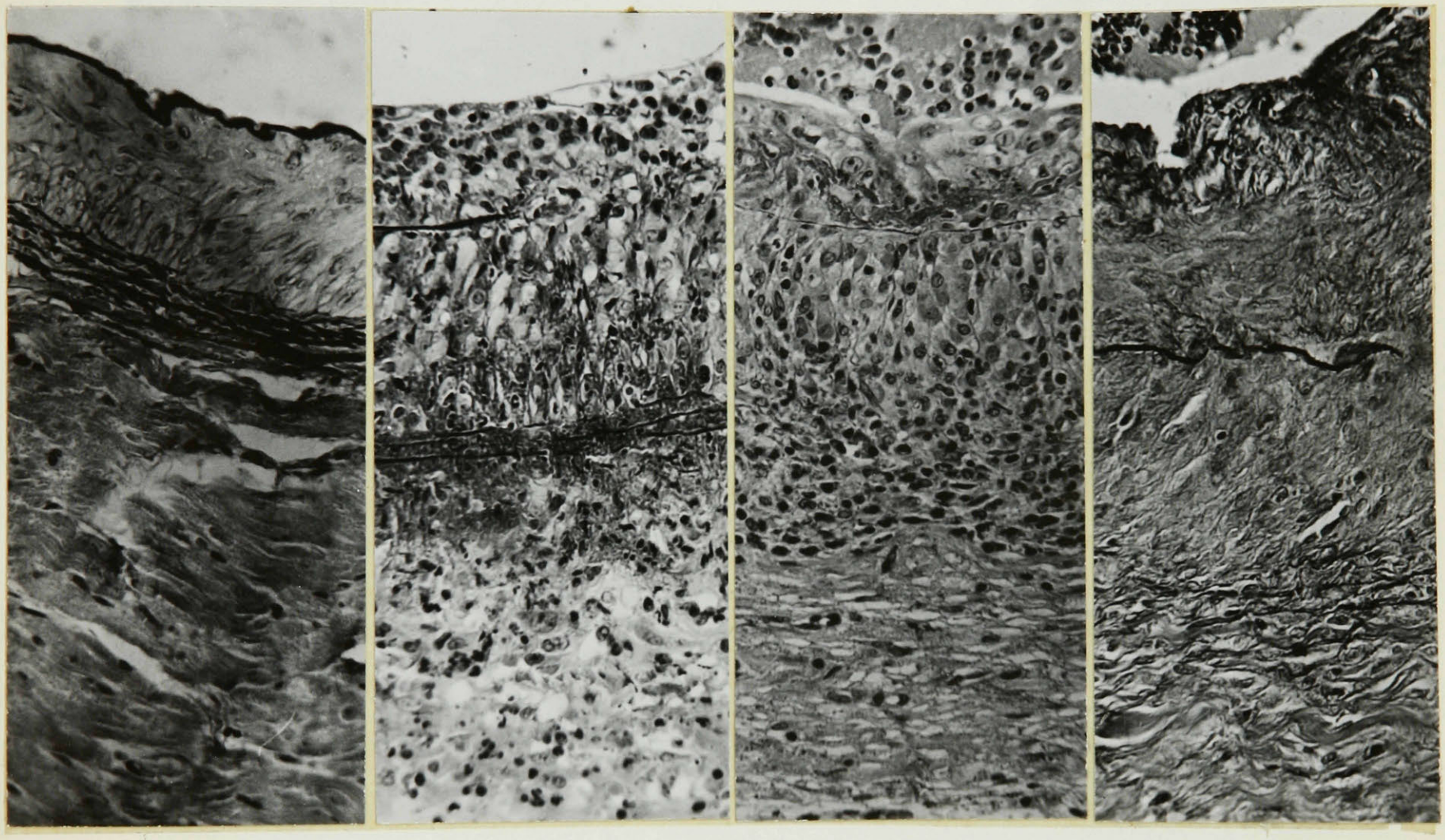


Fig.14 Fig.15 Fig.16 Fig.17

For description see text below.

Figure 14 shows a normal artery. It should be noted that the arterial wall is thin; the internal elastica is immediately beneath the intima of which the cells are not seen in the picture, since they are normally far apart and very flat. The external elastica is a loose layer of parallel and interwoven fibers just outside the media. There is very little adventitial collagen, the tissue outside the external elastica being myocardial fibers.

Figure 15 shows acute damage to an artery. The intima, still intact, is lifted away from the internal elastica and the intervening area filled with large mononuclear cells and a few lymphocytes. The internal elastica is interrupted. The medial muscle cells are swollen and vacuolated and between these there is fibrinoid material present which is continuous with the fibrinoid material in the external elastic layer. The fibers of the elastica externa are stretched and separated by dark staining material which takes stains as does fibrin. The elastic fibers appear to be fewer in number than in the normal adventitia. The adventitia is oedematous and infiltrated with large and small round cells.

In Figure 16 a further stage of necrotizing arteritis is shown. Here the intact intima is still lifted away from the internal elastic membrane but the intervening space contains a number of fibroblasts and fibrinoid material is present. The elastica interna is stretched and flattened. The medial muscle cells appear more normal than in the previous figure. The perivascular exudate is concentrated about the external elastic layer, and the adventitia is thickened by formation of collagen fibers and condensation of unidentified material.

Figure 17 illustrates the healed stage of the lesions. The great thickening of the intima by collagenous fibers with some elastic fibers formed next to the lumen is seen. The elastica interna is interrupted. There is an increase in the collagenous tissue in the media but muscle cells are readily evident. The adventitia shows great collagenous thickening.

By comparing the lesions of human periarteritis nodosa and experimental necrotizing arteritis produced by sensitization to foreign proteins,

it becomes evident that the morphological aspects of the lesions are identical.

Glomerulonephritis.

Since the observation of "blood and serum" in the urine of patients with post-scarlatinal "dropsy" by Wells in 1806[92,93] and by Plenciz[94], the clinico-pathological correlation of the gross morphological picture in the kidneys of such patients by Richard Bright in 1827[95], and the description of all the essential elements of the histological lesions of glomerulonephritis by Langerhans in 1879[96], interest in the morphology, etiology and pathogenesis of glomerulonephritis has been high.

Since the earliest work, histological definition of the different types of renal diseases that may give the clinical picture of "dropsy" has been attempted. Great confusion existed until the classical work of Volhard and Fahr[97], who devised a classification that forms the basis of most of the present day systematic approaches. Their classification consisted of grouping the diseases according to the location of the lesions in the nephron:-

A. NEPHROSES (lesions confined to the renal tubules)

1. Simple nephroses.

2. Specifically characterized nephroses
   (a) Bichloride of mercury poisoning
   (b) Amyloidosis (complicated by glomerular lesions)

B. NEPHRITIS (lesions chiefly in the glomeruli)

1. Diffuse glomerulonephritis

2. Focal nephritides
   (a) Focal glomerulonephritis
   (b) Interstitial nephritis
   (c) Embolic focal glomerulonephritis

C. ARTERIOSCLEROSIS (lesions chiefly in the blood vessels)

1. Pure arteriosclerotic kidney

2. Combined forms.

Although the histological work upon which this classification was based was excellent, it is apparent from the illustration that many conditions which have since been described were included under the heading of "combined forms". Such conditions are the lesions of the kidney of lupus erythematosus, essential hypertension and intercapillary glomerulosclerosis, and others.

The early writers on the histopathology of glomerulonephritis[96,97] emphasized the presence of polymorphonuclear leukocytes and the proliferative changes of the epithelial and endothelial cells of the glomeruli. However, it was not until the work of McGregor that the normal basement membrane[98] and the changes in the basement membranes in glomerulonephritis[99] were brought into prominence.

It was not until relatively recent years that the variable clinical course was correlated with the histopathological findings in the kidneys. Much pioneer work was carried out by Bell[101-103], but this work was not productive of any correlation of the histopathological picture with the clinical course, except in the broadest way. However, extensive studies carried out by Ellis[104], and partly confirmed by Davson and Platt[105], have resulted in what appears to be a good clinico-pathological correlation in glomerulonephritis. They have described what they call Type 1 and Type 2 nephritis.

Clinically, Type 1 nephritis was acute in onset, followed one to three weeks after an acute upper respiratory infection and occurred predominantly in the first four decades of life. Grossly visible blood was present in the urine along with marked albuminuria and oliguria. These urinary findings were accompanied by transient hypertension and subcutaneous oedema. Recovery occurred in 82% of the cases. In the remaining 18% death might occur in the acute stage (4%); after a rapidly progressive course of a few months (4%); or the patients might recover from the acute attack and remain with only a moderate, persistent albuminuria which terminated many years later with hypertension, renal failure and contracted kidneys.

The histopathological description of the lesions of Type 1 nephritis of Ellis(104) gives great prominence to the occurrence of "acute fibrinoid necrosis of the arterioles", a lesion not seen by Bell(103) in his extensive series of cases. Furthermore, it has been pointed out by Kimmelstiel and Wilson(106) that proliferation of the cells of the glomerular tuft, though focal, may be as severe in malignant nephrosclerosis as it is in glomerulonephritis. The last named authors have also drawn attention to the fact that necrotizing arteriolitis is common in hypertensives dying in uraemia, but not in hypertensives dying of extra-renal causes. Thus, two possibilities exist for the observation of necrotizing arteriolitis in acute glomerulonephritis by Ellis. They are, firstly, that he has included cases of malignant hypertension with uraemia in his series, erroneously; and secondly, that patients dying in uraemia resulting from acute glomerulo-nephritis may manifest this lesion of the arteries but that this state has

been overlooked by other investigators. Further study of the kidneys of acute glomerulonephritis is required to elucidate this point.

Apart from the emphasis of the arteriolar lesions, Type 1 nephritis is a classical "extracapillary" type of glomerulonephritis, characterized by proliferation of endothelial and epithelial cells of every glomerular tuft and of the epithelium of Bowman's capsule, forming epithelial crescents. Peri-glomerular and inter-tubular round cell infiltration may be extensive. In the cases of long duration, complete hyalinization of the tufts may be evident along with fibrotic and hyaline crescents. In the acute stages, hyaline droplets may be found in the renal tubular cells and dilatation of the tubules may be prominent where uraemia is a feature, or where many glomeruli are hyaline, the presumably functional nephrons may dilate. The state of the basement membranes is not mentioned by the proponents of this classification[104,105] in Type 1 nephritis, though they state that it is thickened in Type 2. However, it is generally conceded by pathologists, and particularly by Bell[103] and McGregor[99] that beading, thickening and splitting of the basement membranes are characteristic of the later stages of glomerulonephritis.

Illustrations taken from Davson and Platt (Figs. 18 and 19) portray the principal findings in acute Type 1 nephritis.

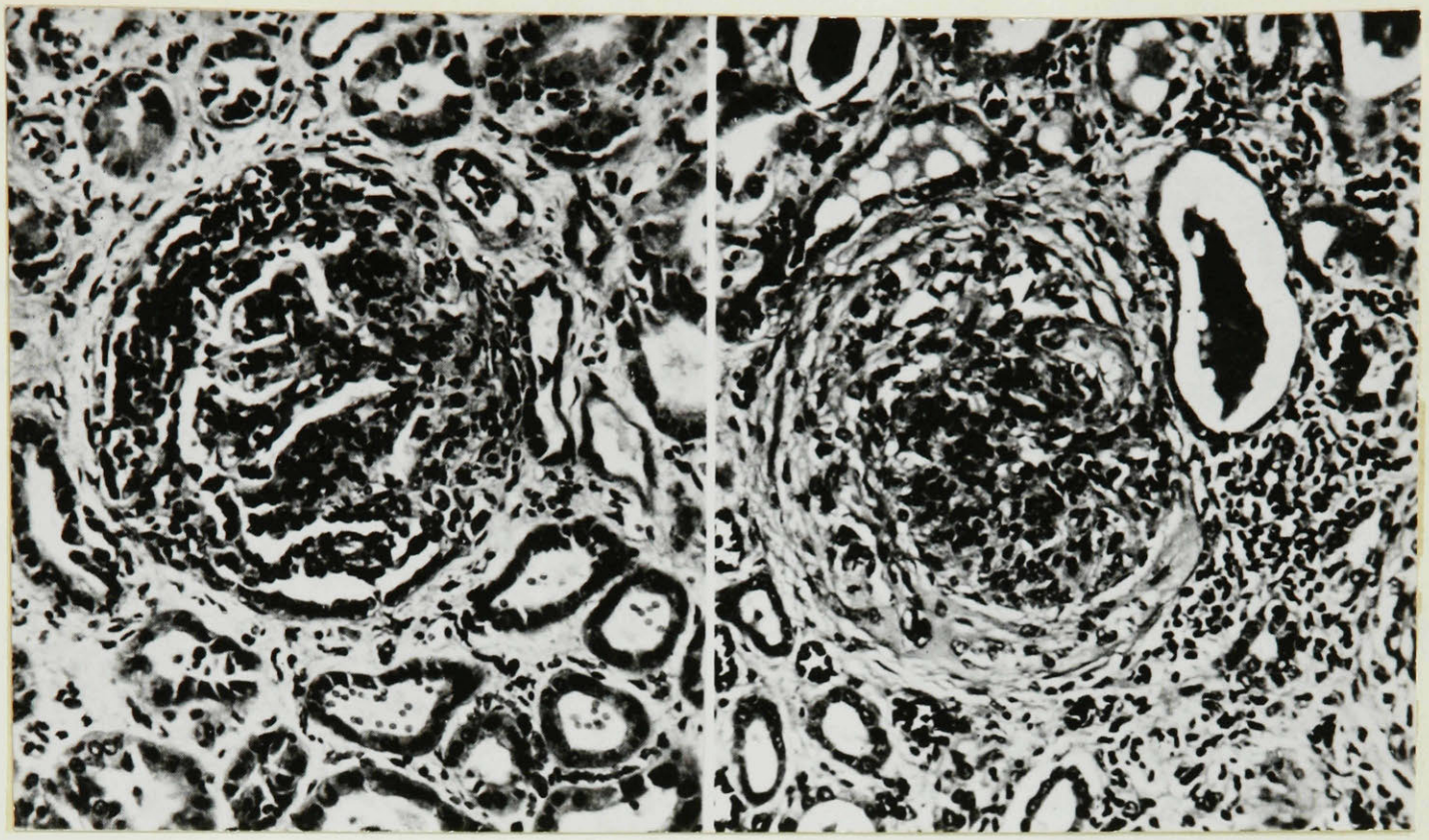


Fig. 18. From the rapidly progressive form of Type 1 nephritis, showing marked proliferation of tuft cells and crescent formation.

Fig. 19. Later stage showing fibrosis of the crescents, peri-glomerular inflammation and dilatation of tubules.

In Type 2 nephritis the onset was insidious, not related to any antecedent infection and occurred from the second to sixth decades. Of all these only 5% recovered, the remainder developing progressive renal failure, hypertension and dying in uraemia or from intercurrent infections, in one to twenty years. Pathologically, these cases were characterized by interstitial thickening of the basement membrane of the glomeruli with very little cellular proliferation or crescent formation.

Thus described, without the arteriolar lesions, Type 1 nephritis is the exact counterpart of the lesions that are produced in rabbits by two massive injections of horse serum, bovine gamma globulin or bovine

albumin. The lesions in rabbits are essentially of the proliferative type, the earliest changes being an increase in the number of polymorphonuclear leukocytes, endothelia and epithelial cells of the tuft. Coincident with this was a swelling of the endothelial cells and partial obstruction of the capillaries. Though it is suspected that swelling of the cells precedes the proliferation, the diagnosis of glomerulonephritis was never made in the absence of proliferation. The tuft also becomes larger and tends to fill the capsular space (Fig.21). Changes in the tubules and casts are usually absent at this stage. This stage has been designated as grade + in this laboratory by Drs. More and Waugh[22], whose classification is used throughout this thesis. In grade ++ the epithelial proliferation of the tuft is more marked, transforming the lobules into solid masses of cells in which

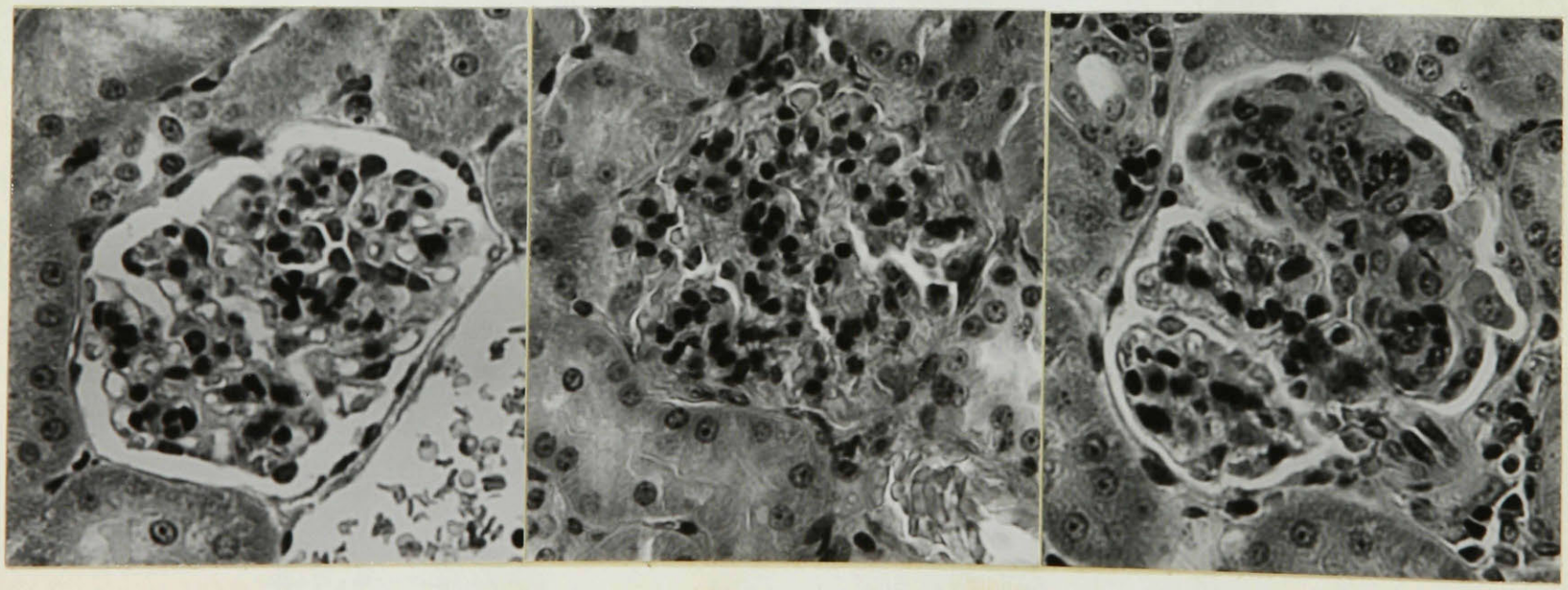


Fig.20 Fig.21 Fig.22

Fig.20 shows a normal glomerulus. Note the patent capillaries and the relative paucity of cells. Most of those present are endothelial (dark nuclei). Fig.21 shows + glomerulonephritis. Note the increase in number of epithelial cells (pale nuclei), polymorphonuclear leukocytes, obliteration of the capillaries and swelling of the tuft (Rabbit L-8, room temperature, horse serum Experiment II, Group II). Fig.22 shows ++ glomerulonephritis with marked epithelial cell proliferation and obstruction of capillaries (Rabbit L-47, Group II). All figures X 400. Haematin, phloxine, saffron.

no capillary lumina can be distinguished, or may only remain at the periphery (Fig.22). The basement membranes and tubules are usually normal in this grade.

In grade +++ glomerulonephritis the epithelial and endothelial proliferation of the tufts is usually more marked and scattered early crescent formation may be seen (Fig.23). The basement membranes often show thickening and beading in the centers of the tufts. The tubules are usually normal in structure, but casts are not infrequently seen.

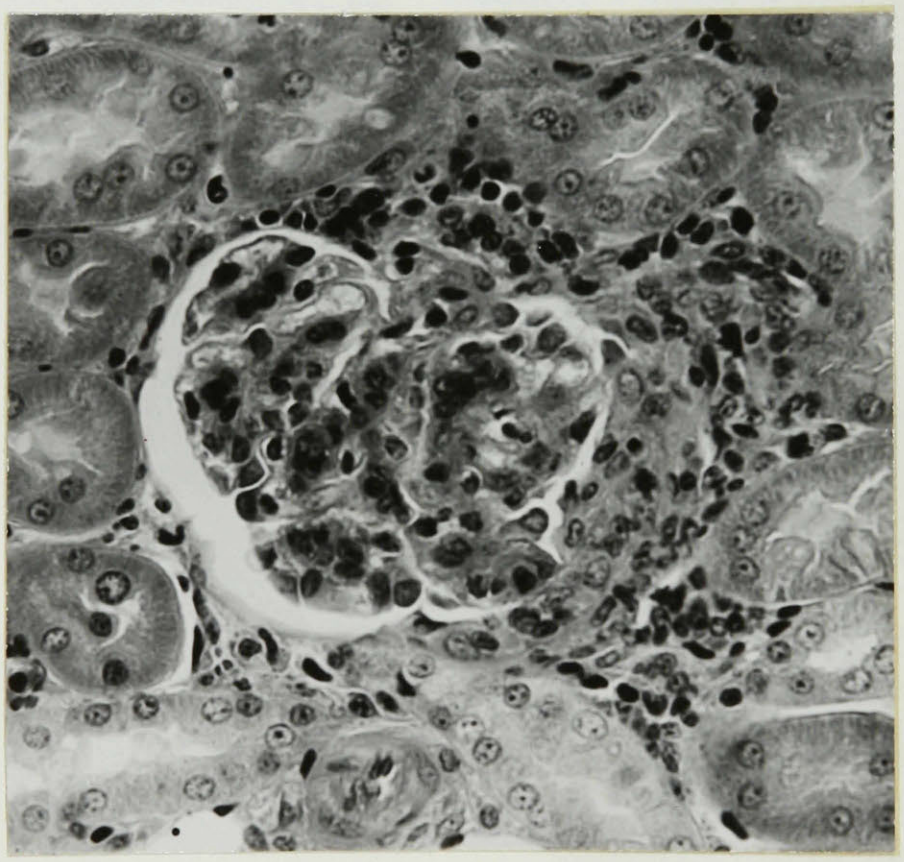


Fig. 23. Shows grade +++ glomerulonephritis with early crescent formation and marked epithelial proliferation of the tuft. There is slight periglomerular inflammation (Rabbit L-61,Experiment II, Group II). H.P.S. X 400.

In grade ++++ glomerulonephritis the epithelial and endothelial proliferation of the tuft is marked and almost every glomerulus shows crescent formation (Fig.24). The basement membranes are extensively thickened and fibrillar (Fig.25). Pericapsular and interstitial round cell in-

filtration is usually marked and tubular hyaline degeneration and cast formation are frequent.

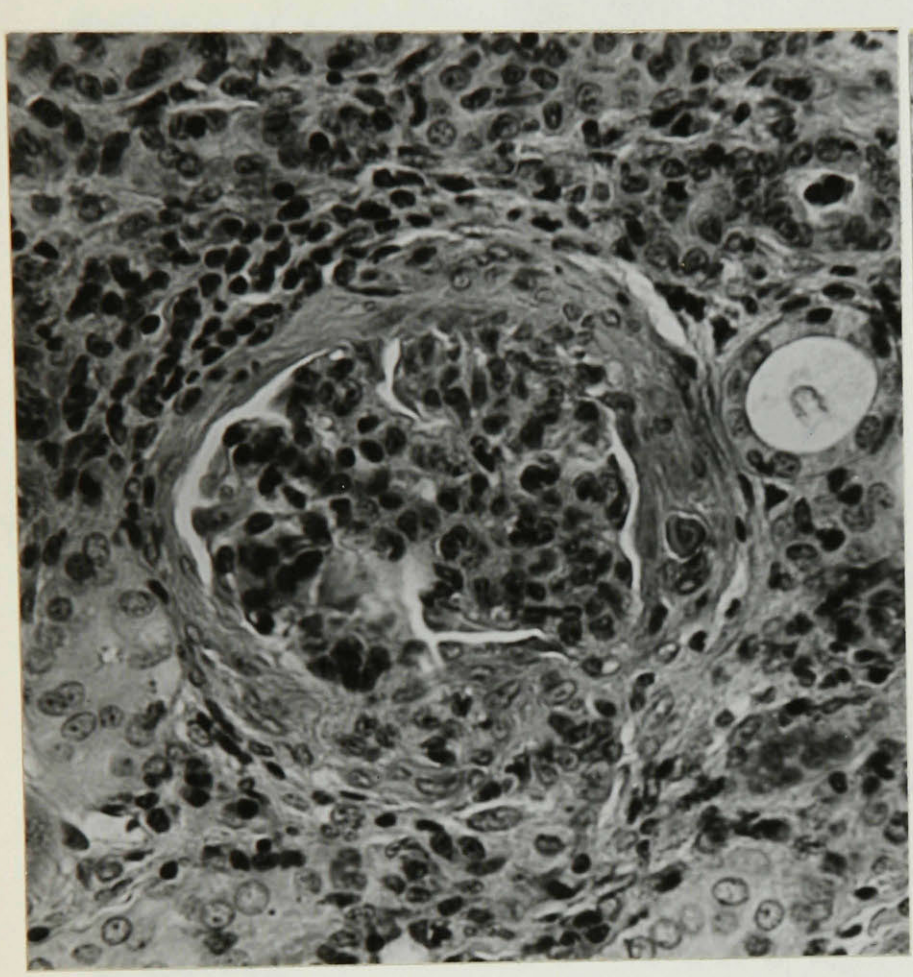


Fig.24. Grade ++++ glomerulonephritis from Rabbit E-29 treated with bovine albumin (Exp.VI). Endothelial and epithelial proliferation in the tuft, well-developed crescent formation, interstitial inflammation and tubular dilatation and atrophy are seen. H.P.S. X 400.



Fig.25. Grade ++++ glomerulonephritis from same rabbit as Fig.24, showing fragmentation of basement membranes, best seen at upper portion of glomerulus. Mallory-Heidenhain stain. X 400.

It is apparent from the comparison between the lesions of human Type 1 nephritis and experimental glomerulonephritis that the lesions may be regarded as identical in their morphological aspects. The identity of clinical behaviour has also been pointed out[107], and will also become apparent from the studies to be reported in the present experiments.

Summary:

It is apparent from the consideration of the morphology of the lesions of human rheumatic fever, polyarteritis nodosa and glomerulonephritis - and comparison of these lesions with those produced in rabbits by sensitizing them to foreign serum proteins - that the lesions produced in the hearts of animals do not resemble those of rheumatic heart disease, but rather the lesions of human serum sickness or sulphonamide allergy; while those in the arteries and kidneys are, on the other hand, identical to human polyarteritis nodosa and glomerulonephritis respectively. It follows that one should refrain from drawing the question of human rheumatic fever into the discussion of the experimental cardiac lesions. On the other hand, it is perfectly justifiable to compare the lesions of arteries and kidneys produced by foreign serum proteins with those of human polyarteritis nodosa and glomerulonephritis.

ETIOLOGICAL AND PATHOGENETICAL FACTORS CONCERNED IN THE EXPERIMENTAL PRODUCTION OF TISSUE LESIONS BY FOREIGN SERUM PROTEINS.

Introduction:

Although most of the interest in the lesions of animals stems from an interest in human disease and many of the reasons for embarking on certain experiments have originated from evidence derived from patients, it is considered not to be practicable to attempt to review all of the literature on human disease in a thesis dealing with a strictly experimental subject. To attempt such a review would extend the length of the thesis beyond reasonable bounds and the labour beyond the possibility of accomplishment in a reasonable time. Furthermore, as with any disease whose etiology is unknown, there are many theories of etiology and pathogenesis in the literature, each with its adherents and its opponents, and based on evidence of varying creditability requiring critical analysis. These theories of the etiology and pathogenesis of serum carditis, polyarteritis nodosa and glomerulonephritis will only be mentioned in passing, and only those theories and factors that bear directly on the experimental work, to be reported, will be reviewed in detail. The suggestion has been made by most investigators that "allergy" or "hypersensitivity" to foreign proteins is important in the genesis of the lesions. It has been further suggested

that in the pathogenesis of both the human and experimentally-induced diseases, the adrenal cortex plays an important part. Accordingly, the historical review concerning the etiology and pathogenesis will aim to examine, critically, the evidence for and against these concepts. As far as possible, consideration of the literature will be confined to that concerning experimental disease, with evidence from human cases being alluded to only where absolutely necessary.

Definition of hypersensitivity and its principal types:

The definition of hypersensitivity (hypersensitiveness, allergy) was constructed from a number of observations in which a delayed deleterious effect could be induced in animals after infecting them with living bacteria[108], after repeated injections of foreign proteins[109-113,154], and also from observations in certain diseases of man[114,115]. Wilson and Miles[116] state that "numerous observations, both clinical and experimental, have afforded instances of the contrary condition, in which an individual is, or may be rendered, more susceptible to some noxious agent than are the generality of his own species. In some cases an individual may become highly sensitive to an agent which is entirely innocuous to his normal fellows. The phenomenon of hypersensitiveness is presented in its clearest and least ambiguous form when the exciting agent is some non-living material, so that there is no question of multiplication within the body of the complex and variable tissue reactions that are associated with bacterial infections". This definition may be differently stated with respect to experimental animals to the effect that hypersensitivity is a type of (usually deleterious)

reactivity developing in animals after the first exposure to materials, usually protein in nature, that were not harmful to the animals in the first instance.

Hypersensitivity is divided into two major types, on the basis of the effect produced in animals, namely the anaphylactic or foreign protein type, and the tuberculin or delayed type. Other forms of hypersensitivity that differ in minor respects from these two principal types are also distinguished. The manifestations of the different types of hypersensitivity have been summarized by Rich(100) in the following table which is reproduced below because of its particular clarity and completeness.

It is apparent from Table I that there are several important differences between the anaphylactic or foreign protein type of hypersensitivity and the tuberculin type. In the foreign protein type, specific antibodies to the injected antigens are present in the blood, and the state of hypersensitivity is passively transferable by means of the serum. The transferability is in direct proportion to the amount of antibody in the blood(163). Furthermore, the type of reaction developed when small doses are injected intradermally is of the immediate type: evidence of oedema appearing in one hour and reaching a maximum in 24 hours. In the tuberculin type of sensitivity, no circulating antibodies can be demonstrated, the sensitivity is not passively transferable by means of the serum and the skin sensitivity to intradermal injection is of the delayed type: the first signs appearing after a lapse of at least 48 hours and reaching a maximum in 96 hours. Other differences may be seen in the Table.

TABLE I +.

THE CHARACTERISTICS OF THE TYPES OF HYPERSENSITIVITY
Illustrating the similarities of the anaphylactic, Arthus and pollen types, and their differences from the tuberculin type.

			Type of Hypersensitivity			
			Arthus	Anaphylactic	Pollen	Tuberculin
Usual *minimum* requirement for establishment of sensitivity.	Single parenteral contact with small amount of free antigen		0	+	+?	0*
	Parenteral contact with repeated small amounts, or with a single large amount, of free antigen		+	0	0	0
	Parenteral contact with intact bacteria or virus		0	0	0	+*
Some of the clinical effects that may result from contact of the previously sensitized body with the specific antigen	Prompt local oedema, erythema, foll. by haemorrhage, inflammation, necrosis		+	0	0	0
	Prompt, evanescent, local oedema and erythema		+‡	+	+	0
	Generalized oedema		+	+	+	0
	Generalized urticaria		+	+	+	0
	Symptoms of asthma (dysp.) or hay fever (nas. fulness; lacrymation)		+	+	+	0
	Prompt, fatal anaphylactic shock		+	+	+	0
	Prompt, non-fatal constitutional reaction		+	+	+	0
	Delayed constitutional reaction, fatal or non-fatal		0	0	0	+
	Delayed and prolonged local inflammation, and necrosis.		0	0	0	+
Evidence for site of action of antigen in sensitized body	Action directly on extravascular tissue cells	Necrosis of cells on contact with antigen *in vitro* (tissue culture)	0	0	0	+
		Necrosis of cells in avascular tissue *in vivo* (cornea)	0	0	0?†	+
	Action on capillaries	Increased capillary permeability (local oedema)	+	+	+	+
		Necrosis of capillaries with haemorrhage	+	0	0	+
	Action on involuntary muscle	Spasmodic contraction of involuntary muscle *in vivo* and *in vitro* (Dale bath)	+	+	+?†	0
Body sensitized to a bacterial protein reacts hypersensitively to the intact bacteria			0	0	−	+
Passive transfer of sensitivity readily accomplished			+	+	+	0

* See text for possible exceptions.
† Not tested.
‡ With small dose of antigen.

+ From Rich, A.R. (100), pg. 340, Table XVIII.

It has been amply demonstrated by serological tests[4,11-13,17,22], intradermal tests[7,8,13,14,22], and by passive transfer of anaphylactic shock[47] that the type of hypersensitivity conferred on the animals by two massive injections of foreign serum proteins is of the anaphylactic type.

The role of hypersensitivity to foreign proteins in the pathogenesis of the lesions:

In experimental animals many investigators have produced lesions of the heart valves, valve rings, myocardium and arteries of the heart by injecting large amounts of foreign proteins[12-18]. The protein solution used most often was horse serum[2-12,14-18,24], although pork serum[3], duck serum[11], bovine serum[16,21] or its fractions[13,21], and egg albumen[1,117] have also been employed. Horse serum has also been fractionated and the protein fractions used individually[12].

To produce the lesions, the early workers were accustomed to administer a course of small subcutaneous injections of antigen to sensitize the animals then to challenge them with an intravenous injection [2-6,16] of variable size on one[5] or more[3,4,6,16] occasions. Others of these challenged with an intra-articular injection[2,3]. Various combinations of intravenous and subcutaneous injections were used as well in different groups of animals[4]. It appeared from this work that the instances in which lesions of the heart and arteries were produced were those in which large amounts of antigen had also been used. Accordingly, the later workers have employed large doses of antigen given intravenously [7-18]. Usually two doses were used of 10 c.c. of horse serum per kilo of

body weight in rabbits given at an interval of 17 days between injections. However, shorter intervals have been used[118,119]. Where bovine serum or fractions were used, a dose of 1 gram per kilo has been given in a single injection[21], or two injections at an interval of 9 to 10 days[13].

Although the state of anaphylactic hypersensitivity has been shown to be present in all animals sensitized to foreign proteins, it is quite usual to find that in any group of animals similarly treated, some animals develop lesions while others do not. This is true of any of the above-quoted studies. The question is thus raised as to the exact role played by the state of hypersensitivity in the pathogenesis of the lesions.

The association of the state of hypersensitivity with lesions is so constant in cases of human polyarteritis nodosa[74-88] and in experimental animals[2-19] that it is difficult to conceive of an absence of pathogenetical relationship. However, it has been difficult to adduce positive evidence in favour of this relationship.

Such positive evidence has been sought by attempting to inhibit the lesions with agents known to inhibit some aspects of the state of hypersensitivity. One such agent has been sodium salycilate which is supposed to inhibit antibody formation[126]. The use of sodium salycilate has given rise to conflicting results. Some of the studies have failed to demonstrate a statistically significant alteration in the incidence[120-122,125]. On the other hand, a careful study by Smull, Wissler and Watson[124] appears to have resulted in a significant diminution in the incidence of lesions under salycilate treatment. In the last-cited study

larger doses of salycilates were used than in any similar investigation. A decrease in the antibody titers (collodion particle technique) was also demonstrated[124]. It may be that further studies should be undertaken using large doses of salycilates.

Other substances such as colchicine[125], dicoumarol[125] and the antihistaminic drugs[120,121,127,128] were uniformly ineffective in preventing the lesions. Nitrogen mustard has been shown to diminish antibody formation[128] and skin sensitivity[128,129]. However, the inhibition of lesions is not quite statistically significant if their doubtful cases are considered positive with both experiments taken together, and the results applied to standard fourfold contingency tables[123] (where P - .0349 significant - P + .025). However, in their analysis of the content of antibodies in the serum (antibody nitrogen methods) with respect to each animal, they were able to state that the animals with the terminal amount of antibody in excess of 0.06 mg. of nitrogen per ml. exhibited vascular lesions, and those with values below this did not show lesions. Whether or not the inhibition of the lesions is significant, the correlation of the amount of antibody with the occurrence of lesions would constitute important evidence of the essential role of the state of hypersensitivity as indicated by this criterion.

Other methods of attempting inhibition of lesions utilizing adrenal or pituitary hormones will be discussed later under the role of the adrenal in the pathogenesis of the lesions.

Considering the attempts made to inhibit the lesions with salyicilates, antihistaminic drugs and nitrogen mustard, it appears admissable to consider that there is suggestive evidence that tissue lesions produced by foreign serum proteins administration are related to the state of hypersensitivity, as expressed by the development of precipitins in the blood. The difficulty experienced in evaluating the question of inhibiting the lesions by any given agent is related to the incidence of the lesions that may be expected in the control serum-treated groups. If the incidence of lesions in the latter groups is low, then it is difficult to obtain figures that are statistically different in the groups treated with the inhibiting substances. It is inherent in this type of experiment that the incidence of lesions induced by foreign proteins varies from group to group, but it is desirable to utilize modifications of the serum administration that allow one to expect a high incidence in most groups. This has been our objective in the experiments to be reported in this thesis. Furthermore, it is desirable that the methods of inhibition of the lesions be such that can consistently accomplish this end. Our efforts have been directed towards the study of agents that appeared to offer this possibility, as will be discussed in a later section.

It has been alleged that the characteristic fibrinoid necrosis affecting the collagen in various parts of the body, including that around and in blood vessels, a common morphological manifestation of many diseases(35,62,67-69,71,77,91,112,113), is an indication of a common

allergic pathogenesis[35,67-69,70]. However, it has been pointed out[46,71,72,89-91] that one cannot take one characteristic of a morphological reaction out of context and regard it as of particular significance in pathogenesis in the absence of other supporting evidence and this criterion alone is not sufficient to allow one to consider an allergic pathogenesis of either human or experimental disease. However, there is supporting evidence of associated allergy in serum carditis[51,52], sulphonamide carditis and arteritis[50], polyarteritis nodosa, both human and experimental[2-18,50-52,80-88].

Thus far, the discussion has been confined to the cardiac and arterial lesions occurring in animals treated with injections of foreign proteins. However, in many of the experiments previously discussed, the administration of the antigens has given rise to glomerulonephritis in the same animals[7,13,20-22,118], and much of the discussion of pathogenesis centers about the occurrence of the three lesions. It is thus pertinent to introduce consideration of the etiology and pathogenesis of experimental glomerulonephritis at this point, and to attempt an integration with the discussion of the factors concerned in the production of the lesions of the heart and arteries.

Although attempts have been made to produce glomerulonephritis in animals by injection of various types of bacteria, or their products, [20,136-138,149,150] or bacteria combined with suspensions of homologus kidney[131], these methods have not, in most instances[136-138,149,150], given rise to convincing lesions. On the other hand, very striking lesions

corresponding to glomerulonephritis in man have been produced by injection of sterile foreign protein solutions. Two large injections of horse serum (7,19,47), repeated injections of small doses of horse serum to rabbits(118) or horse serum and egg albumin(139) to rabbits, guinea pigs, dogs and cats; rabbit anti-rat-kidney serum to rats(1940-145,151); duck anti-rabbit kidney serum to rabbits(135,146,147); rabbit anti-rat-placenta serum to rats(148); or by one(21) or two(22) massive injections of bovine gamma globulin to rabbits, have all given rise to a typical glomerulonephritis.

Although much has been said about the specificity of the anti-kidney serums(140-147) to the extent that they were called by most authors "nephrotoxins", it should be noted that all the sera administered to animals were heterologous, and thus foreign to the animals receiving them, and subsequently developing glomerulonephritis. Furthermore, it is evident that anti-placenta serum(148) must also be considered to be a nephrotoxic serum and one, indeed, of more nephrotoxic potency than the anti-kidney serum prepared at the same time in the same investigation(148). However, in all the nephrotoxic studies, the control groups injected with comparable doses of normal heterologous sera appear to have evidenced a lower incidence of glomerulonephritis than in the nephrotoxic serum treated groups, but nevertheless, the lesions did occur. Also, where the titers of the nephrotoxic sera were measured(140), the titers were higher against the injected serum than against kidney tissue. It has also been stated that the urinary signs appeared at the same time as in the glomerulonephritis appearing after bovine gamma globulin sensitization(132). Moreover, it has been pointed out that

the antigen-antibody mechanism of the recipient animal must be intact in order to develop glomerulonephritis(135). It seems probable, therefore, that the anti-kidney sera act, at least in part, in the same manner as ordinary serum proteins that are not carrying anti-kidney antibodies. That is to say that some active participation of the injected animal's antigen-antibody, or other physiological, mechanism, must be involved. However, it is not discordant with the facts to consider that the anti-kidney property of the so-called nephrotoxic sera may have some localizing power whereby a high incidence of glomerulonephritis is consistently produced.

The role of the hypersensitive state in the development of glomerulonephritis must be assessed in the same way as its role in the development of the lesions of the heart and arteries previously discussed.

Again, investigations have been made using agents designed to inhibit the state of hypersensitivity. Kay(135) showed that when animals were subjected to total body X-radiation, both antibody formation to the injected sera and the lesions of the kidney failed to occur. Schwab and associates(130), utilizing a single injection of bovine gamma globulin to produce glomerulonephritis in rabbits, have demonstrated an association between the absence of tissue lesions and the absence or reduction of precipitins in the blood, and the failure to depress complement at the 9th day as is characteristic of animals developing a serological response to bovine gamma globulin. The most credible part of their study is the comparison of the titers with the presence or absence of lesions in individual animals for the method of producing the lesions by a single

injection of globulin is a hazardous one, and does not permit one to rely on the difference in incidence of lesions between groups. The methods used to inhibit the antibodies (and lesions?) were nitrogen mustard and X-radiation of the animals.

It appears from these studies that there is an association between the state of hypersensitivity and the development of experimental glomerulonephritis.

It has been suggested as a result of the occurrence of the three types of lesions, carditis, necrotizing arteritis and glomerulonephritis, in animals treated with whole serum, that the development of each type of lesion might depend on a different antigenic component of the serum[17,21], each having different physical and chemical properties. Accordingly, investigations were undertaken utilizing the purified fractions of bovine serum[21] and horse serum[22]. The first such study was that of Hawn and Janeway[21] who, with a single injection of whole bovine serum, obtained lesions predominantly of the arteries; with bovine albumin predominantly of the arteries and heart valves; and with bovine globulin, predominantly of the kidney. However, it is clear from perusal of their results that this separation of lesions was by no means absolute, and that all three types of lesions occurred in the albumin treated groups, and lesions of the heart and kidneys followed the injection of the gamma globulin. Similar results were obtained by Wissler, Smull and Lesh[12] with rather impure fractions of horse serum. Experience in this laboratory[13,22] with bovine gamma globulin - obtained

from the same sources as the previous investigators[21] but administered twice to unilaterally nephrectomized animals - that all three lesions, cardiac valvulitis, necrotizing arteritis and glomerulonephritis can result from sensitization to this single antigen. Further studies, utilizing bovine albumin will be reported later in this thesis.

It appears from the literature that the physical or chemical constitution of the antigenic substance cannot be of major importance in the induction of either cardiac valvulitis, necrotizing arteritis or of glomerulonephritis.

In summary of the material considered thus far, it appears that the state of hypersensitivity to injected foreign proteins is of importance in the development of the lesions of heart, arteries and kidneys, but that this is not established beyond all doubt. The physical or chemical nature of the antigen does not determine the occurrence of any specific lesion. There is suggestive evidence that a quantitative alteration of the intensity of the state of hypersensitivity is of importance in determining whether or not a given animal will develop lesions or not, but this is not firmly established. Even assuming the firm establishment of the above facts, the factors that influence the development of the state of hypersensitivity in the animal to the extent that one animal might and another might not develop lesions, would still remain to be discovered. If, however, the rather tenuous positive evidence should not be upheld by further work, one would be forced to search for alternative or at least auxiliary factors to explain the pathogenesis of the lesions.

That there are factors, other than the state of hypersensitivity itself, involved in the pathogenesis of the lesions of the heart, arteries and kidneys resulting from sensitization to large doses of foreign serum proteins, is indicated by certain studies that have been carried out. Smadel and Farr[152] demonstrated the inhibition of nephrotoxic nephritis in one strain of rate on a low protein-high carbohydrate diet, but emphasized that two other strains of rats failed to demonstrate this effect[151]. It was observed that exposing animals to a cold environment while they were given two massive injections of bovine gamma globulin increased the incidence of glomerulonephritis[134], and animals similarly treated failed to develop glomerulonephritis when their total food intake was restricted[153]. The incidence of severe glomerulonephritis was increased when horse serum was combined with killed and dried group A streptococci[47]. Unilateral nephrectomy appears to increase the incidence of glomerulonephritis induced by two massive injections of horse serum[19, 118]. Shortening the time interval between injections of horse serum from 17 to 11 days also appears to enhance the lesions[118]. The reasons for these alterations in severity of the lesions are not clear. It may be that they act by quantitative alteration of the state of hypersensitivity, but this has not yet been demonstrated.

Most investigators of the experimental diseases produced by rendering animals hypersensitive to foreign proteins, believe that their lesions are related in some way to the state of hypersensitivity. However, one other major theory has come into prominence with regard to these lesions

and in view of the general implications of this theory, it appeared important to consider it in our experiments and to review the theory itself at some length, as will be done below.

The role of the adreno-hypophyseal mechanism in the pathogenesis of the lesions:

In 1943 it was demonstrated[155] that lesions can be produced in the tissues of rats given large doses of desoxycorticosterone acetate (DCA) and desoxycortisone mineralo-corticoid[165] after unilateral nephrectomy, and while being maintained on a high sodium chloride intake. The lesions they produced were a myocarditis that they compared to the Aschoff nodules occurring in rheumatic fever, arteritis compared to periarteritis nodosa and a renal lesion compared to human "nephrosclerosis". These lesions appeared in the same animals and were stated to be accompanied by a rise in blood pressure. Although, with the exception of the lesions of the arteries, the analogies to the lesions of human diseases appears not to have been completely justified on the grounds of morphological comparison, the production of tissue lesions aroused the interest of investigators in the field of experimental pathology. Furthermore, the similarity between the lesions produced by Selye by various means have been likened by him to the lesions produced in rabbits by foreign serum proteins[156,157]. Apart from consideration of the lesions in which we are interested, Selye has embraced the etiology of nearly all diseases, of otherwise unknown origin, under the all-inclusive heading of "diseases of adaptation". The inclusive nature of a list of these diseases may be gleaned from perusal of his latest book "Stress"[156].

The concept of "diseases of adaptation" is based on experimental observations made by Selye in innumerable experiments and reported in an even greater number of publications. These observations led him first to define the "alarm reaction" and then the "general adaptation syndrome". It is not easy to summarize his concepts, since they involve changes which may occur in opposite directions depending on the intensity and duration of the stimuli and of the observations. Accordingly, a desire to remain faithful to the facts as presented by their author has impelled the writer to utilize Selye's[156] own summary in extenso.

"The alarm reaction" (A-R) is defined as the sum of all non-specific phenomena elicited by sudden exposure to stimuli. The alarm reaction of the nervous system, decrease in muscular tone, haemoconcentration, deranged capillary and cell membrane permeability, generalized tissue breakdown ("catabolic impulse"), hypochloremia, hyperkalemia, acidosis, a transitory rise followed by a decrease in blood sugar, leucopenia, followed by leukocytosis, eosinopenia and acute gastro-intestinal erosions. A discharge of adrenaline, corticotrophin and corticoids (with secondary metabolic and organ changes caused by these hormones) are early defense reactions which begin during the shock phase but are actually characteristic of counter-shock. The shock phase may vary from a few minutes to about 24 hours, depending on the intensity of the damage inflicted, but (unless death ensues), it is always followed by the counter-shock phase.

The phase of counter-shock: This phase is characterized by phenomena of defense against shock. There is an enlargement of the adrenal

cortex with signs of increased activity, acute involution of the thymico-lymphatic apparatus and generally speaking, the reversal of most of the changes seen during the shock phase (e.g. a rise in blood pressure, hyperchloremia, hyperglycemia, rise in blood volume, alkalosis, increased diuresis and often hyperthermia). These changes are not only synchronous with the stimulation of adrenal cortical activity but largely dependent upon the discharge of corticoids into the blood. Counter-shock phenomena may explain cases in which a distinct period of "primary shock" was followed by "secondary shock", after an intermediate stage of relative well-being. It is possible that here the intermediate shock-free period is merely the equivalent of the counter-shock phase which cannot be maintained (c.f. "exhaustion" below) and gives way to fatal shock. The counter-shock phase of the A-R represents a transition to the stage of resistance.

The stage of resistance represents the sum of all non-specific systemic reactions elicited by prolonged exposure to stimuli to which the organism has acquired adaptation. It is mainly characterized by an increased resistance to the particular stressor agent to which the body has been exposed, and a decreased resistance to other stimuli. Thus, the impression is gained that during the stage of resistance, adaptation to one agent is acquired "at the expense of" resistance to other agents. Most of the morphologic and biochemical changes of the A-R disappear during the stage of resistance and, indeed, in some instances, the direction of the deviations from the normal is reversed (e.g. hypochloremia during the A-R, hyperchloremia during the stage of resistance; loss of lipids from the adrenal cortex during the A-R, deposition of lipids into this gland during

the stage of resistance).

The stage of exhaustion represents the sum of all non-specific systemic reactions which ultimately develop as a result of prolonged over-exposure to stimuli to which adaptation has been developed but could no longer be sustained. It was found that even a perfectly adapted organism cannot indefinitely maintain itself in a state of resistance. If exposure to abnormal conditions continues, adaptation wears out and many lesions characteristic of the A-R (e.g. involution of the thymico-lymphatic apparatus, loss of adrenal lipids, gastro-intestinal ulcers) reappear as a stage of exhaustion develops and further resistance becomes impossible.

The "General Adaptation Syndrome" is an inclusive term used by Selye to denote "the whole response of which the A-R, the stage of resistance and the stage of exhaustion are merely three successive phases".

The latest version of the mechanism of operation of the General Adaptation Syndrome is presented in Fig.26

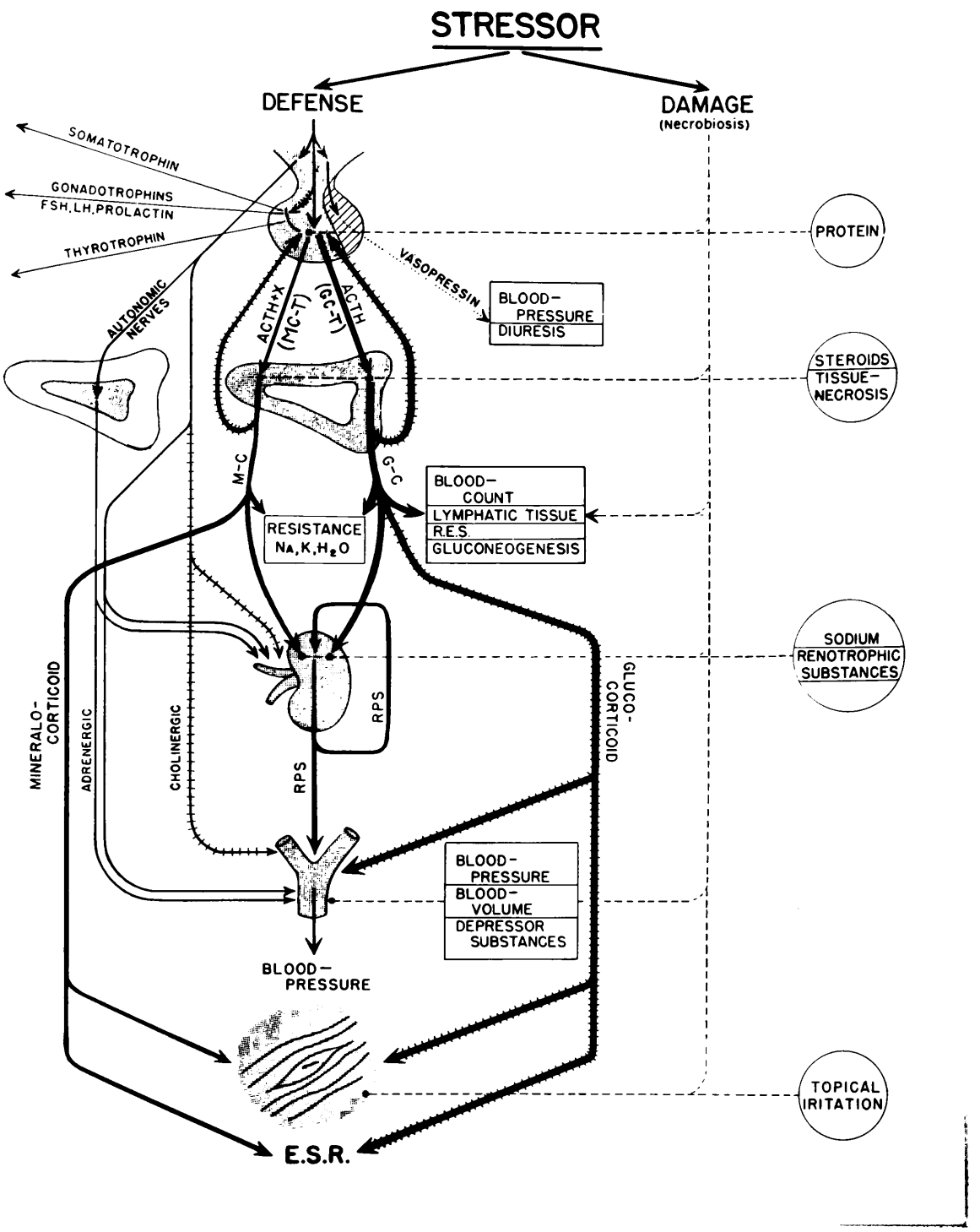


Fig. 26. *

* from Selye, H.:(156)

Legend:- R.P.S. = renal pressor substance; GC-T = glucocorticotrophic;
MC-T = mineralocorticotrophic; GC = glucocorticoids;
MC = mineralocorticoids; E.S.R. = erythrocyte sedimentation rate;
++++++++ = lines indicate inhibitors with respect to the solid lines.

In general, it may be stated with regard to the mechanism, envisaged by Selye at the present time, that non-specific stress stimuli act via the nervous system to stimulate the pituitary to produce both mineralocorticotrophic hormones which in turn stimulate the adrenal cortex to produce mineralocorticoids (typified by DCA); and/or glucocorticotrophins (ACTH) which in turn stimulate the adrenal cortex to produce glucocorticoids, (typified by Cortisone). It is the balance between these two sets of hormones that determine the occurrence of disease processes in the tissues, the DCA-like substance producing lesions and the ACTH or Cortisone-like substances providing protection against or cure of the diseases.

As may be seen from the summary of this theory, the changes that manifest themselves in animals exposed to stress are manifold and affect every tissue of the body and every conceivable metabolic activity. Thus, there are many aspects that one could study in order to determine the association between the general adaptation syndrome and the lesions produced by sensitizing animals to foreign serum proteins. The main difficulty experienced is to know what change is significant, since the changes may be in opposite directions. Thus it was our approach to determine whether there were any consistent changes in any of the aspects under study (in any direction) that could be correlated with the occurrence of the lesions. To discuss all the factors to be studied at once would make a very confusing account. Accordingly, the changes induced in the general adaptation syndrome will be discussed with the appropriate portions of the experimental results to be described.

Considerable force has been added to the arguments of Selye regarding the role of the adrenal and pituitary in the pathogenesis of disease processes by the availability of relatively large quantities of purified adrenocorticotrophic hormone (ACTH) and the synthetic adrenal glucocorticoid Cortisone (Compound E of Kendall) for clinical trial. Since the demonstration that ACTH and Cortisone were, at least temporarily effective in alleviating the clinical manifestations of rheumatoid arthritis[164], a great impetus has been lent to investigations utilizing these substances in almost every human and experimental condition known to man. Since this first demonstration of clinical effectiveness, the literature on this subject has grown to unbelievable proportions[166]. It is of particular interest to us that favourable therapeutic effects of these agents have been reported in periarteritis nodosa[166], acute disseminated lupus erythematosus[167,168], acute rheumatic fever[168], dermatomyositis[168], and possibly in acute and subacute glomerulonephritis[169]. It is interesting to speculate as to the pathogenetical relationship between these diseases which have been grouped together on morphological grounds as "collagen diseases" on the basis of this response to ACTH and Cortisone. Also favourably affected by these agents were conditions long associated with the state of hypersensitivity such as drug hypersensitivity[170] and bronchial asthma[171,172].

Although ACTH and adrenal cortical extract (ACE) have been reported to increase the production of antibodies[173-175] in rats, this work has failed to find confirmation in man[166,177] or in animals[178,179,182]. Indeed, where the studies have indicated any effect at all, they have been

to demonstrate a diminution of antibody formation in animals treated with these hormones[179-181].

The administration of ACTH appears not to have been effective in preventing anaphylactic shock in passively[178,184] or actively[183] sensitized animals. However, large amounts of serum from animals sensitized but simultaneously-treated with Cortisone failed to transfer sensitivity passively to other animals. On the other hand, much smaller amounts of serum from non-Cortisone-treated, but similarly sensitized controls and ACTH-treated and sensitized animals, were effective in transmitting their sensitivity passively[180]. These results may be correlated with the almost complete supression of antibodies observed by these investigators[180] in their Cortisone-treated animals in contrast with the moderate diminution of the ACTH group.

The skin reactivity to foreign serum proteins of animals anaphylactically sensitized to them has not been inhibited by ACTH[185-187] or Cortisone[181,188], although an effect amounting to almost complete inhibition has been reported as resulting from treatment with Cortisone[181,187]. However, these conclusions are not supported by critical examination of the data which may perhaps indicate some quantitative diminution in the size of the reactions. On the other hand, there appears to be agreement on the fact that the tuberculin type of skin reaction may be inhibited by Cortisone[181,189].

The effect of ACTH or Cortisone on the tissue lesions resulting from anaphylactic sensitization of animals to foreign serum proteins has not been studied extensively and, indeed, no reports on this subject existed

in the literature prior to the commencement of the present experiments, although several studies have since been reported. Knowlton and associates[190] noted that Cortisone failed to prevent the occurrence of glomerulonephritis in rats treated with rabbit anti-rat-kidney serum. On the other hand, Clampit[119] reported the prevention of horse serum arteritis in rabbits by ACTH, but did not give details of his experiments. Berthong, Rich and Griffith[185], and Bennet, Berthong and Rich[188] reported the inhibition of necrotizing arteritis and cardiac lesions in rabbits (given a single injection of 10 c.c. of horse serum per kilo of body weight) by ACTH[185,186] and by Cortisone[188]. The development of lesions in individual animals under Cortisone or ACTH treatment occurred, and the presence or absence of tissue lesions was not paralleled by the degree of skin or serological reactivity. They also reported complete suppression of the lesions of the kidneys. However, it should be noted that giving a single injection of horse serum to rabbits is not a reliable method of producing glomerulonephritis or arteritis and carditis, and the results of this latter phase of the study should be cautiously interpreted, as will be discussed in connection with our own results. Furthermore, More and associates[191] have reported that Cortisone prevented the occurrence of arteritis but not of glomerulonephritis in unilaterally nephrectomized rabbits given two injections of horse serum at an eleven-day interval.

Other observations bearing on the role of the adrenal in the pathogenesis of the lesions, made in these studies, will be discussed in the appropriate places while considering the results of the experiments to be reported.

ORIGINAL EXPERIMENTS.

General Introduction:

The following series of experiments was undertaken in order to study the role of the state of hypersensitivity, the role of the adrenal-pituitary mechanism and the role of specific antigens in the pathogenesis of the lesions induced in rabbits by means of two massive injections of foreign serum proteins. Since there are several experiments to be discussed, and the objective of each one is different, it appears appropriate to introduce each experiment separately and to discuss each as a unit with its specific experimental procedures and results.

Materials and Methods - General:

Male albino rabbits weighing 1800 to 2300 grams were used throughout the experiments. These rabbits were obtained from Sunset Farm, St. Rose, P.Q. No attempt was made to get rabbits of uniform pedigree, but they were all within a small range of parentage, which is known and may be analyzed in the future. They were received in the animal house within 300 grams of the weight at which the experiments were usually started, and kept untreated until they reached the required weight, and at the same time became aclimatized to the conditions, food, etc., in the animal house.

During this period they were weighed at intervals and the animals that gained weight satisfactorily were regarded as healthy animals and were used in the experiments. Those that did not gain weight were discarded. From the healthy animals groups were selected at random by placing each rabbit number on an identical card, mixing the numbers in a hat and having a disinterested person draw the tage from the hat. The experimenter placed the animals in the experimental and control groups alternately as their numbers were drawn.

After the experiment was over, the animals were killed at first by a blow on the head or by injecting 30 c.c. of air into the ear vein. Latterly, the method was changed so that the animals were killed by an intravenous injection of 3 to 5 c.c. of nembutal given quickly into the ear vein, which induced death painlessly and instantaneously. A complete autopsy was performed, and gross observations recorded. The animals were weighed before and after removal of the gastro-intestinal tract. The latter weight will be referred to as the "carcass weight" throughout. Each organ was carefully weighed to the nearest decigram, with the exception of the pituitary and adrenal glands, which were weighed on a very accurate beam balance to the nearest tenth of a milligram in the fresh state. Thin slices of the organs were fixed in Zenker-formol solution (20% formalin) for 18 hours, after which they were trimmed, dehydrated, embedded in paraffin and stained with a variety of stains, as required, including Haematin, phloxine and saffron, Masson's trichrome, Mallory-Heidenhain aniline blue azo-carmine, phosphotungstic acid haematoxylin,

Weigert's elastic stain; and McManus' periodic acid technique(159); Glynn's modification of Gram's stain; and a stain for acid fast bacilli, utilizing a detergent(158).

Microscopical sections were made of heart, kidney, adrenal, pituitary, spleen, liver, lung, thymus, thyroid and testis of most of the animals. Only the results of the histological study of the hearts and kidneys are presented in this thesis. The method of taking the blocks of heart is considered in detail, since it is necessary to see the coronary arteries at or near their origin in the aorta in order to be sure that necrotizing arteritis is not present for, occasionally, the lesions are confined to an area very near the coronary orifices. Thus, a transverse section was taken in the plane of the atrioventricular sulcus, including not more than 1 to 2 mm. of tissue on either side. When this block was sectioned, the orifices of the arteries almost invariably appeared or could be found by repeated sectioning. No heart was regarded as not having arteritis unless this portion of the coronary arteries was observed. Routinely, the next block was taken of the next 3 to 4 mm. of the thickness of both ventricles. Study of both sections allowed observation of the leaflets of all four valves of the heart and their points of attachment to the fibrous ring of the myocardium.

It will be observed that valvulitis and arteritis were graded from + to ++++ in the Tables of the results. This grading is of such a subjective and approximate nature that it was not considered in the analysis of the results where only the presence or absence of the lesions

was used in the correlations. On the other hand, the grading of the lesions of the kidney is based on fairly well-defined criteria, as indicated in the section on morphology, and is considered in making the correlations with other results.

In the animal house, the animals were singly, caged and fed on Purina Rabbit Chow. The food and water were available in individual dishes, the rations from individual animals never being mixed as far as possible. When it was not desired to study the urine of the animals, they were kept in their original cages throughout the experiment with the cage grills and pans being changed at least once a week or more often if required. If it was desired to study the urine, a special grill, composed of 1/16th inch copper mosquito netting formed the bottom of the cage. Beneath this a galvanized iron funnel was fitted exactly into the bottom of the cage so that no urine was lost, and the urine collected in a 1000 c.c. bottle. The grills and funnels were cleaned daily with hot water and brush, and the urine bottles, similarly cleaned, were replaced daily at approximately the same time, so that a 24 hour sample of urine was available for analysis. The daily urinalysis was performed within 2 hours of collection of the samples. The electrolyte studies were made on samples collected in clean tubes and kept frozen at -20°C. until the analysis could be made.

The measurement of food and water intake was made by placing a weighed amount of food (300 gm.) in the feeding bucket and weighing the residue 24 hours later. Spilling of the food, wetting of the food with urine or water was recorded, and the food weight, for the day on which these events occurred, was not included in the calculations. Similarly, a measured

amount of water (700 c.c) was placed in the water container, and the residue measured 24 hours later in a 1000 c.c. graduate cylinder. Any contamination of the water with urine excluded the measurement from the calculations. It was rather difficult to assess the water spilled into the urine, but where this was suspected, special precautions were taken to ensure that this did not occur. However, it was recognized that such precautions could not but have their limitations, and that the results would have to be carefully considered along with those of the control groups before the significance could be properly evaluated.

Many of the groups of animals were kept in the cold room almost throughout the duration of the experiment. The cold room is 8 X 8 X 8 feet (512 cu.ft.), and able to accomodate 24 animals in ordinary cages or 12 in metabolism cages. It is insulated with 6 inch blocks of cork cemented together, sized and painted with aluminum paint. Air vents are present in two walls to ensure exchange of air. Three walls are each lined by a bank of four 12 X 40 inch cooling plates. The temperature of the room is thermostatically controlled. During the present experiments, the temperature was maintained between 0 and -20°C. The temperature rose to the upper limits during the day when the door was opened by the experimenters to make their observations, collect samples etc., and by the animal room attendants who cleaned the cages. The grills and funnels of the cages in the cold room were changed daily. Urine was always frozen in the morning and was thawed, before it could be measured and the samples collected, by placing the bottles in a bath of warm water.

Water was placed in the cold room in the mornings but was thawed at 5 p.m. each day. The result of this routine was that the animals were certain to have liquid water for at least 8 hours of the day. At other times the animals were observed to lick the ice so that they were never without water. All animals in the cold room were shaved at the beginning and mid-way through the period of cold exposure. This was done with an electric clipper over a large area, comprising most of the back and flanks of the animals.

The horse serum used in the experiments was obtained from the Institute of Microbiology of the University of Montreal. It was pooled serum from non-immunized (normal) horses, Seitz filtered and bottled in sterile 100 c.c. bottles without preservative. The serum was stored at $4^{o}C.$ or at freezing temperatures until used. After storage, the serum often developed a fine flocculent precipitate of lipoprotein. The practice was adopted of shaking the bottle and drawing the serum and precipitate into the syringe and injecting the whole into the animal. No ill-effects have been observed to result from this procedure. Injecting the complete horse serum has the advantage of exposing the animal to all the antigenic factors in the serum, some of which might be extracted if the precipitate were eliminated. Horse serum, in general, has a high content of globulin, and this is borne out by the analysis of the serum obtained through the courtesy of Dr. D. Waugh:-

Batch 1 Albumen 2.9 gm.%, Globulin 4.0 gm.%
Total 6.9 gm.%

Batch 2 Albumen 1.15 gm.%, Globulin 3.8 gm.%
Total 5.05 gm.%

The bovine gamma globulin used in these experiments was obtained from Armour and Company, Chicago, as a dry powder (Lots 904, 1823B, C-188B and C-188A). This was reconstituted to a 10% solution in normal saline and sterilized by Seitz filtration. No preservative was added. Tests for sterility after filtration revealed no growth.

The bovine albumen was obtained in crystalline form from Armour and Company, Chicago, (Lots J-5005, 41-4802). It was prepared for injection in the same way as the bovine gamma globulin.

Injections were made in various ways. Intravenous injections were given slowly into the marginal ear veins of the rabbits. The massive injections were of whole horse serum, 10% bovine gamma globulin or 10% crystalline bovine albumen. The desensitizing doses were given the day before the second massive injection and consisted of 10 c.c. of tenfold dilution of the massive dose in normal saline. Intradermal injections were given for the purpose of observing the reaction in the skin to the sensitizing agents employed in the experiments. They consisted of injecting 1/10 of 1 c.c. of the antigenic solution in its original strength and in dilutions of 1/10, 1/100, 1/1000 along with a control dose of normal saline into discrete areas on the shaved back of the animal. The reactions were read one hour, 24 hours, and in some instances, 48 and 72 hours, after the injections. The material was injected using a tuberculin syringe to ensure accurate measurement of the dose and a fine, 25 or 26, guage needle. Intramuscular injections of the antigens suspended in Freund's adjuvant, or of the ACTH or Cortisone were made into the flexor and extensor muscles

of the thigh. No local infections were observed to occur as a result of these injections.

Reading of skin tests was carried out by placing the animal in a good light, wetting the skin-test area with 70% alcohol to wet the hairs and measuring the maximum and minimum diameters of the zones of redness, oedema, haemorrhage and necrosis. The height of the oedema was not recorded since it could be, at best, a very inaccurate recording and also since the height of the oedema was always proportional to the area. The only instance where the absence of this measurement was found to be a disadvantage was in the Cortisone-treated group, where it will be necessary to comment on this point in consideration of the results. When the measurements were finally compiled, the average diameter of the various phases of the skin reactions was computed, and it is this figure that forms the basis for the statistical evaluation of the results.

Blood was collected from the rabbits from the central artery of the ear. This was accomplished by dilating the vessel with Xylol painted onto the underside of the ear. This was then washed off with 70% alcohol and dried with cotton. Careful treatment of the ears resulted in a minimum of skin burns. When the artery was maximally dilated, a slit was made obliquely across it as near the tip as possible with a sharp razor blade. If the procedure was carried out correctly, a spurting stream of blood was released from which it was possible to obtain the sample without much difficulty. Occasionally a second cut might be required, but most of the time gentle stroking of the artery caused the bleeding to be adequately maintained. At least 12 c.c. and sometimes 22 c.c. of blood were obtained

from a rabbit at a single bleeding. Usually, 2 c.c. of blood were collected in a tube coated with potassium oxalate and sodium fluoride at the same time. This coating was applied to the inside of the tube by evaporating 1 c.c. of a solution containing 16 mg. of potassium oxalate and 4 mg. of sodium fluoride per c.c. Agitation of the blood in this tube caused the salts to be dissolved in the blood and prevented it from clotting. This sample of blood was used to determine the blood sugar and the blood urea nitrogen values. The former were usually determined on the day of collection of the blood, but the latter were stored in the frozen state until the completion of the experiment. On the day the blood was collected the oxalated-fluorided sample was shaken well and the sedimentation rate, packed red cell and packed white cell volumes were determined in Wintrobe tubes. The refractive index of the plasma was also estimated by measuring its ability to rotate a beam of light in a refractometer.

For the collection of serum, the blood from the ear artery was allowed to run into a 10 c.c. culture tube containing 3/4 c.c. of paraffin oil, so that the latter formed a layer over the blood in the tube. This prevented continuous oxidation of the red cells. The tubes were tightly stoppered and the clot cllowed to retract. The serum was separated from the clot and suspended red cells by repeated centrifugation. Since the determination of electrolytes in the serum demands that the serum be free from the products of haemolysis, it was found (after considerable adverse experience) that one of the most important factors in the occurrence of

haemolysis was trauma to the clot. Accordingly, the clot was not touched until retraction was almost complete, after which it was freed from the walls of the tube by running a fine wire around the rim of the clot. Where it was desired to determine the levels of serum lipids or quantitative antibody levels, an additional tube of blood was collected and the serum obtained without the use of the paraffin oil.

Ring tests were made with the serum of the rabbits by placing a small amount of the rabbit serum in the bottom of a glass tube 4 mm. in diameter. The antigen solution in appropriate dilution was placed above the rabbit serum carefully with a capillary pipette so that the two solutions did not mix. When antibodies were present in the rabbit serum, a precipitate formed at the interface of the two fluids which gave the appearance of an opalescent ring. The presence or absence of a ring was noted immediately after the two fluids were placed in contact and after incubation at 37°C. for one hour. The one hour reading is the one recorded in the results, unless otherwise specified.

The blood sugars were determined on samples of blood obtained from the animals without having fasted them for any period. Accordingly, they are random samples. The method employed is one which is supposed to measure the true glucose value and was a modification of the Somogyi method for photometric use by Nelson(159).

The blood urea nitrogen was determined by the methods described by Hoffman(162).

Urinalysis was done daily in certain of the experiments and consisted of qualitative determination of sugar by Benedict's qualitative method(160). The quantitative estimation was also made in some instances by Benedict's quantitative method(161). Quantitative determination of albumen in the urine was made by the sulphosalycilic method of Kingsbury-Clark, using the set of standards recommended by these authors*. The approximate pH of the samples was determined by immersing strips of treated paper into the urine and noting the colour produced.

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* Marketed by A.S.Aloe Co., St. Louis, Mo., and Standard Reagents Co., Park Lane Street, Philadelphia, Pa.

Experiment I.

The effect of cold on lesions induced in rabbits by two massive intravenous injections of horse serum.

Interest in the effect of cold on the lesions induced by injection of foreign proteins has stemmed from a variety of sources. For instance, it has been common experience among clinicians that exposure to cold accentuates the symptoms and signs of many diseases. It has been demonstrated by Selye that cold is a stimulus which by itself may produce pathological alterations in animals(157). It has been pointed out, in the general consideration of the role of hypersensitivity in the pathogenesis of lesions produced by sensitization to foreign proteins, that in any group of animals a certain percentage of them fail to develop lesions, making the evaluation of any attempt to inhibit the lesions very difficult to interpret. In fact, there are reports where groups of animals treated in a manner usually effective in producing lesions have revealed no lesions at all(200). It was therefore of interest to us to find methods of modifying the administration of the proteins or using them in association with synergistic conditions that might consistently increase the incidence of lesions to a point where unreasonably large numbers of animals would not be required to make a clear statistical evaluation of the effect of any inhibiting substance or condition. It was thought that cold might be such a synergistic stimulus. Furthermore, at the time these experiments were begun, it was reasoned that if the lesions of the heart produced by foreign serum proteins bore any relation in structure to the lesions of the heart in human rheumatic fever, subjecting the animals to conditions that might cause the lesions to

become more florid, might also reveal a structure more like the lesions of rheumatic fever. Among several modifications made of the mode of foreign serum administration(47) was the exposure of the animals to cold which is reported below. Further interest in the effects of cold was manifest from sources remote from the laboratory in the possibility of warfare occurring under conditions of extreme cold, and it is to the Defence Research Board that we are indebted for building the cold room in our laboratory.

In the past, few attempts have been made to allow animals to live under extreme conditions that were at the same time compatible with life for a relatively long period of time. Most of the experiments on the effects of cold were very acute and drastic, such as chilling the ears of rabbits with dry ice(193,194), immersing animals in ice water baths momentarily(195,196). In one of these studies(196) it was reported that chilling of rabbits twice daily in cold baths (0 to -8°C.) produced a slight rise in the titer of anti-typhoid agglutinins but no effect was observed on the production of haemolysin against sheep's red blood cells. McKay and associates(197) exposed rats to cold (5 to 10°C.) for 90 days and found soft, pale kidneys with tubular necrosis and agglutination of red blood cells in glomerular and other capillaries. Renal lesions were also reported by Dugal and Therien(198) in rats kept at -2°C. that died after one month or longer. These lesions consisted of necrosis and dilatation of renal tubules. It was also demonstrated that there was an increase of adrenal weight(199), kidney weight(199) and heart weight(198)

as a result of exposure to cold. Selye[157,206] has described changes in the adrenals, thymus, blood sugars and blood chlorides of rats exposed to cold. However, there were no experiments in the literature which could indicate what could be expected in this experiment. Accordingly, it may be regarded as an empirical experiment.

Experimental procedure:

Twenty-four rabbits were placed in the cold room after a large area of their backs was shaved. Of these, six were designated as controls and were not treated. The remaining sixteen received two intravenous injections of normal horse serum in a dose of 10 cc. per kilo of body weight on the 4th and 21st days after the beginning of their exposure to cold. The rectal temperature of the animals was measured daily. All the animals were killed one week after the second injection, and a complete autopsy performed.

Animals composing this experiment were:-

Serum treated: B-10, B-27, C-22, C-25, C-26, C-73, C-74, C-81, C-76, D-3, D-4, D-5, D-6, D-8, D-10.

Controls: D-2, D-7, D-9, C-80, C-77, B-8.

Results:

Table 2 indicates the incidence of the lesions of the heart, arteries and kidneys. The incidence of valvular lesions (91.6%) and glomerulonephritis (91.6%) was higher than had ever been observed in this laboratory resulting from two injections of horse serum. Since one must have standards of comparison, and since there is some confusion in

Table 2.

INCIDENCE OF LESIONS IN EXPERIMENT I.

Mode of death	No. of animals	Valve lesions	Valve ring lesions	Artery lesions	Glomerulonephritis + ++	Glomerulonephritis +++ ++++	Glomerulonephritis Total
a) Died after 1st. serum injection	4	0	0	0	0	0	0
b) Died after 2nd. serum injection	0	-	-	-	-	-	-
c) Killed at end of experiment	12	11 (91.6%)	4 (33.3%)	8 (66.6%)	8 (66.6%)	3 (25%)	11 (91.6%)

the literature concerning the designation of the lesions of the heart[13] and the grading of glomerulonephritis is peculiar to this laboratory[22], the incidence of lesions will always be compared to the incidence of lesions in other experiments performed in this laboratory. Accordingly, the incidence of lesions in groups of animals receiving two massive injections of foreign proteins, under the conditions designated, have been compiled graphically in Figs. 26, 27, 28 and 29. Although the results of the experiment under discussion have been omitted from the Figs., it is evident that the incidence of valve lesions and glomerulonephritis was higher in this experiment than that observed in any other experiment in which two injections of horse serum were administered. The incidence of valve ring lesions and necrotizing arteritis was not unusually high.

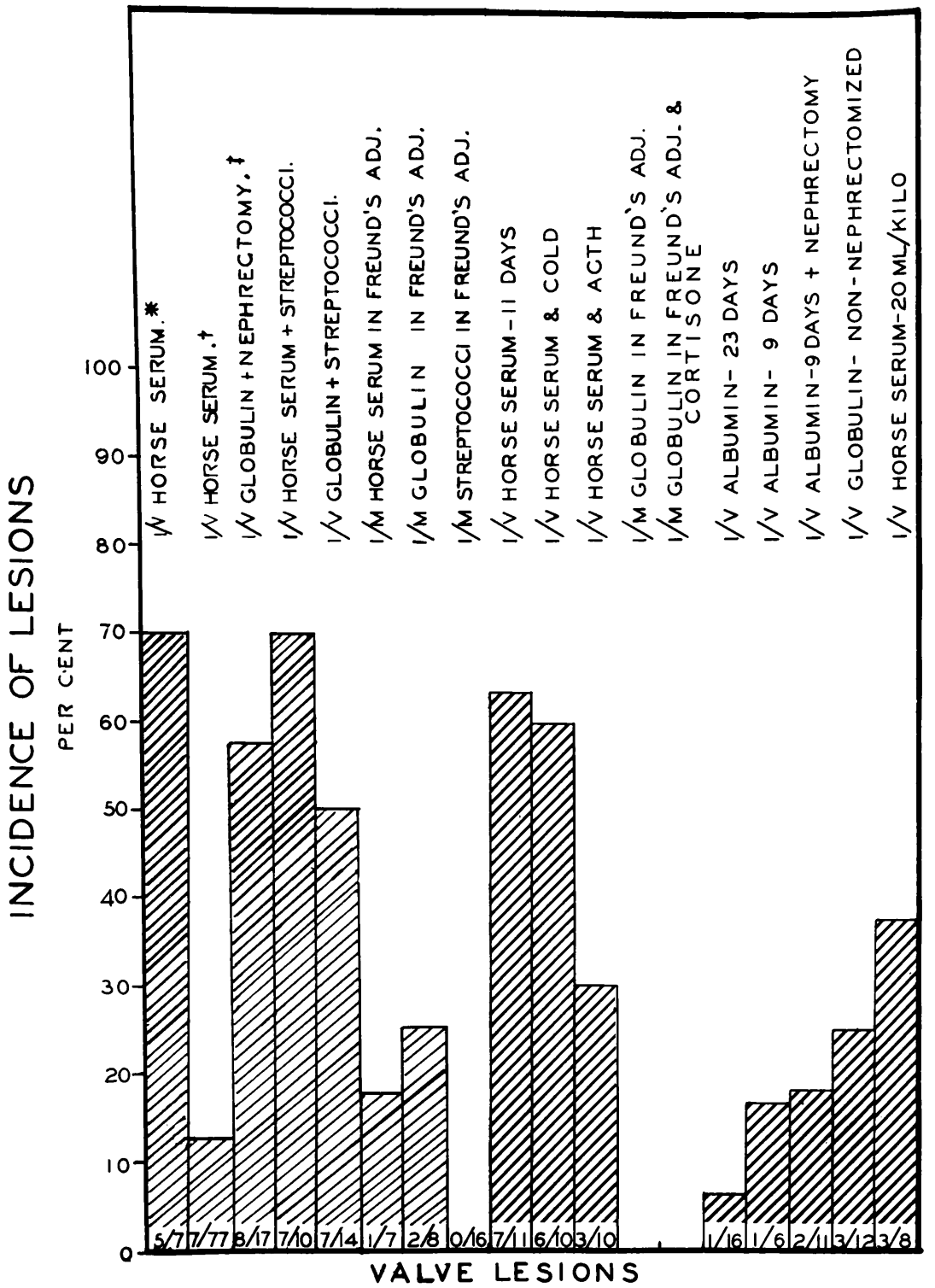


Fig. 26

Legend for Figs. 26, 27, 28 and 29.

Results of Experiment I are not shown in the Fig.

* = Horse serum at room temperature 17 day interval (47)
\+ = Horse serum at room temperature 17 day interval (10)
‡ = Reference (22)
Columns 3, 4, 5, 6, 7, 8 (47)
Columns 9, 10, 11, 12, 13 - Experiments 2, 3, 4, 5.
Column 14 - Experiment 6.
Column 15 - Experiment 7.
Column 16 - Experiment 8.
Column 17 - Experiment 9.
Column 18 - Experiment 10.

Figures at base of columns indicate number of animals with lesions/ number ~~treated.~~

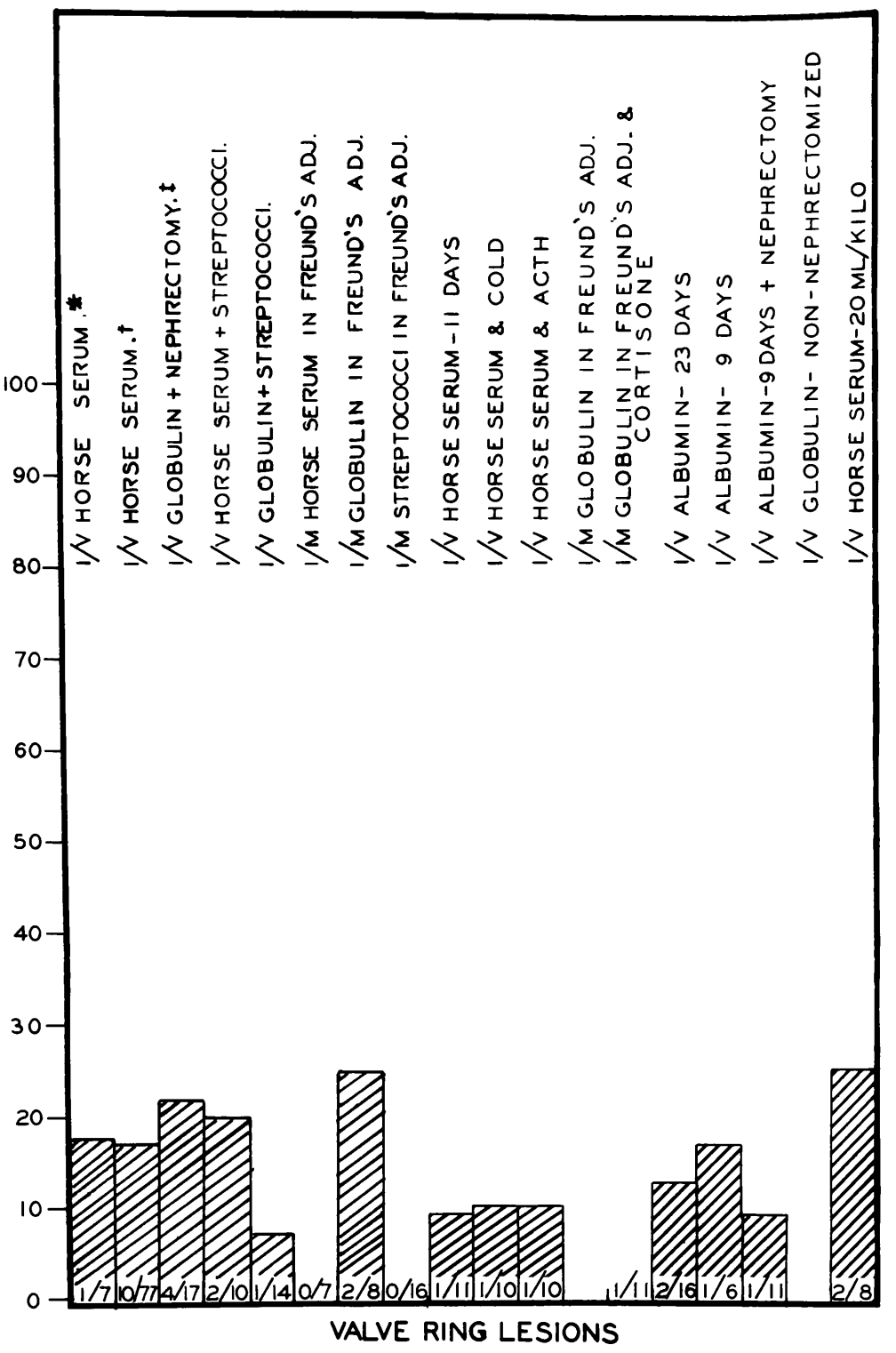

INCIDENCE OF LESIONS
PER CENT
100
90
80
70
60
50
40
30
20
10
0
I/V HORSE SERUM.*
I/V HORSE SERUM.†
I/V GLOBULIN + NEPHRECTOMY.‡
I/V HORSE SERUM + STREPTOCOCCI.
I/V GLOBULIN+STREPTOCOCCI.
I/M HORSE SERUM IN FREUND'S ADJ.
I/M GLOBULIN IN FREUND'S ADJ.
I/M STREPTOCOCCI IN FREUND'S ADJ.
I/V HORSE SERUM-11 DAYS
I/V HORSE SERUM & COLD
I/V HORSE SERUM & ACTH
I/M GLOBULIN IN FREUND'S ADJ.
I/M GLOBULIN IN FREUND'S ADJ. & CORTISONE
I/V ALBUMIN- 23 DAYS
I/V ALBUMIN- 9 DAYS
I/V ALBUMIN-9 DAYS + NEPHRECTOMY
I/V GLOBULIN- NON-NEPHRECTOMIZED
I/V HORSE SERUM-20 ML/KILO
1/7
10/77
4/17
2/10
1/14
0/7
2/8
0/16
1/11
1/10
1/10
1/11
2/16
1/6
1/11
2/8
VALVE RING LESIONS

Fig. 27.

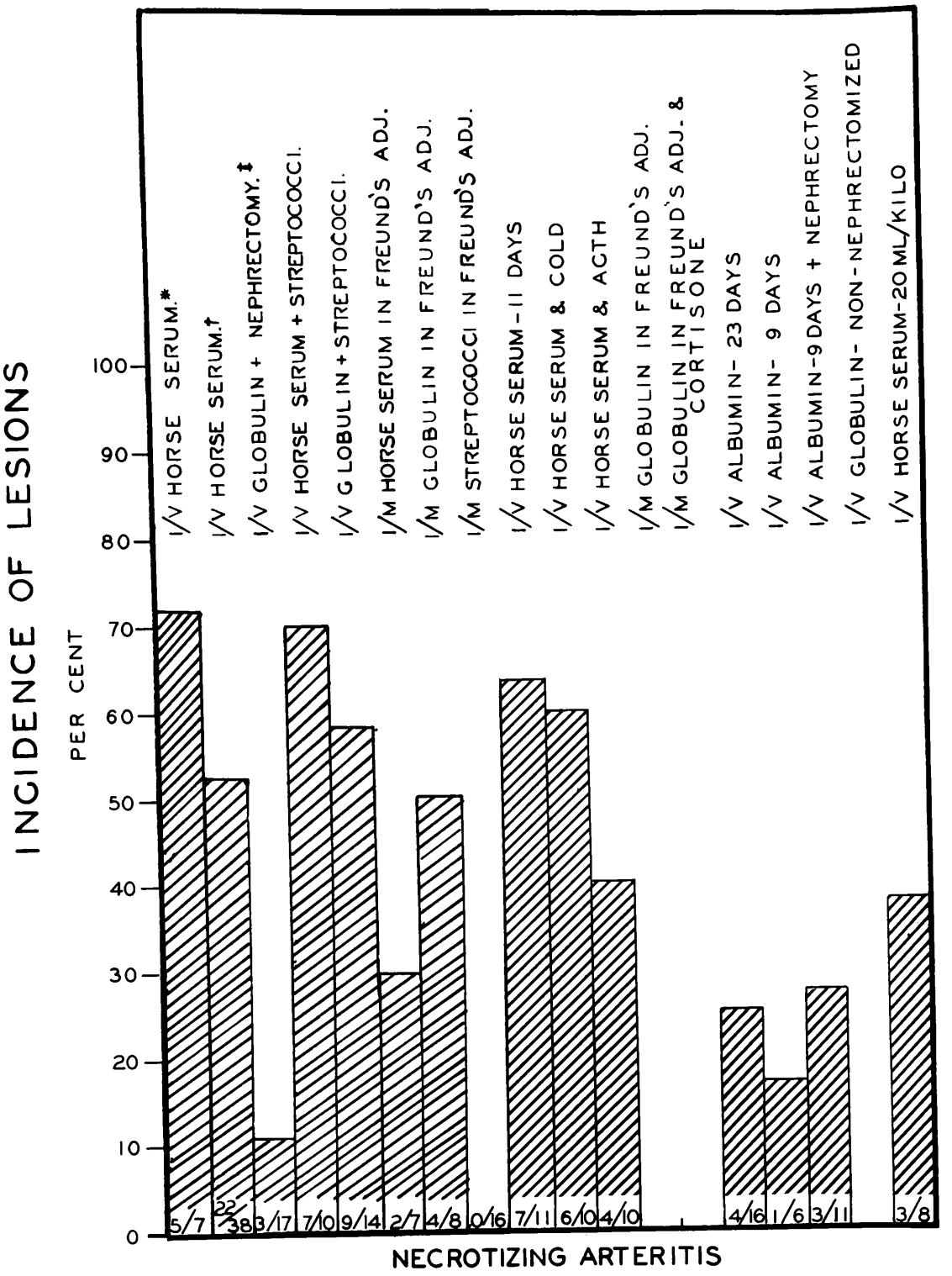

INCIDENCE OF LESIONS
PER CENT
100
90
80
70
60
50
40
30
20
10
0
I/V HORSE SERUM.*
I/V HORSE SERUM.†
I/V GLOBULIN + NEPHRECTOMY.‡
I/V HORSE SERUM + STREPTOCOCCI.
I/V GLOBULIN + STREPTOCOCCI.
I/M HORSE SERUM IN FREUND'S ADJ.
I/M GLOBULIN IN FREUND'S ADJ.
I/M STREPTOCOCCI IN FREUND'S ADJ.
I/V HORSE SERUM-11 DAYS
I/V HORSE SERUM & COLD
I/V HORSE SERUM & ACTH
I/M GLOBULIN IN FREUND'S ADJ.
I/M GLOBULIN IN FREUND'S ADJ. & CORTISONE
I/V ALBUMIN- 23 DAYS
I/V ALBUMIN- 9 DAYS
I/V ALBUMIN-9 DAYS + NEPHRECTOMY
I/V GLOBULIN- NON-NEPHRECTOMIZED
I/V HORSE SERUM-20 ML/KILO
5/7
22/38
3/17
7/10
9/14
2/7
4/8
0/16
7/11
6/10
4/10
4/16
1/6
3/11
3/8
NECROTIZING ARTERITIS

Fig. 28.

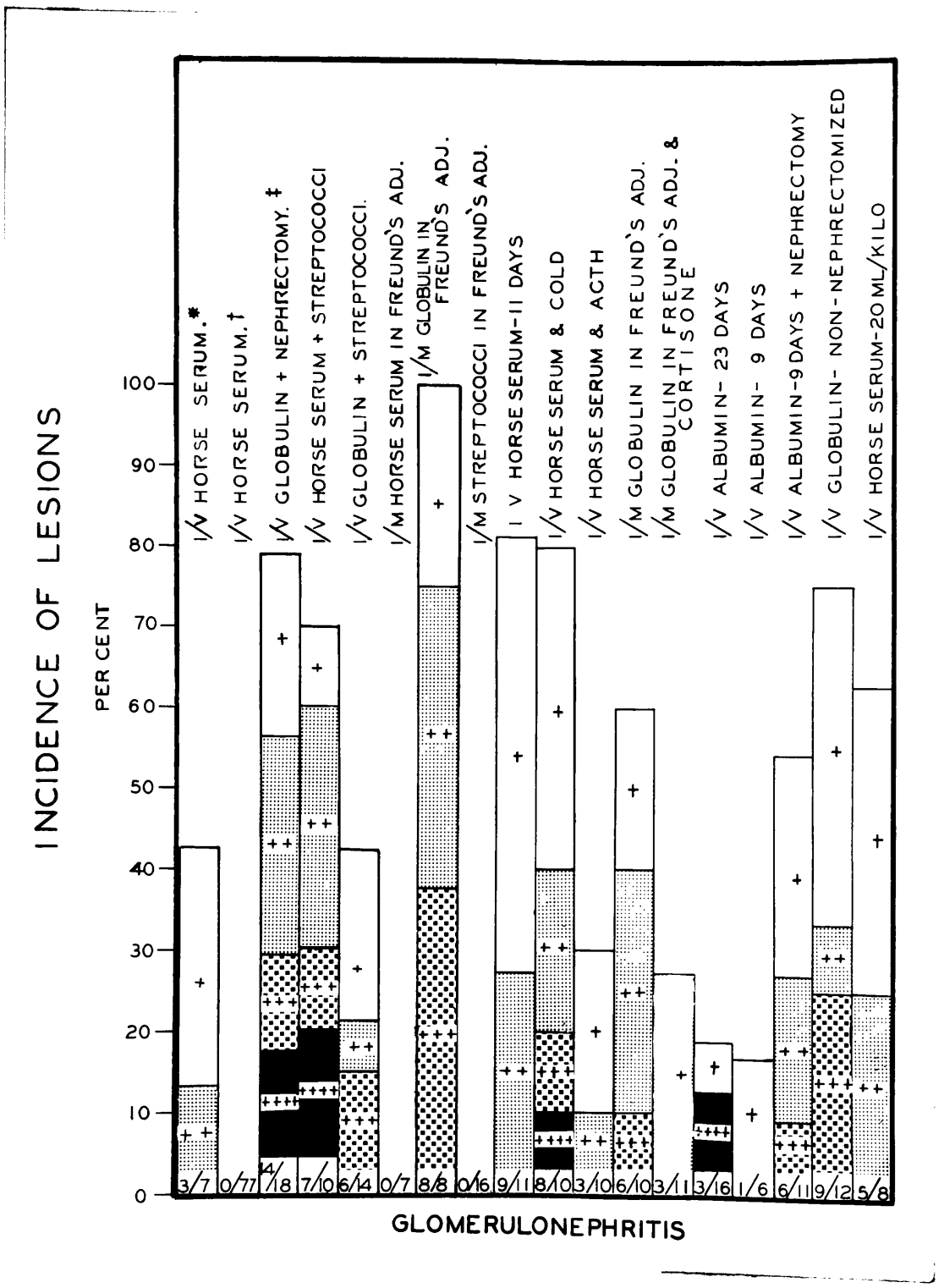

INCIDENCE OF LESIONS
PER CENT
100
90
80
70
60
50
40
30
20
10
0
I/V HORSE SERUM.*
I/V HORSE SERUM.†
I/V GLOBULIN + NEPHRECTOMY.‡
I/V HORSE SERUM + STREPTOCOCCI
I/V GLOBULIN + STREPTOCOCCI.
I/M HORSE SERUM IN FREUND'S ADJ.
I/M GLOBULIN IN FREUND'S ADJ.
I/M STREPTOCOCCI IN FREUND'S ADJ.
I V HORSE SERUM-II DAYS
I/V HORSE SERUM & COLD
I/V HORSE SERUM & ACTH
I/M GLOBULIN IN FREUND'S ADJ.
I/M GLOBULIN IN FREUND'S ADJ. & CORTISONE
I/V ALBUMIN- 23 DAYS
I/V ALBUMIN- 9 DAYS
I/V ALBUMIN-9 DAYS + NEPHRECTOMY
I/V GLOBULIN- NON-NEPHRECTOMIZED
I/V HORSE SERUM-20 ML/KILO
3/7
0/77
14/18
7/10
6/14
0/7
8/8
0/16
9/11
8/10
3/10
6/10
3/11
3/16
1/6
6/11
9/12
5/8
GLOMERULONEPHRITIS

Fig. 29.

TABLE 3.

RECTAL TEMPERATURES IN DEGREES FAHRENHEIT OF RABBITS IN EXPERIMENT 1.

	Day of Experiment																			Lesions		
	-3	-2	-1	0	1	2	3	4	5	6	7	10	12	14	17	18	19	20	22	Valve	Artery	Kidney
					Cold Room																	
								i/v HS I								i/v Desens.			i/v HS II			
B-27	103.2	102.8	101.4	103.2	102.5	100.8	101.3	102.2	103.2	103.4	102.4	100.6	102.7	102.9	101.6	100.4	108.2	102.	104.	+	0	++
D-3	101.6	102.2	103.	102.1	103.2	103.	103.4	103.	100.5	100.4	97.	Died								0	0	0
D-4	101.4	102.8	102.6	102.5	103.5	102.6	103.5	103.2	103.7	102.2	102.6	103.6	103.	103.6	101.6	103.	103.2	103.7	104.	+	+	++
C-72	101.6	101.8	100.6	102.	102.5	99.6	102.4	100.8	103.	101.2	101.	100.	103.	102.5	100.8	102.6	102.2	102.4	104.	+	+	+
D-5	103.	103.	102.4	103.3	103.6	102.2	102.4	103.1	103.4	103.5	103.	101.5	102.8	103.2	102.2	103.	103.2	103.	104.	+	++	++
D-6	103.	102.6	102.6	100.4	103.5	100.8	102.9	102.5	103.5	103.4	101.4	101.8	103.5	103.3	102.	102.4	102.4	103.8	103.7	+++	++	++
D-8	103.4	102.	102.7	102.8	104.4	102.4	102.3	104.4	104.1	103.9	102.	102.6	103.4	103.7	104.	103.5	103.5	101.2	104.3	++	++	+++
D-10	102.6	103.4	102.6	103.2	103.1	101.9	104.1	104.	103.7	102.3	103.	102.6	100.7	Died						0	0	0
C-81	104.	101.6	103.8	104.	103.9	101.5	102.6	103.3	103.9	103.5	100.	101.8	104.	103.2	102.4	103.	101.	103.	104.5	0	0	+++
C-74		101.4	102.3	103.3	103.2	101.6	102.2	103.3	103.4	102.9	101.	Sick	Died							0	0	0
C-22	101.8	102.2	101.6	104.	103.4	101.	101.5	103.5	102.8	102.8	102.4	100.	102.5	Died						0	0	0
C-25	101.	101.4	101.6	103.	102.9	100.9	102.2	105.	103.4	103.2	101.6	101.2	104.	102.5	101.6	103.	103.	103.8	103.8	++	+	+
C-26	101.6	102.2	102.	102.6	101.4	100.6	100.6	104.2	102.5	102.8	102.4	101.4	103.8	104.	100.8	102.9	103.	102.6	105.2	++	0	+
C-76	102.	102.4	102.2	103.6	102.9	106.	103.5	104.	103.4	103.7	102.4	103.2	103.2	103.5	101.2	102.5	103.2	102.6	102.6	++	+	+
C-73		101.8	100.6	102.9	102.4	100.4	100.6	103.2	103.6	102.8	103.6	101.	102.8	102.6	101.2	103.	102.8	103.4	102.9	+	0	+
B-10	101.8	101.6	101.9	103.	101.5	100.8	102.	101.4		104.5	102.2	101.6	103.4	102.	101.6	103.2	103.6	103.2	103.5	0	0	0
Controls																						
D-2	103.2	102.6	102.2	103.	103.4	102.	104.	103.5	103.	104.	102.4	102.	101.4	102.6	102.2	103.4	103.2	103.6	103.5	0	0	0
D-7	103.	104.	102.6	104.	103.8	101.	103.6	102.5	104.	103.8	102.8	101.8	103.1	103.	102.6	103.	103.5	103.4	103.	0	0	0
D-9	102.8	103.	102.4	103.3	102.4	101.2	101.7	104.	104.4	103.8	103.	102.8	103.8	103.6	100.8	103.5	104.6	103.	103.	0	0	0
C-80	100.6	101.8	101.2	102.8	102.6	100.4	102.8	102.2	103.	102.8	101.2	101.5	102.9	102.5	101.	103.	102.8	103.	103.4	0	0	0
C-77	101.8	102.2	100.8	102.6	102.5	100.3	101.8	103.6	102.3	103.6	101.4	101.4	103.5	103.5	101.	102.	103.	103.4	102.8	0	0	0
B-8	101.8	102.4	100.9	102.5	102.1	101.1	101.5	103.2	104.3	102.5	101.2	101.	101.7	102.8	99.8	102.2	102.5	103.6	102.4	0	0	0

One further interesting fact was that in addition to the occurrence of a high incidence of glomerulonephritis, the severity of the glomerulonephritis was such as had never been seen before resulting from two injections of horse serum alone in this laboratory (see Fig.29). Indeed, glomerulonephritis reported as a result of horse serum injections to intact animals[7,11,47] has always been of the mild type graded by us as + or ++. The more severe grades of glomerulonephritis had been previously reported to result from horse serum only when the animals had been unilaterally nephrectomized before the experiment[19,118], after repeated small injections over a long period of time[1,118], or combined with killed and dried haemolytic streptococci[47].

The results of the body temperature determinations are presented in Table 3. It may be seen from this Table that the range of normal temperature in rabbits is from 100.6 to 104.°F. and that there was no significant rise or lowering of the body temperature in relation to exposure to cold, or to the serum injections. Exceptional readings of 105. and 106. degrees were recorded, but these did not bear any relationship to the treatment. Furthermore, there was no alteration in body temperature that could be correlated with the occurrence of lesions of the heart. The question of the normal body temperature in rabbits was reviewed by Hagebusch[201], who noted a great discrepancy in the literature where single temperatures are frequently reported as the normal, ranging from 101.6 to 104.°F. He made 258 readings on 6 rabbits over a period of 21 days and found the range of normal to be 101.3 to 104.°F.

These figures correspond fairly well with our own, but a considerable number of our readings were below 101.°F. Hagebusch also reports that 53% of his rabbits treated with horse serum manifested a rise in temperature. However, this effect was not noted in our experiments. Whether it was masked by the cold or not is not clear from this experiment.

The structure of the lesions of the animals that survived the whole experiment was similar to that seen when horse serum was given at room temperature(10), and may be seen in the appropriate portions of the section on the morphological aspects of the lesions. The lesions of the heart did not bear any closer resemblance to those of rheumatic carditis than those previously observed, and the glomerulonephritis was of the extracapillary variety.

No lesions appeared in the hearts, arteries or kidneys of the control animals that survived the experiment. The lesions observed in the animals dying spontaneously in the cold room will be discussed with the results of Experiment III.

In summary, it appeared from this experiment that placing animals in a cold room (0 to -20°C.) with their backs shaved while they were being treated with two injections of horse serum at a 17 day interval, intensified the lesions of the heart valve and induced the lesions to occur in a high incidence. The lesions of the valve rings and of the arteries were not increased in incidence over those seen in animals treated at room temperature. Glomerulonephritis was produced in high incidence and in a severity not seen when two injections of horse serum are given unless the animals were first

unilaterally nephrectomized[19,118], or the antigen combined with killed streptococci[47]. No effect on the body temperature referable to the exposure to cold or to the injection of horse serum was evident in this experiment. This intensification of the lesions and high incidence of valvulitis and glomerulonephritis induced by exposure to cold led us to carry out many of the experiments, designed to study the pathogenesis of the lesions, in the cold room.

Experiment 2.

Studies in the pathogenesis of lesions of the heart, arteries and kidneys, produced in rabbits by two massive injections of horse serum.

Experiment 3.

Studies on the influence of cold in the pathogenesis of lesions produced in rabbits by two massive injections of foreign serum proteins.

Experiment 4.

The effect of ACTH on the lesions induced in rabbits by two massive injections of horse serum while in a cold environment.

Experiment 5.

The effect of Cortisone on the lesions produced in rabbits by two intramuscular injections of bovine gamma globulin in Freund's adjuvant.

Introduction:

As has been previously indicated, the factors in the pathogenesis of interest to us are the role of the state of hypersensitivity and the role

of the adrenal-pituitary mechanism. All four experiments listed above were designed to elucidate aspects of these factors.

It has been previously noted that in all the reported experiments in which animals have been anaphylactically sensitized to large doses of foreign serum proteins, lesions have failed to occur in some of the treated animals. One obvious mode of study of the factors involved, therefore, is to observe the differences between the reactions of those having lesions and those not evidencing them. Prior to the commencement of the present experiments, such observations had been attempted but, except in a few instances(128-130,135) where attempts had been made to inhibit the lesions, no correlation of the state of hypersensitivity with the occurrence of lesions had been observed. In the studies where a correlation was shown to exist between the state of hypersensitivity and the occurrence of lesions, this demonstration was made by demonstrating a decreased production of precipitins(128,129), the failure to develop a typical drop in complement titer(130), or the inactivation of the antigen-antibody mechanism in animals not developing lesions. In some instances(128-130) it was not clearly indicated that the treatment employed to inhibit the lesions had been the effective agent in producing the diminution of antibody response, but the diminished antibody production appears to have occurred in association with the absence of lesions. Further evidence would have been provided in favour of the hypothesis, that hypersensitivity was important in the pathogenesis of the lesions, could a statistically significant inhibition of the lesions have been effected in association with a reduction (or accentuation) in the

intensity of the state of hypersensitivity. However, statistically significant results of this type have been difficult to obtain because the methods employed to produce the lesions failed to result in a high enough incidence of lesions to make an inhibiting effect observable in experiments of practicable size. While it was our intention at all times to study the differences between animals with and without lesions, the results of Experiment 1 led to the hope that a method existed whereby a high incidence of lesions could be obtained. This impression was confirmed by the experiments of Drs. Waugh and More utilizing two massive injections of bovine gamma globulin in the cold room[132]. Accordingly, Experiments 3 and 4 were carried out in the cold room. Experiment 2, performed simultaneously with 3 and 4 served as a control for untreated animals and those treated with horse serum at room temperature, and also was designed to be a study of the differences between animals with and without experimentally-induced lesions.

When first planned, and as first attempted, Experiment 5 was conducted in the cold room in exactly the same manner as Experiment 4, but the mortality from Cortisone was so great in the cold room that the experiment had to be abandoned. An alternative method of obtaining a high incidence of lesions - in this case of the kidneys[47] - was adopted, namely, that of incorporating bovine gamma globulin in Freund's adjuvant[46,47, 133].

The methods chosen to inhibit the lesions - for reasons already discussed in the role of the adreno-hypophyseal mechanism - ACTH and

Cortisone were chosen. Although the originality of this idea may be challenged at this time, when these experiments were planned during the latter half of 1949, no publications had appeared dealing with a similar study.

The evaluation of the intensity of the state of hypersensitivity obviously requires quantitative methods. Thus far, where skin tests were observed, a single dose of the test antigen was given. Such studies have not been productive of any correlation with the development of the lesions of the heart, arteries or kidneys. In the present experiments graded dilutions of the antigens were used, and the reactions carefully measured and charted. In the studies where correlation of the antibody titer and the development of lesions was indicated (see above), the methods of determination of the antibody titers were refined and accurate. Other studies utilizing ring tests and precipitin tests of doubtful value were not productive of any positive correlations. In these experiments ring tests were used to detect the presence or absence of antibodies only. For this purpose, they are very sensitive. However, further evaluation of the amount of antibody present should be done by accurate quantitative techniques. Since the latter are complicated and time-consuming, the results are not yet available for discussion.

To evaluate the role of the adreno-hypophyseal mechanism certain changes reported by Selye and his group to be associated with the General Adaptation Syndrome have been selected for evaluation with respect to the occurrence of lesions in our animals. They are the changes

in adrenal weight[202], thymus weight[202], and the pituitary weight. Variations in the blood sugar as a response to non-specific stress has also been described[207,208], and this will be evaluated in relation to our lesions. Furthermore, the influence of ACTH and Cortisone on the incidence of the lesions produced by foreign serum proteins will be evaluated in terms of the influence of the adreno-hypophyseal mechanism in the pathogenesis of our lesions.

Experimental procedures in Experiments 2, 3 and 4:

These three experiments are considered together since they were performed at the same time and the groups that compose them serve as controls for one another. Thus, the groups included in Experiment 1 are untreated normal animals kept at ordinary room temperature, Group I, and animals given two intravenous doses of horse serum while at ordinary room temperatures, Group II. In Experiment 2, it is necessary to consider the two groups of Experiment 1, and two further groups:- animals kept in the cold room but otherwise untreated, Group III, and animals given two intravenous injections of horse serum while in the cold room, Group IV. In Experiment 3, the groups outlined above are compared with the group given ACTH daily in addition to the two intravenous injections of horse serum while in the cold room, Group V.

These experiments were conducted in small groups in the manner outlined below. Two or three animals in each of the five groups were treated simultaneously, and the whole experiment carried out. After the animals were killed and autopsied. The experiments were repeated until a sufficient number of animals was available in each group so that the results

could be studied. Thus, although all the animals in any given group were not studied simultaneously, each portion of the group had corresponding controls in all the other groups, so that the composition of every group was comparable with that of every other one. While attempts were made to carry out each section of the experiment in an identical manner, minor variations inevitably crept in that were, however, represented in all the groups. For instance, in one portion of the experiment, frequent large bleedings resulted in a high mortality, but this mortality from the effect of bleeding was equally represented in all the groups under study. The total duration of the actual experimental procedures was from March to December 1950 (July and August excluded). The differences in climatic conditions of the seasons was also represented equally in all the groups. It has, therefore, been considered justifiable to regard the groups as comparable and homogeneous and the results are presented accordingly.

The general plan of Experiments 2, 3 and 4 is indicated below.

Group I Untreated room temperature controls.

Group II Horse serum at room temperature.

Group III Untreated cold room controls.

Group IV Horse serum in the cold room.

Group V ACTH and horse serum in the cold room.

Procedures.

Day of Experiment	
-3	Body weight. Bleeding A (12 c.c.) Animals placed in metabolism cages.
-2	Urine specimen A collected and stored (Urine A).
-1	
0	
1	ACTH injections 20 mg. per day in 2 divided doses begun for Group V and continued throughout the experiment.
2	Bleeding B (12 c.c.) Urine B.
3	
4	Groups III, IV and V placed in the cold room.
5	Bleeding C (12 c.c.) Urine C.
6	
7	Massive intravenous injection of horse serum 10 c.c. per kilo of body weight to Groups II, IV and V. Body weight.
8	Bleeding D (12 c.c.) Urine D.
9	
10	Bleeding D_1.
11	Bleeding D_2.
12	Bleeding D_3.
13	Bleeding E (12 c.c.) Urine E.
14	Bleeding E_1.
15	Bleeding E_2.
16	Bleeding F (12 c.c.) Urine F.

Bleedings with subscript number were optional in different portions of the experiment; 1 c.c. for ring test.

Procedures (Cont'd.)

Day of Experiment	
17	Desensitizing dose, intravenous injection of 10 c.c. of 1:10 dilution of horse serum to Groups II, IV and V. Body weight.
18	Massive intravenous injection of horse serum to Groups II, IV and V.
19	Bleeding G (12 c.c.) Urine G.
20	
21	
22	
23	Intradermal injections given.
24	Skin reactions read.
25	Skin reactions read. Half the animals killed and autopsied. Bleeding H, Urine H.
26	Half the animals killed and autopsied. Bleeding H, Urine H.

Daily determinations were made of food and water intake and urinary output. Complete urinalysis was performed daily.

The animals comprising the experimental groups were:-

Group I K-38, K-40, L-3, L-17, L-19, L-43, L-48, L-55, N-85, N-91, O-74.

Group II K-29, K-27, L-7, L-8, L-12, L-46, L-47, L-61, N-83, N-95, O-58, O-55.

Group III K-28, K-31, L-2, L-4, L-16, L-52, L-63, N-86, N-9[illegible], N-93, O-42, O-48, O-51, O-63, O-83.

Group IV K-26, K-32, L-5, L-6, L-13, L-51, L-57, L-64, N-79, N-82, N-92.

Group V K-30, K-36, L-1, L-18, L-20, L-44, L-49, L-67, N-80, N-96, N-97, O-54, O-43, O-66, O-53, O-76, O-80.

Experimental procedure in Experiment 5:

The results of Experiment 5 will be considered together with those of Experiments 2, 3 and 4, since the morphological and metabolic studies were the same although the experimental plan, outlined below, was somewhat different.

Twenty-two rabbits were used, of which 10 were designated as controls (Group VIII) and 12 were treated with Cortisone (Group IX) throughout the course of the experiment. The control animals received two intramuscular injections consisting of 1 gram of bovine gamma globulin incorporated in Freund's adjuvant. The globulin was first suspended in saline and the suspension incorporated in Falba then combined with paraffin oil containing 2.5 mg. of M. tuberculosis in a manner already described in detail in publications from this laboratory(47), and by the originators of the method(133). The injections were given into the thigh muscles on the 4th and 14th day of the experiment. Cortisone was given to Group IX from day 1 to the end of the experiment. A control period of detailed observation was included in the experiment of which the general plan is outlined in the following summary:-

General plan of Experiment 5.

Group VIII Bovine gamma globulin in Freund's adjuvant.

Group IX Bovine gamma globulin in Freund's adjuvant and Cortisone.

Day of Experiment		
-3	Animals placed in metabolism cages. Body weight. Bleeding A (12 c.c.)	
-2	Urine sample collected and stored (Urine A).	
-1		
0		
1	Cortisone injections begun to Group IX, 20 mg. per day in two divided doses.	
2	Bleeding B. Urine B.	
3		
4	First intramuscular injection of bovine gamma globulin in Freund's adjuvant (1 gm. globulin per kilo of body weight). Body weight.	
5	Bleeding C. Urine C.	
6		
7	Bleeding C_1.	Bleedings with subscripts were of 1 c.c. of blood taken for ring tests.
8	Bleeding C_2.	
9	Bleeding C_3.	
10	Bleeding C_4. Urine D.	
11	Bleeding C_5.	
12	Bleeding C_6.	
13		
14	Second adjuvant globulin injection. Body weight.	
15	Bleeding D (22 c.c.) Urine E.	
16		

Day of Experiment	
17	
18	
19	Intradermal injections given.
20	Skin reactions read.
21	Skin reactions read.
22	Skin reactions read.
23	Bleeding E. Urine E. Killed and autopsied half the animals.
24	Bleeding E. Urine E. Killed and autopsied half the animals.

Daily determinations were made of the food and water intake and urinary output. Urinalysis was performed daily.

The animals comprising the experimental groups were:-

Group VIII. 0-46, 0-50, 0-52, 0-64, 0-67, 0-69, 0-71, 0-79, 0-81, 0-82.

Group IX. 0-44, 0-47, 0-49, 0-56, 0-59, 0-60, 0-61, 0-65, 0-68, 0-70, 0-73, 0-78.

Results of Experiments 2, 3, 4 and 5.

Tissue lesions:

Tissue lesions appeared in all the treated groups of animals, but were absent in the untreated, warm room controls and in the untreated cold room controls. The incidence of the lesions occurring in each group is indicated in Table 4. The lesions of the heart, arteries and kidneys were similar to those described in the first section of this treatise, with the exception of certain lesions of the kidney that occurred in Group IX.

TABLE 4.

INCIDENCE OF MORPHOLOGICAL LESIONS IN EXPERIMENTS 2, 3, 4 and 5.

Group	Category	No. of animals	Valvulitis	Valve ring lesions	Necrotizing arteritis.	Glomerulonephritis / & //	Glomerulonephritis /// & ////	Total
I	Died before end of experiment	1	0	0	0	0	0	0
	Killed at end of experiment	10	0	0	0	0	0	0
II	Died after 1st. injection	2	1 (50%)	0	1 (50%)	0	0	0
	Died after 2nd. injection	0	0	0	0	0	0	0
	Killed at end of experiment	11	7 (63.5%)	1 (9.09%)	7 (63.5%)	8 (72.7%)	1 (9.09%)	9 (81.8%)
III	Died before end of experiment	9	0	0	0	0	0	0
	Killed at end of experiment	13	0	0	0	0	0	0
IV	Died after 1st. injection	1	0	0	0	0	0	0
	Died after 2nd. injection	0	0	0	0	0	0	0
	Killed at end of experiment	10	6 (60%)	1 (10%)	6 (60%)	6 (60%)	2 (20%)	8 (80%)
V	Died after 1st. injection	3	0	0	0	0	0	0
	Died after 2nd. injection	4	0	0	0	1	0	0
	Killed at end of experiment	10	3 (30%) /21.3/	1 (10%) /7.1/	4 (40%) /28.5/	3 (30%) /28.4/	0	3 (30%)
VIII	Died after 1st. injection	0	0	0	0	0	0	0
	Died after 2nd. injection	0	0	0	0	0	0	0
	Killed at end of experiment	10	1 (10%)	1 (10%)	1 (10%)	5 (50%)	1 (10%)	6 (60%)
IX	Died after 1st injection	1	0	0	0	0	0	0
	Died after 2nd. injection	6	0	0	0	0	0	0
	Killed at end of experiment	5	0	1	0	3 (60%) /27.2/	0	3 (60%)

/ / = % applies to animals killed or died after 2nd. injection.

It is of interest that no lesions appeared in the hearts or kidneys of animals exposed to cold that survived the whole experimental period (Group III). This result is confirmed by other experiments performed in this laboratory(134), and by the experience with the controls to Experiment 1. However, placing the animals in the cold with their backs shaved for a month was attended by a considerable mortality (9/22 = 40.9%). The animals that died of the effects of cold often manifested an ulcerative duodenitis which sometimes perforated and caused fibrinous peritonitis (Fig.30).

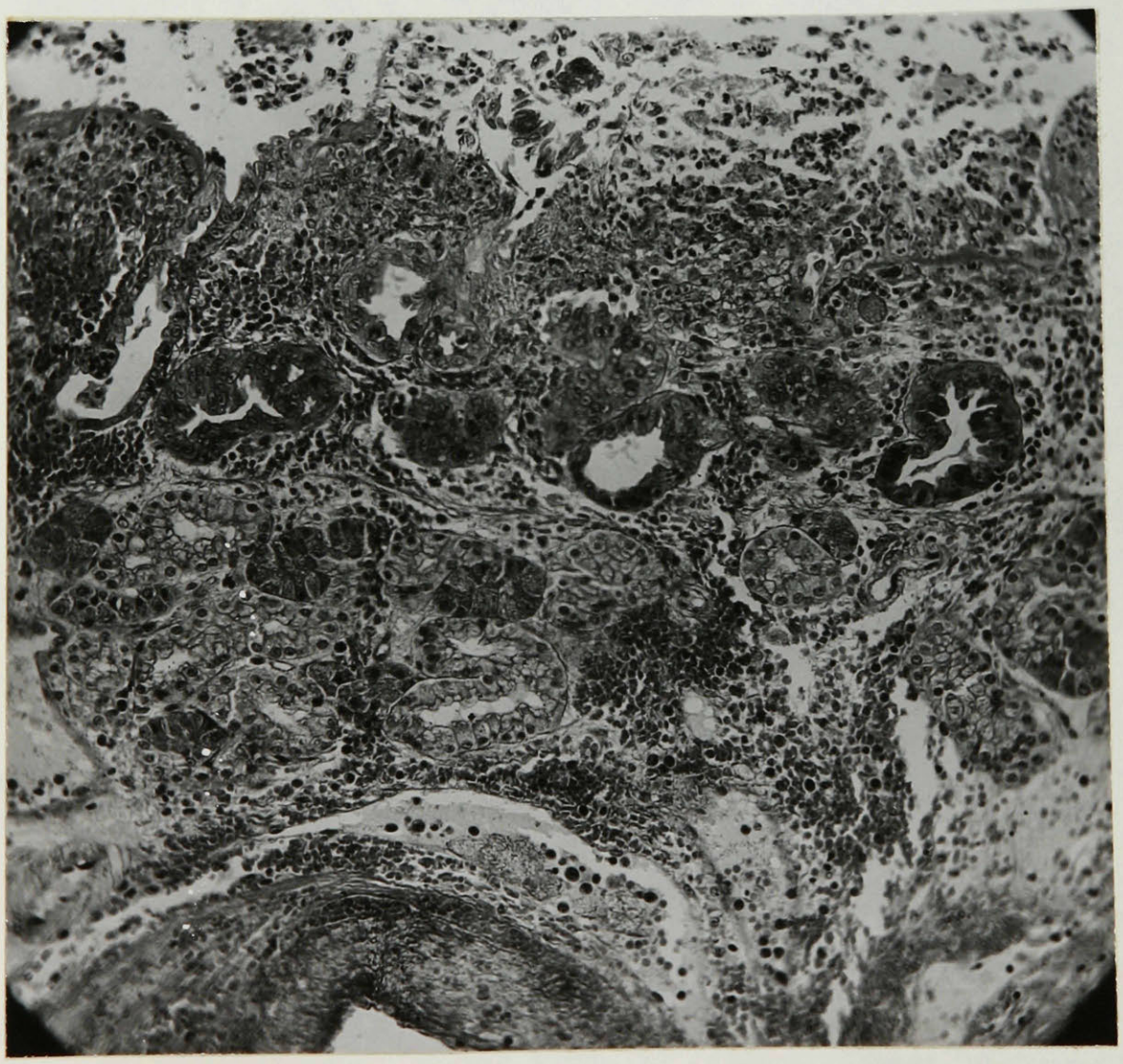


Fig.30. Section through a portion of the duodenum of rabbit C-74 that died after 11 days of exposure to cold. Ulceration of the mucosa, the fibrinopurulent and necrotic exudate is seen in the base of the ulcer. The wall is oedematous and infiltrated with polymorphonuclear leukocytes. A small portion of the serosa is shown at the bottom of the picture, covered with fibrinous exudate.

In some of the animals dying of exposure to cold, the kidneys sometimes showed vacuolization of the convoluted tubules, but the glomeruli, arteries and arterioles of the kidneys showed no morphological evidence of damage (Fig.31).

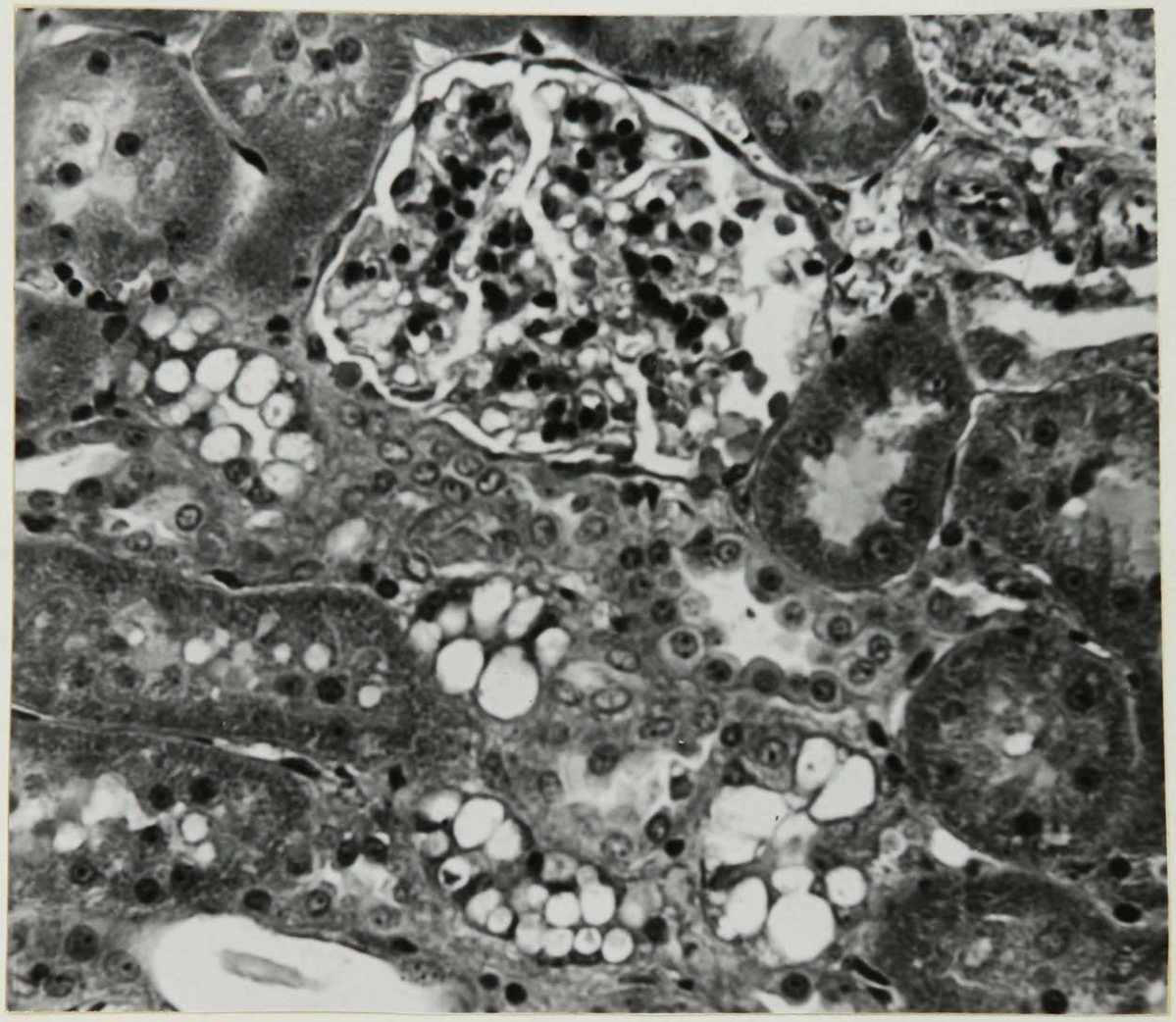


Fig. 31. Vacuolization of renal tubules of control animal B-8 (Experiment 1) after prolonged exposure to cold. Note the normality of the glomerulus and arterioles, upper right.

The animals treated with horse serum in the cold, Group IV, did not manifest any significant difference in incidence of the lesions of heart or kidneys from Group II treated with horse serum at room temperature, nor were the lesions of the kidney significantly more severe. This was at

variance with the results of Experiment 1, and vitiated the hope held at the beginning of the experiments that the increase in incidence and severity of the lesions would occur consistently as a result of exposure to cold.

In comparing the results obtained in Groups II and IV, it is of interest that the high incidence of glomerulonephritis that occurred with horse serum at room temperature (Group II) is at variance with previous experience in this laboratory (Fig.29). This may, perhaps, be explained by the fact that while it had always been our practice in the past to administer the horse serum in the same dosage at an interval of 16 to 17 days, in the present experiments the horse serum was given at an interval of 11 days. Thus, shortening the interval between injections may exert some effect on producing a high incidence of glomerulonephritis. McLean[118] has noted such an effect of the time interval of injection. However, when the animals were exposed to cold, those injected at the 17-day interval (Experiment 1), manifested more severe lesions in high incidence than those injected at the 11-day interval (Group IV). These results are inexplicable at this stage of the discussion, and they may be an expression of the variability in response between individual animals and groups observed so frequently in this type of experiment. It is also possible that the improvements in histological technique in the laboratory during the past two years allowed of more accurate evaluation of the kidney lesions, since glomerulonephritis had been detected in animals treated with two injections of horse serum at the 17 day interval in 1950[47] but not in 1948[10] (see also Fig.29).

Re-evaluation of the earlier material may now be in order.

The animals receiving ACTH in addition to horse serum in the cold room, Group V, showed a small incidence of all the lesions in which we are interested, as may be seen from Table 4. When compared with the incidence of lesions of the appropriate control group (Group IV, cold and horse serum), it is evident that there can be no question of a significant difference in incidence between the lesions of the hearts and arteries of both groups. In the case of the kidney lesions, however, there is a considerable incidence. Applying the results to fourfold contingency tables for equal samples[123] shows that the P value is .0349 where the level of significance is a value less than .025, and the value for highly significant is less than .005. With these numbers of animals and an 8/10 incidence in the control group, it would require that no animals in the ACTH series show lesions before the result could be regarded as statistically significant. However, the glomerular lesions were of milder intensity in the ACTH-treated group, and taken together, the severity and incidence indicate a probable inhibiting effect of ACTH.

The results of this study have been subjected to the criticism that the ACTH was inadequately administered, having been given only twice daily instead of four times[203]. This possibility is admitted. However, it is evident from detailed analysis of the work of those who have given the hormone four times daily[185,187] that lesions of arteries and valves can develop under ACTH treatment, and that it may only be a question of additional animals to demonstrate significance. The small number of animals

used in the experiment was the result of a considerable mortality, limited capacity of the cold room and the large number of observations being made on the animals. On the other hand, the method of giving a single injection used by these authors[185,187] is not a reliable method of producing necrotizing arteritis in our experience.

It is also exceptional in our laboratory to obtain an incidence of arteritis exceeding 72%, even when two injections are given and adjuvants are used to increase the incidence (c.f. Fig.28). It is, therefore, difficult to accept the results without seeing the actual lesions that were counted. It may be that some of the lesions were those described by us as "spontaneous"[47]. The question of the adequacy of this experiment will also be discussed in connection with other results. Nevertheless, it is obviously necessary to meet these objections to the mode of administration by repeating the experiment giving the hormone four times daily. If the results are then similar to the ones of the present experiment, larger numbers of animals will have to be used. At the present time, however, the results of this experiment indicate that there is a possible inhibitory effect exerted on the lesions by ACTH. It should also be mentioned that under the conditions of the experiment the dosage of ACTH was at the maximum level of tolerance, as may be seen from the considerable mortality that was experienced in this group. This mortality may have been, in part, due to the exposure to cold, since the mortality in the cold room controls (Group III) was also high.

In the group of animals receiving bovine gamma globulin in Freund's adjuvant, six developed glomerulonephritis, and only one animal had lesions

of the valves and small arteries. The incidence of lesions was again rather lower than was expected from previous results(47 and Fig.29).

By comparison, the animals receiving Cortisone (Group IX) in addition to the bovine gamma globulin, also manifested lesions of the kidneys. Glomerulonephritis developed in three of the 11 that survived beyond the second globulin injection. Comparing the incidence of lesions merely as to the presence or absence of lesions reveals that the incidence is not statistically significant (fourfold contingency tables for unequal samples indicated that this incidence in Group IX would be statistically significant only if all the animals in the control group were to have manifested lesions). However, it is evident from perusal of Fig.29 and Table 4 that the severity of the lesions was much less severe in the Cortisone group than in the control group. Actually, this decreased severity was more marked than is indicated in Fig.29, for even though the lesions were graded as +, the cellular proliferation involved only portions of scattered glomeruli (Fig.32) which were difficult to find. On the whole, one must reach the conclusion that the lesions were less severe as well as fewer in the Cortisone animals than in the control. Although the glomerulonephritis can develop under Cortisone treatment, there appears to have been some inhibiting effect. Enlarging the series would help the statistical interpretation of the changes. However, the series are of the present size due to the number of observations attempted on the animals to date. Rich et al(188) reported complete suppression of glomerulonephritis with Cortisone. However, their method of producing the

lesion is unreliable. McLean et al[191] obtained results similar to ours.

The lesion that developed under Cortisone treatment that was not observed in animals not receiving this hormone, was the lesion illustrated in Fig.33. It consisted essentially of a dilatation of the capillary bed



Fig.32. Section of kidney from rabbit O-78 showing cellular proliferation of the glomerular tuft and dilatation of some of the capillaries. The tubule below shown marked vacuolization of the glycogen type. Trichrome X 400.



Fig.33. Glomerulus from rabbit O-47 showing the marked dilatation of the capillaries of the glomerulus and the relative acellularity. The tubules are normal in this animal. Trichrome X 400.

of the glomerulus, giving it the appearance of possessing a decreased number of endothelial and epithelial cells, and attenuation of the basement membranes.

In some instances, fragmentation and fibrillation of the basement membranes was noted, but without having controls treated with Cortisone alone, and since this effect on basement membranes is one that may be exerted by globulin and the ensuing glomerulonephritic process, the specificity of this aspect of the lesion to the Cortisone treatment cannot be evaluated. The incidence of these lesions is indicated in Table 5.

Table 5.

INCIDENCE OF CORTISONE LESIONS OF THE KIDNEY IN EXPERIMENT 5.

Mode of death	No. of animals	Number of animals with Cortisone lesions and percent.
Died after 1 globulin injection	1	1 (100%)
Died after 2 globulin injections	6	5 (83.6%)
Killed at end of experiment	5	4 (80%)
Total	12	10 (83.6%)

Although the fragmentation of the basement membrane was seen in animals not showing the proliferative changes by which the diagnosis of glomerulonephritis was made, and the basement membrane changes do not usually precede the cellular proliferation in experimental glomerulonephritis, it is still possible to conceive of a prevention by Cortisone of proliferation due to globulin without the effect on the basement membranes having been altered. This can be clarified easily by treating a series of animals with Cortisone alone. However, this was not done in the first instance because the objectives

of the study did not include a study of the effects of Cortisone per se nor was there enough Cortisone available for such a study. This aspect will be studied further in the future, since in addition to the effects on the renal glomerulus, a diabetic state with glycogen vacuolization of the beta cells of the islets of Langerhans of the pancreas was observed to result from administration of Cortisone, in this laboratory[205]. Thrombosis of capillaries of the glomerulus was not observed in these animals,as has been reported by others, to result from the administration of Cortisone[191].

On morphological grounds alone, the effect of ACTH and Cortisone on the lesions appears to indicate that secretions of the adrenal and pituitary glands exert some influence on the development of the lesions. However, whether this implies an essential role of these hormones in the pathogenesis of the lesions is a question that cannot be answered at this stage, and will be discussed again later. It is also of interest, in view of the contentions of Selye[156,157], that cold does not induce lesions in intact or unilaterally nephrectomized, but otherwise untreated, animals[134] if the animals survive the experimental period. His animals[213-215] treated with DCA were all unilaterally nephrectomized and given large amounts of sodium chloride at the same time. Furthermore, lesions of the kidney have been produced in animals by giving sodium chloride alone[216]. The essentiality of the hormone to the lesions and, by inference "stress", must be questioned. Certainly in our lesions sodium chloride intake or nephrectomy played no part, and it has been pointed out that there are no changes in sodium chloride levels in the blood that parallel the development of the lesions produced by foreign

serum proteins[217], nor are the lesions prevented by administration of ammonium chloride[217], as is the case in Selye's lesions[218]. Thus, it must be concluded that from consideration of the morphological aspects of the lesions, that the lesions produced by foreign serum proteins cannot be regarded as having a similar pathogenesis to those produced by Selye.

Summary:

Two intravenous injections of 10 c.c. of horse serum per kilo of body weight given at an interval of 11 days at room temperature were capable of inducing a mild glomerulonephritis. The incidence of cardiac, valvulitis and necrotizing arteritis was comparable to that seen when an interval of 16 to 17 days elapsed between injections.

Exposing animals to cold does not induce glomerulonephritis, arteritis or cardiac valvulitis. However, some animals dying spontaneously during the exposure to cold manifest an ulcerative duodenitis and degeneration of the kidney tubules.

Two intravenous injections of 10 c.c. of horse serum per kilo of body weight given to rabbits while in a cold environment does not increase the incidence of any of the lesions, but the glomerulonephritis induced in the cold appears to be more severe than that induced when the animals were maintained at ordinary room temperatures.

In Experiment 4 where 20 mg. of ACTH were given to the animals in two divided doses throughout the period of the experiment, the hormone failed to prevent the development of cardiac valvulitis or necrotizing arteritis. The glomerulonephritis that developed in these animals occurred in a smaller incidence, but the reduction in incidence alone was not

statistically significant. However, the lesions of the ACTH animals were also milder in intensity so that regarding both factors, severity and incidence, together, one may state that ACTH did exert some inhibitory effect on the lesions of the kidney. Larger numbers of animals are required to establish these results firmly. The question of the adequacy of the mode of administration of ACTH was discussed.

The results of Experiment 5 indicate that the glomerulonephritis was also milder in intensity and smaller in incidence in the Cortisone-treated group than in the control group. Again, larger numbers of animals are required to establish the inhibitory effect of Cortisone on these lesions beyond all question. A lesion peculiar to the Cortisone-treated animals was observed in the kidneys consisting of a marked dilatation of the glomerular capillaries, degeneration of renal tubules and fragmentation of the basement membranes.

Organ Weights.

When the animals used in this experiment were killed, the organs were removed, freed from blood clot and extraneous tissue and carefully weighed. The heart, kidney, thymus, spleen and liver were weighed to the nearest tenth of a gram, and the adrenal and pituitary to the nearest tenth of a milligram. The "carcass weight" refers to the body weight with the gastro-intestinal tract removed. The calculations of the organ to carcass weight ratios were made by expressing the carcass weight in a unit of weight that allowed the final value of the ratio to approximate unity and all the ratios to be compared on a single scale. However, this method introduces the factor of the weight of the animal. While it is quite logical to consider the weight of the animal as a measure of size in relation to the size of the organs to be considered, where abnormal loss of weight occurs the ratio would tend to be higher than in animals experiencing the ordinary gain of weight expected in normal animals. To overcome this objection, it would seem necessary to have some measurement of size other than weight, e.g. body length, femur length, so that one might calculate the expected weight of an animal for a gizen size or the expected weight of the organs for the weight, size and age. It would require large numbers of animals to accumulate the necessary data. The present methods were chosen since a study on a large number of rabbits comparing the organ weights to the carcass weight (net body weight) in normal animals had been made by Brown, Pearce and van Allen(209). These authors also established that obscure lesions of the organs did not influence the organ weights, unless the animal manifested evidence of

illness[210,211]. It appeared at the outset that it might be possible to use the figures of the above-quoted authors as a means of evaluating our experimental material. However, the average weight of many of the organs differed in our control series (see Tables 7 to 13), which may be due to a difference in the strains of rabbits, or to the fact that our experiments contained fewer animals.

It is interesting, in view of what is to follow, that these investigators were not a little troubled by the influence of the loss of body weight on the ratios, particularly with reference to the small organs.

Body weight changes in Experiments 2, 3, 4 and 5:

Since the problem of evaluating the influence of the loss of body weight, in the organ to carcass weight ratios, looms so large, it was considered desirable to analyse the changes in body weight before entering upon the consideration of the organ weights. The data regarding the body weight changes are summarized in Table 6.

It may be seen from Table 6 that in Group I (room temperature controls) all but one animal gained weight; this gain amounting to 29.9% of the weight at the beginning of the experiment. The animal that lost weight lost 3.3%. In Group II (horse serum at room temperature), the weight gain was similar to Group I; the weight loss in one animal was slightly greater. In Group II (cold controls), although seven animals lost weight, the percentage loss of weight was not much greater than in the first, and less than in the second group. The gain in weight was similar for the surviving animals. Group IV (cold and horse serum)

TABLE 6.

BODY WEIGHT CHANGES IN ANIMALS IN EXPERIMENTS 2, 3, 4 and 5.

Average weight:-	Group I animals	Group I Av.Wt. gm.	II animals	II Av.Wt. gm.	III animals	III Av.Wt. gm.	IV animals	IV Av.Wt. gm.	V animals	V Av.Wt. gm.	VIII animals	VIII Av.Wt. gm.	IX animals	IX Av.Wt. gm.
at start of all animals in group	11	2058.4	13	2044.2	16	2107.7	11	2071.1	17	2073.3	10	2211	12	2153.3
at end of all animals in group	11	2638.2	13	2448.5	16	2305.2	11	2297.9	17	2022.5	10	2541	12	1865.4
at end of all animals with wt. loss	1	1665	1	1355	7	1801	2	1530	11	1799.3	0	0	9	1688.8
Av. wt. loss		-68		-305		-303		-82.5		-239.5		0		-438.9
% wt. loss		3.3		14.9		9.6		4.0		11.5		0		20.4
% of all with lesions		(0)		(0)		(0)		(50)		(18.1)		(0)		(22.2)
at end of animals with wt. gain	10	2735.5	12	2539.6	9	2398	9	2468.3	6	2431.6	10	2541	3	2385
Av. wt. gain		617.0		609.7		700.2		278.5		304.6		330.2		155
% wt. gain		29.9		29.8		33.2		13.4		14.9		14.9		7.2
% of all with lesions		(0)		(83.3)		(0)		(87.5)		(66.6)		(60)		(66.6)
of carcass of all animals in group	11	2192.6		2051.1	16	1798.6	11	1854.8	17	1593.6	10	1916.7	12	1413.7
of carcass of animals with wt. loss	1	1435	1	1058	7	1436.7	2	1242.5	11	1421.5	0	0	9	132.8
of carcass of animals with wt. gain	10	2268.3	12	2133.9	9	2080.1	9	1990.9	6	1900.8	10	1916.7	3	1689.3

% wt. gain or loss computed as % of wt. at start.

showed a gain that was smaller, but a loss of weight that was within the range of the previous three groups. In Group V (cold, horse serum and ACTH), the loss of weight was approximately equal to that of the cold controls, but the gain in weight was equal to the cold and horse serum Group IV. In Group VIII there was no animal that lost weight, and the gain of the group was the same as that of Groups IV and V. Of all the experimental groups, the Cortisone

group (IX) showed the most marked loss of weight, and this loss occurred in a majority of the animals. The gain in weight was also the smallest observed in any group.

The carcass weight corresponded to the gain or loss of weight. In considering the animals that gained weight, a roughly descending scale in order of magnitude is observed from Group I to Group IX. The first six are within 16.236% of one another, while the last group is much lower and out of the range of the previous groups. It may be observed, by examining the data for the individual animals in Tables I to VII of Appendix B, that the animals that gained weight in the first six groups correspond, with exceptions, to those that survived the whole experiment. Thus, in considering the organ to carcass weight ratios, it is necessary to consider only the ratios of those that survived the experiment as valid expressions of changes in the organ weights (within 16.236%). In the last group, it will be necessary to consider the actual organ weights in comparison of those of the other groups. Thus, in all the groups, the organ weights will be analyzed in three ways, namely, as organ to carcass weight ratios; as average actual weights compared with average ratios, and as actual weights correlated with gain or loss of weight. It is hoped by these methods to arrive at a true estimate of the weight changes in the organs of the animals in these experiments.

Heart weight:

The distribution of the heart to carcass ratios correlated with lesions of the heart and kidneys is indicated in Fig.34. The distribution

of the ratios correlated with the lesions of the heart or kidneys, individually, may be seen in Text-figure I and II of Appendix A. However, they do not show any substantial difference from Fig.34

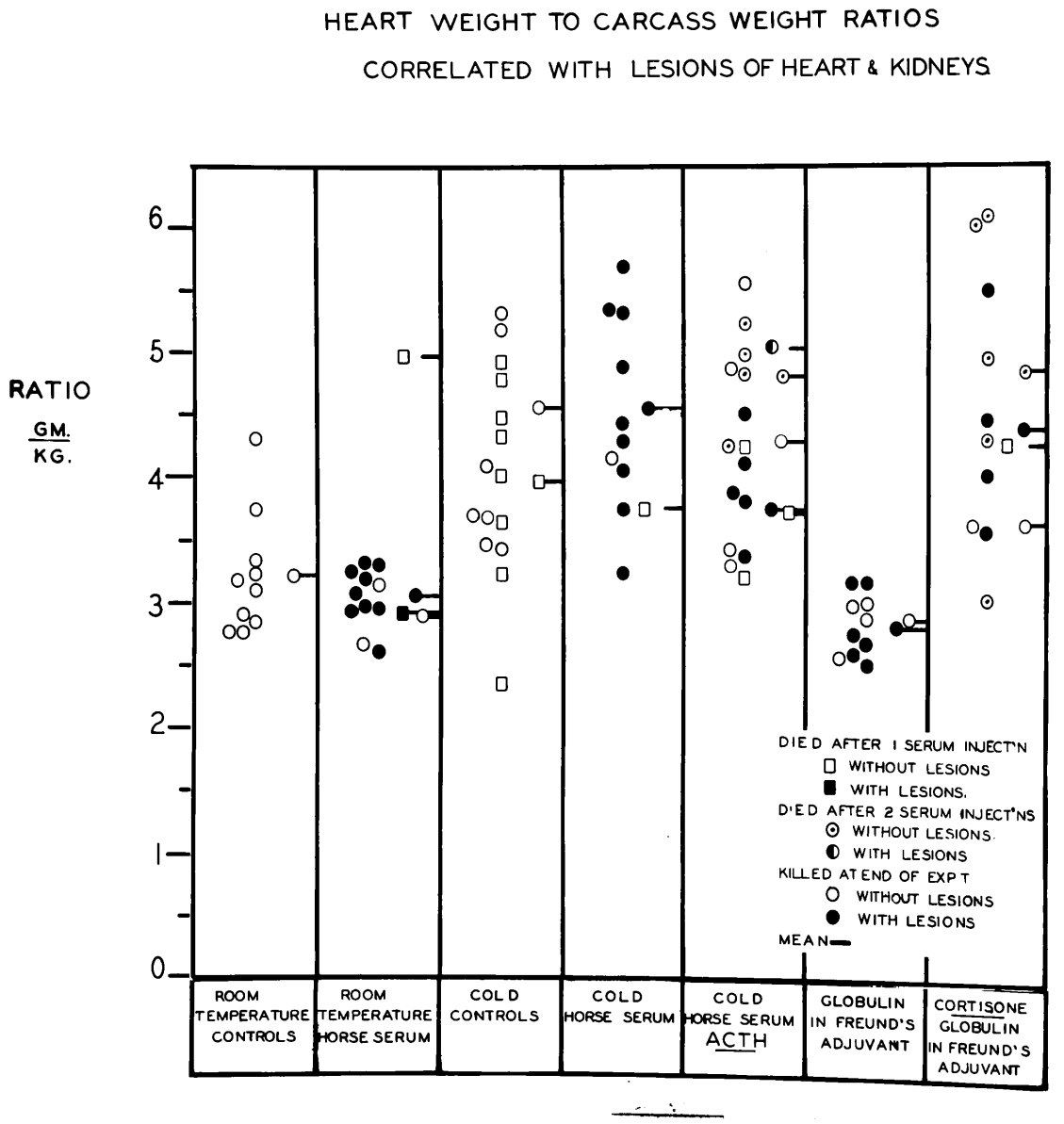


Fig.34

It is evident that the heart to carcass weight ratios have a similar distribution in the room temperature control group, room temperature horse serum group, and in the group receiving globulin in Freund's adjuvant. In all the cold room groups the ratio is elevated as it is in the Cortisone-

treated group. There does not appear to be any relationship between the occurrence of lesions and the size of the ratios.

Since the magnitude of the ratios is affected by the carcass weight as well as by the organ weight, a comparison was made between the actual heart weight and the organ to carcass weight ratios, with respect to the loss or gain of body weight during the experiment. This correlation is detailed in Table 7.

TABLE 7.

COMPARISON OF ACTUAL HEART WT.
AND HEART WT./CARCASS WT. RATIOS
WITH RESPECT TO LOSS OR GAIN IN BODY WT. DURING THE EXPERIMENT

	Group I		II		III		IV		V		VIII		IX	
Mean of:-	Wt. gm.	Ratio $\frac{gm.}{kg.}$	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio
all animals in group	7.1 (10)	3.235	6.35 (13)	3.016	7.25 (16)	4.068	8.04 (11)	4.479	6.86 (17)	4.402	6.17 (10)	2.847	6.32 (12)	4.55
animals with wt. loss	0	0	5.28 (1)	4.99	6.64 (7)	4.448	6.6 (2)	4.543	6.33 (11)	4.434	0	0	6.14 (9)	4.74
animals with wt. gain	7.1 (10)	3.235	6.44 (12)	2.85	7.8 (9)	3.815	8.34 (9)	4.288	7.75 (6)	4.110	6.17 (10)	2.847	6.85 (3)	4.07
Ref.(209)	5.26	2.85												

Numbers in brackets = Number of animals.

The ratios in Group I and all but one animal in Groups III and IX represent values for animals that gained weight. In Group III, seven of the sixteen animals lost weight, and the average ratio of these animals is higher than the average of the nine that gained weight, even though the average actual heart weight was lower in the former group. However, the average actual

heart weight being somewhat higher,in the animals that gained weight than in the warm room controls, indicates a tendency to a greater heart weight in animals exposed to cold. This is confirmed by the fact that the ratios are also higher than those of the warm room controls when the animals that gained weight are considered alone. The latter animals correspond to those surviving the whole experimental period, and are represented as such in Fig.34.

The distribution of the ratios in Group IV (cold room horse serum) is similar to that of the cold controls. Since only two animals of the eleven lost weight, they are comparable with the survivors of Group III. It is again evident that there is an increase in the ratios similar to that of the cold room controls (Fig.34), and that this increase in ratios represents an increase in the actual heart weight may be seen from Table 7. It is also seen that the ratios in animals that have lesions are both higher, equal to and lower than those that did not have lesions. The similarity of the distribution between the ratios of Groups III and IV and the similarity between the distributions of the ratios in I, II and VIII makes it possible to say that an increase in the heart to carcass weight ratio may be accounted for by exposure to cold, but not by sensitization to horse serum or bovine gamma globulin. There is no relation between cold or sensitization and the occurrence of lesions.

In the ACTH-treated group (V), six of the animals that survived the experiment gained weight (Fig.35). The distribution of the lesions and of the ratios (Fig.34) is similar to those of the other cold room

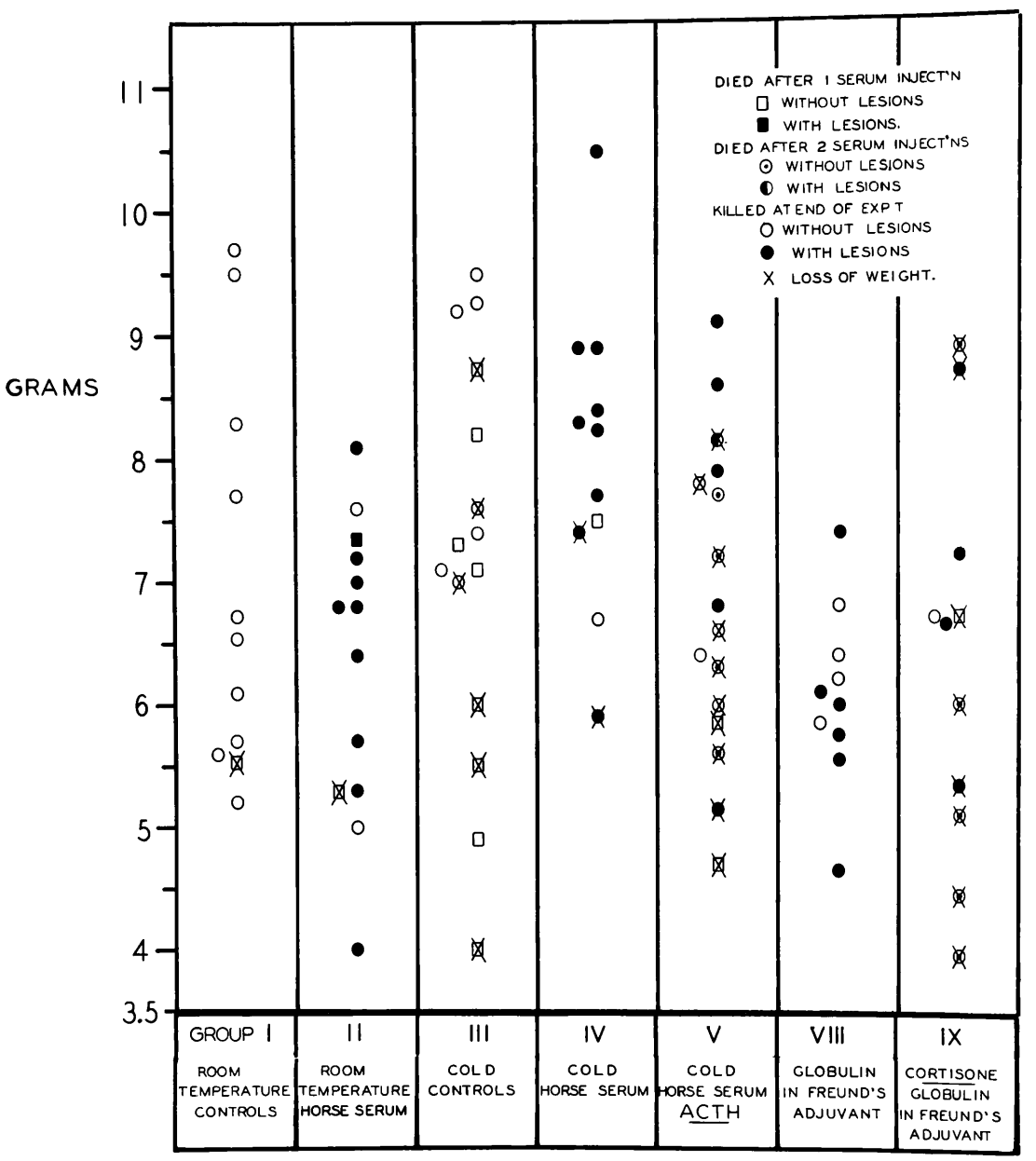


Fig. 35.

groups, indicating that ACTH had no separate or additive effect on the heart weight to carcass weight ratios. The distribution of the actual heart weights (Fig.35) is slightly lower in the ACTH group. However, the average actual heart weights (Table 7) were similar to those of the cold room controls, although the ratios were somewhat higher, probably

due to the slightly smaller average increase in body weight.

In the Cortisone-treated group, the greatest difficulty was encountered in evaluating the ratios, since all but three animals lost considerable weight and those that gained weight gained much less than the animals in all the other groups. From consideration of the actual heart weight (Fig.35), it is evident that the distribution of the weights was not much different from those of other groups. The average values of the actual weights (Table 7) indicates that there is no difference between the weight of the hearts in the Cortisone-treated group and those of the other groups treated at room temperature. Furthermore, the occurrence of lesions was not associated with any constant increase or decrease of heart weight.

It may be concluded from the data presented, that the average heart weight of animals in the cold, as represented by either the heart or carcass weight ratios or by the actual weights of the hearts of animals surviving the whole experimental period, is increased after exposure to cold. However, the administration of horse serum, bovine gamma globulin, ACTH or Cortisone does not appear to influence the weight of the heart. There is no correlation between the weight of the heart and the occurrence of experimental cardiac valvulitis, necrotizing arteritis or glomerulonephritis.

The increase of heart weight due to exposure to cold is in accord with the results of previous investigators(134,198,199). No studies on the effect of ACTH and Cortisone on heart weight have come to our attention to

date. The lack of influence of sensitization by massive doses of foreign serum proteins on heart weight is also in accord with other experiments(132).

Kidney weight:

The distribution of kidney weight to carcass weight ratios correlated with lesions of the heart and kidneys, may be seen from Fig.36. The correlation of this ratio with the lesions of the heart and kidneys, individually, is shown in Text-figures III and IV of Appendix A, but these do not differ materially from Fig.36.

KIDNEY WEIGHT TO CARCASS WEIGHT RATIOS
CORRELATED WITH LESIONS OF HEART & KIDNEYS

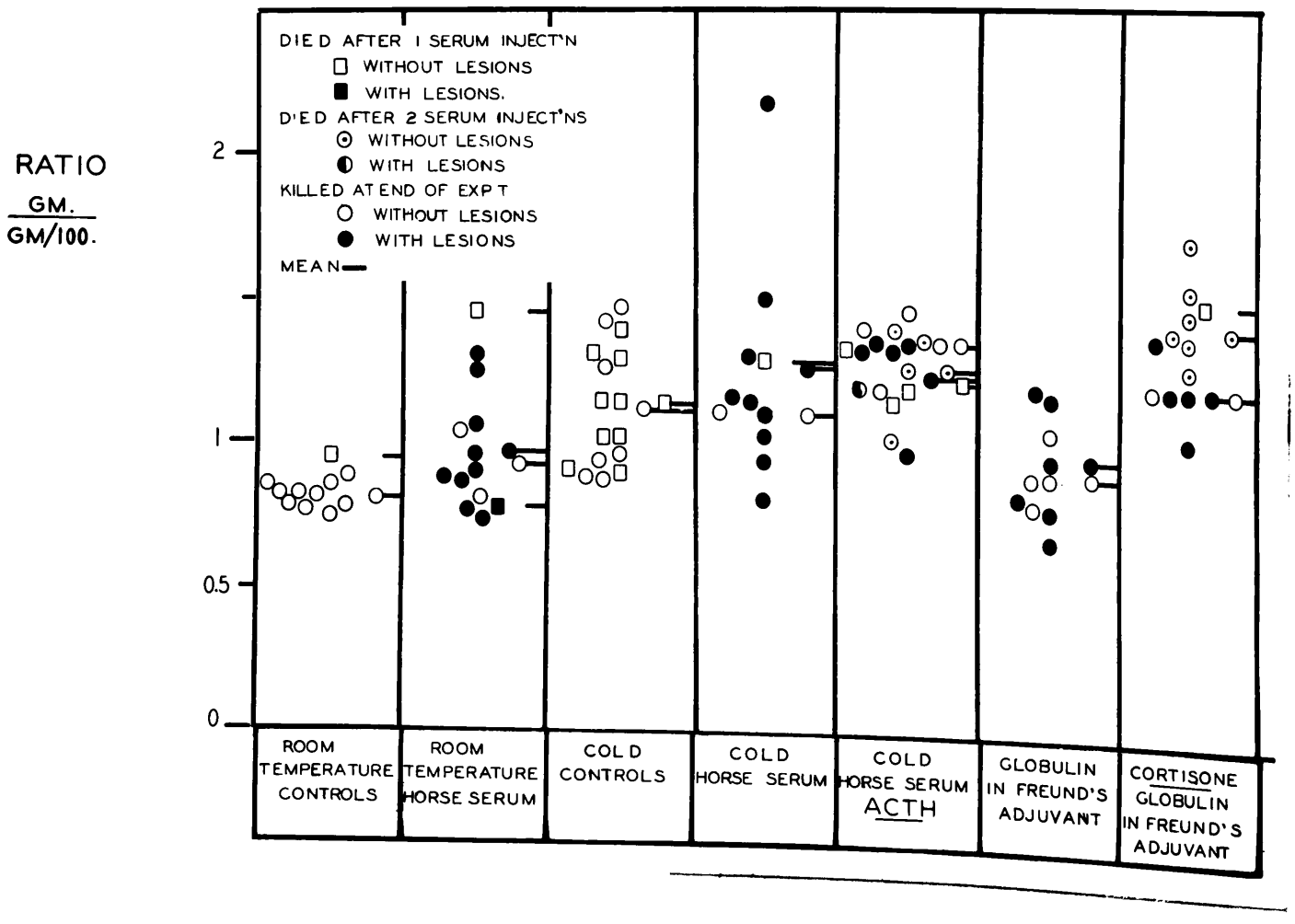


Fig. 36.

The influence of loss of body weight on the ratios may be seen from the comparison of average actual kidney weights and the average spleen to carcass weight ratios, with respect to gain or loss of weight, shown in Table 8. It may be seen from the Table that loss of weight exerts a pronounced effect on the ratio to the extent of reversing the relationship of the ratio and the organ weight. In Groups I, II and VIII, the ratios present an accurate picture of the state of the kidney weight, since the majority of animals and all the survivors gained weight. In Group IV the

Table 8.

COMPARISON OF ACTUAL AVERAGE KIDNEY WT.
AND AVERAGE KIDNEY WT./CARCASS WT. RATIOS
WITH RESPECT TO GAIN OR LOSS OF BODY WT. DURING THE EXPERIMENT.

Mean of:-	Group I Wt. gm.	Ratio gm/100	II Wt.	Ratio	III Wt.	Ratio	IV Wt.	Ratio	V Wt.	Ratio	VIII Wt.	Ratio	IX Wt.	Ratio
all animals in group	17.65 (11)	.816	18.92 (13)	0.9785	19.59 (16)	1.122	22.1 (11)	1.237	19.9 (17)	1.257	18.5 (10)	0.934	18.7 (12)	1.332
animals with wt. loss	13.5 (1)	.940	15.4 (1)	1.455	18.27 (7)	1.269	13.5 (2)	1.123	17.8 (11)	1.254	0	0	17.8 (9)	1.358
animals with wt. gain	18.7 (10)	.8032	19.3 (12)	0.9388	20.73 (9)	1.008	23.9 (9)	1.2625	23.9 (6)	1.264	18.5 (10)	0.934	21.2 (3)	1.255
Ref. (209)	12.7	.697												

situation is also well represented by the ratios, in that only two animals lost weight. In the cold room control group III, all the surviving animals gained weight, as did four of the ones that died before the completion of the experiment. In this group, the ratios belonging to the surviving animals may be safely considered to represent the organ weight. In the remaining animals of this group reference should be made to the chart showing the actual weights (Fig.37).

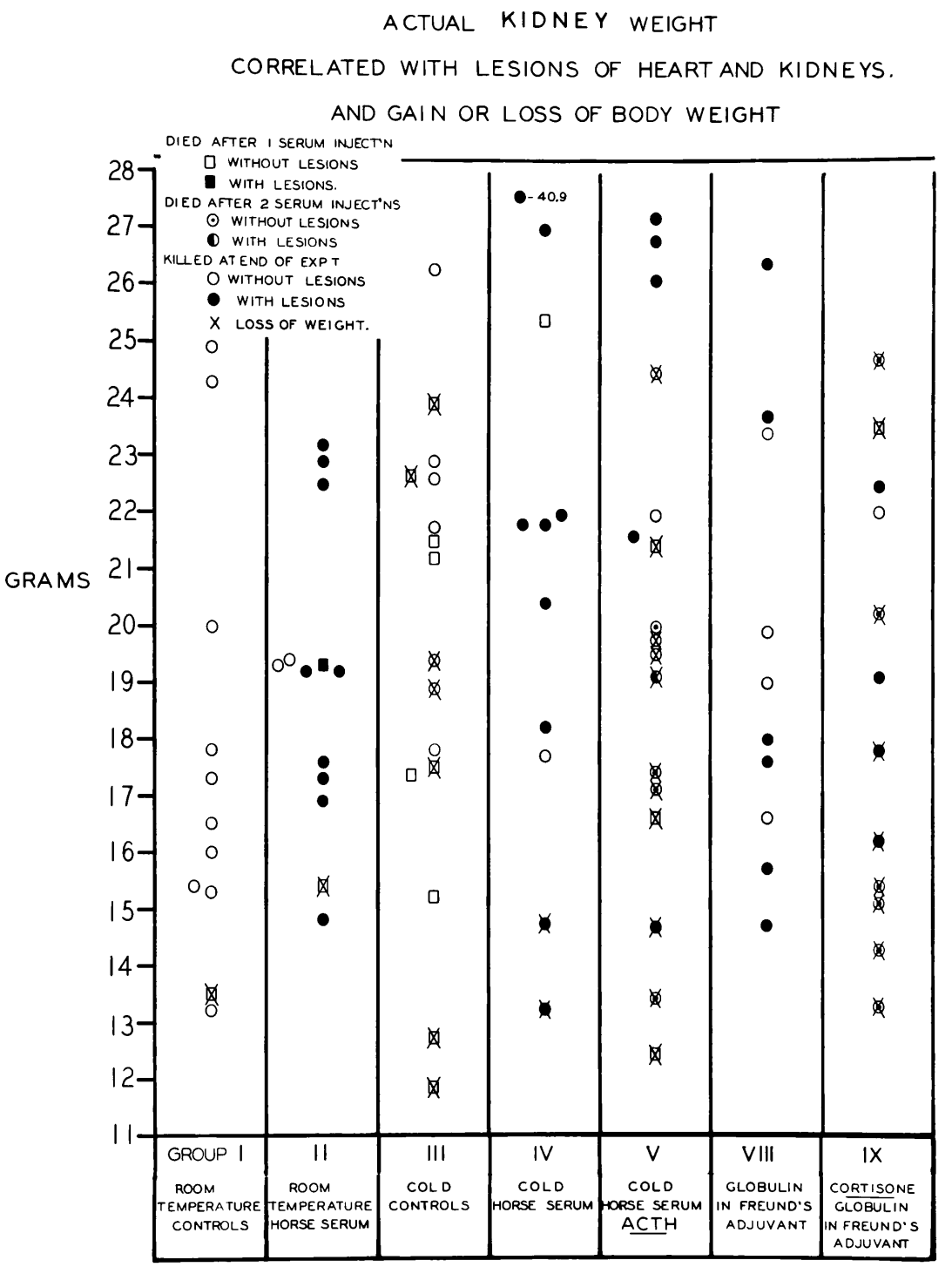


Fig. 37.

In Group V all but two of the surviving animals gained weight, and their ratios reflect the kidney weights. In the others, the actual weights must be considered. In Group IX so many animals lost weight that it is necessary to make most of the deductions from the actual kidney

weights rather than from the ratios.

Considering the kidney weight to carcass weight ratios (Fig. 36) of the animals surviving the whole experiment, it is evident that there does not appear to be any consistent correlation between the presence or absence of lesions, and an increase in the ratio. However, if the more detailed data contained in Tables I to VII of Appendix A are examined, it is apparent that where very high ratios occur, the lesions of the kidney are usually of a severe grade (+++ or ++++). However, where the renal lesions are mild (+ or ++), the kidneys may well be within normal limits of weight. The average ratios and the actual kidney weights reach higher values in the groups exposed to cold than in those treated at room temperature. The ACTH group does not show any difference in weight distribution from the other cold room groups. The distribution of the ratios and the actual kidney weights was similar in the group receiving globulin in Freund's adjuvant and that receiving horse serum at room temperature. In both the latter groups, the kidney weights were somewhat higher than the room temperature controls which is accounted for by the development of mild glomerulo-nephritis in these groups. In the Cortisone-treated groups, the ratios were high, but considering the actual weights of the kidneys, it is evident that the distribution of the weights is similar to the cold room groups, and the average weight of the kidneys (Table 8) was actually higher than the controls. Thus, the increase in the ratios was an expression of the loss of body weight more than anything else.

The distribution of the actual kidney weights (Fig.37) indicates a wider range than the ratios (Fig.36). This is consistent with the results of Brown et al.(209), and is expected for animals gaining weight and growing normally. The animals having lesions of the kidney (see also Text-fig.III, Appendix A) have higher actual weights than those not manifesting lesions, though the opposite state exists with respect to individual animals. Most of the animals with lesions were animals that gained weight. This association is explainable by the fact that the animals manifesting lesions were usually those that survived the whole experimental period and received the full course of treatment. The increase in kidney weight following serum injection and exposure to cold is in accord with the findings of other investigators(134,198).

It may be concluded that the weight of the kidney increases in relation to occurrence of severe lesions of the kidney. Cold and Cortisone appear to cause a slight increase in kidney weight; ACTH does not appear to produce an effect of its own on the kidney weight.

Liver weights:

The distribution of liver to carcass weight ratios may be seen in Fig. 38, correlated with lesions of the heart and kidneys. The ratios correlated with these lesions, individually, are presented in Text-figs. V and VI of Appendix A, and do not differ materially from Fig. 38. It is evident that the distribution of the ratios is extremely close in all the groups but the last. The question of the influence of loss of body weight is taken up in Table 9, where the comparison of average actual liver weights

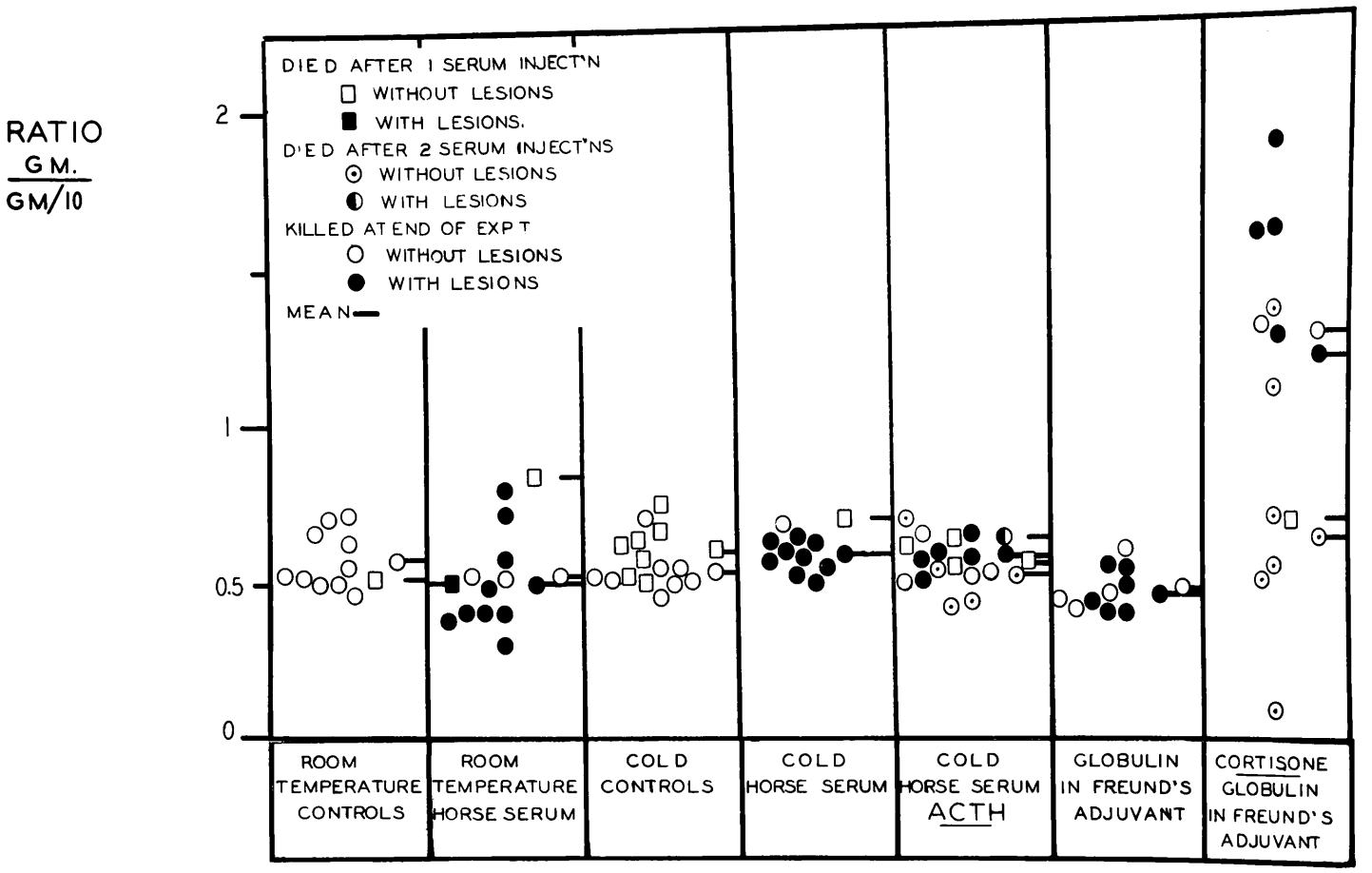


Fig. 38

and the average ratios are compared with respect to gain or loss of body weight. From the Table, it is evident that loss of weight produces an abnormal elevation of the ratio, irrespective of the liver weight, although this tendency is not so marked as has been seen in other organs considered previously. That the animals that gained weight correspond to those that survived the whole experiment with a few exceptions is evident from the Table (also see Tables I to VII of Appendix B). Considering the survivors of the experiment in Fig. 38, the liver weight to carcass weight ratios

TABLE 9.

COMPARISON OF AVERAGE ACTUAL LIVER WT.
AND AVERAGE LIVER WT./CARCASS WT. RATIOS
WITH RESPECT TO GAIN OR LOSS OF BODY WT. DURING THE EXPERIMENT.

	Group I		II		III		IV		V		VIII		IX	
Mean of:-	Wt. gm.	Ratio gm./gm/10	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio
all animals in group	124.3 (11)	.5674	107.6 (13)	.526	102.6 (16)	.567	111.4 (11)	.598	92.2 (17)	.564	100.4 (10)	.4704	154.8 (12)	0.946
animals with wt.loss	73.5 (1)	.512	88.7 (1)	.838	90.4 (7)	.610	76.7 (2)	.633	69.8 (11)	.545	0	0	128.5 (9)	0.799
animals with wt.gain	129.3 (10)	.5729	109.2 (12)	.499	110.6 (9)	.538	119.1 (9)	.5053	113.7 (6)	.599	100.4 (10)	.4704	233.6 (3)	1.388
Ref. (209)	84.3	.4646												

Numbers in brackets = Number of animals.

show a very close grouping about the averages. There are no differences in the ratios that can be correlated with the presence or absence of lesions; exposure to cold; serum or ACTH treatment. However, there is a marked increase in the liver weight to carcass weight ratios in the surviving animals under Cortisone treatment. Best's carmine stain showed that this increase in liver weight was associated with a marked increase in the amount of glycogen in the hepatic cells. This great increase in the ratio is due to an increase in the actual weight of the liver, as can be seen from Table 9 and from Fig. 39, in which the distribution of the actual liver weights is shown. It is an interesting fact that the increase in weight of the liver occurred only in those animals that gained weight and ate well during the experiment. Those that were ill, lost weight or that did not eat well, failed to show the increase. It is also apparent from both Figs. 38 and 39 that there is no correlation

between the weight of the liver and the presence or absence of lesions. Furthermore, the distribution of the actual weights (Fig.39) indicates a striking parallelism between the loss of weight and small size of the liver in all the groups.

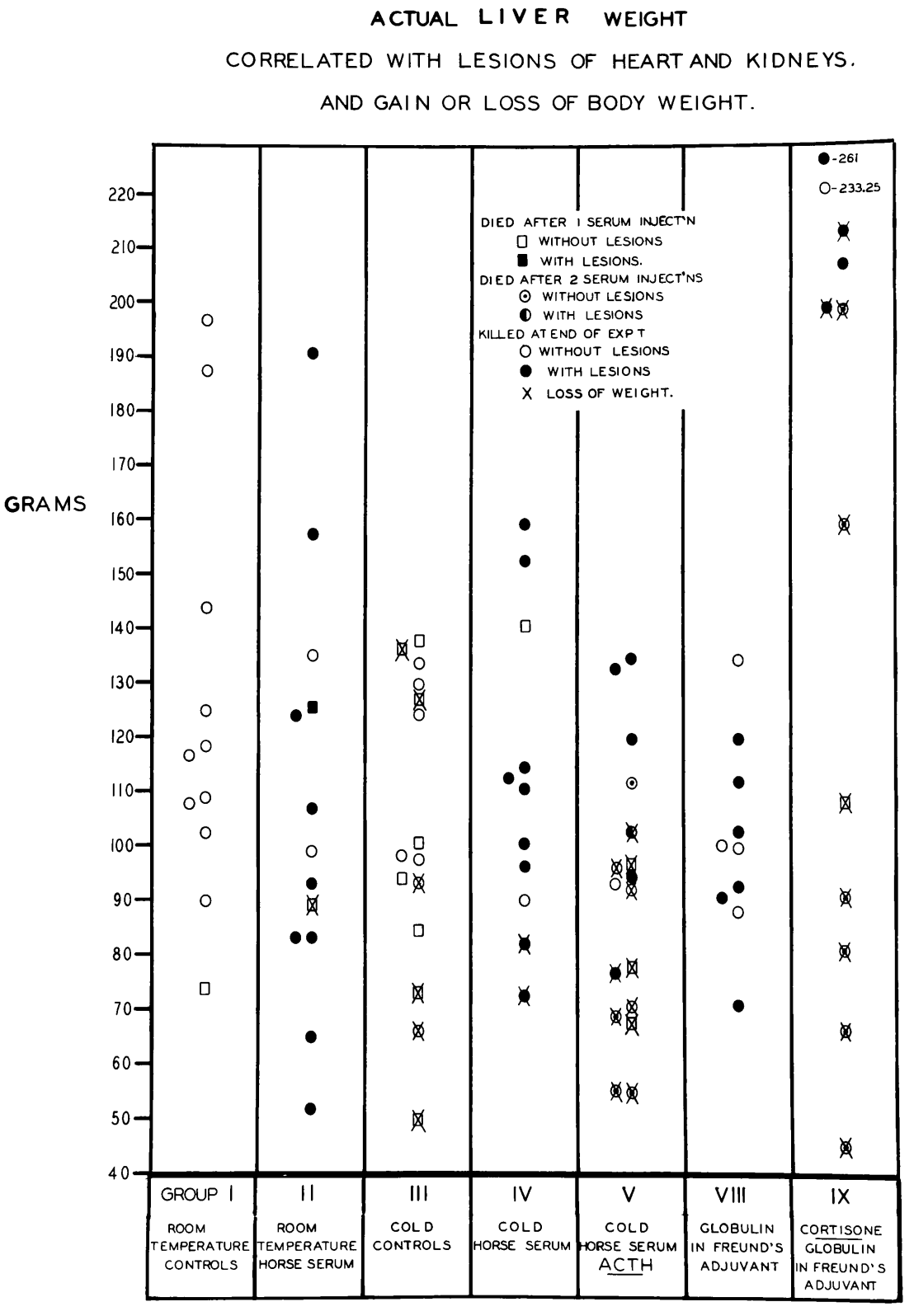


Fig. 39.

Thus, it may be concluded that the liver weight remains in relatively good proportion to the body weight under all circumstances, except under treatment with Cortisone. These conclusions are similar to the results of Brown et al.(211), where it was observed that the liver weight of rabbits tended to increase somewhat in chronic progressive maladies, and to decrease somewhat in acutely fatal illnesses, and the ratios and the actual weights varied in an identical manner. The increased weight of the liver, and the increased glycogen content as a result of Cortisone treatment is in accord with other reports(187,191). There is no correlation between the liver weight and the presence or absence of lesions of the heart, arteries and kidneys; exposure to cold or treatment with ACTH.

Spleen weights:

The distribution of the spleen weight to carcass weight ratios, correlated with lesions of the heart and kidneys, may be seen from Fig. 40. The correlation of the ratios with these lesions, individually, is shown in Text-figs. VII and VIII of Appendix A, but these do not differ materially from Fig.40.

The influence of the loss of body weight on the ratios may be seen in Table 10, where the comparison is made between the average actual spleen weights and the average spleen weight to carcass weight ratios with respect to gain or loss of weight. It is apparent from the Table that loss of body weight is accompanied by a small spleen, while gain in body weight is associated with a larger spleen. The spleen weights and the ratios vary

SPLEEN WEIGHT TO CARCASS WEIGHT RATIOS

CORRELATED WITH LESIONS OF HEART & KIDNEYS

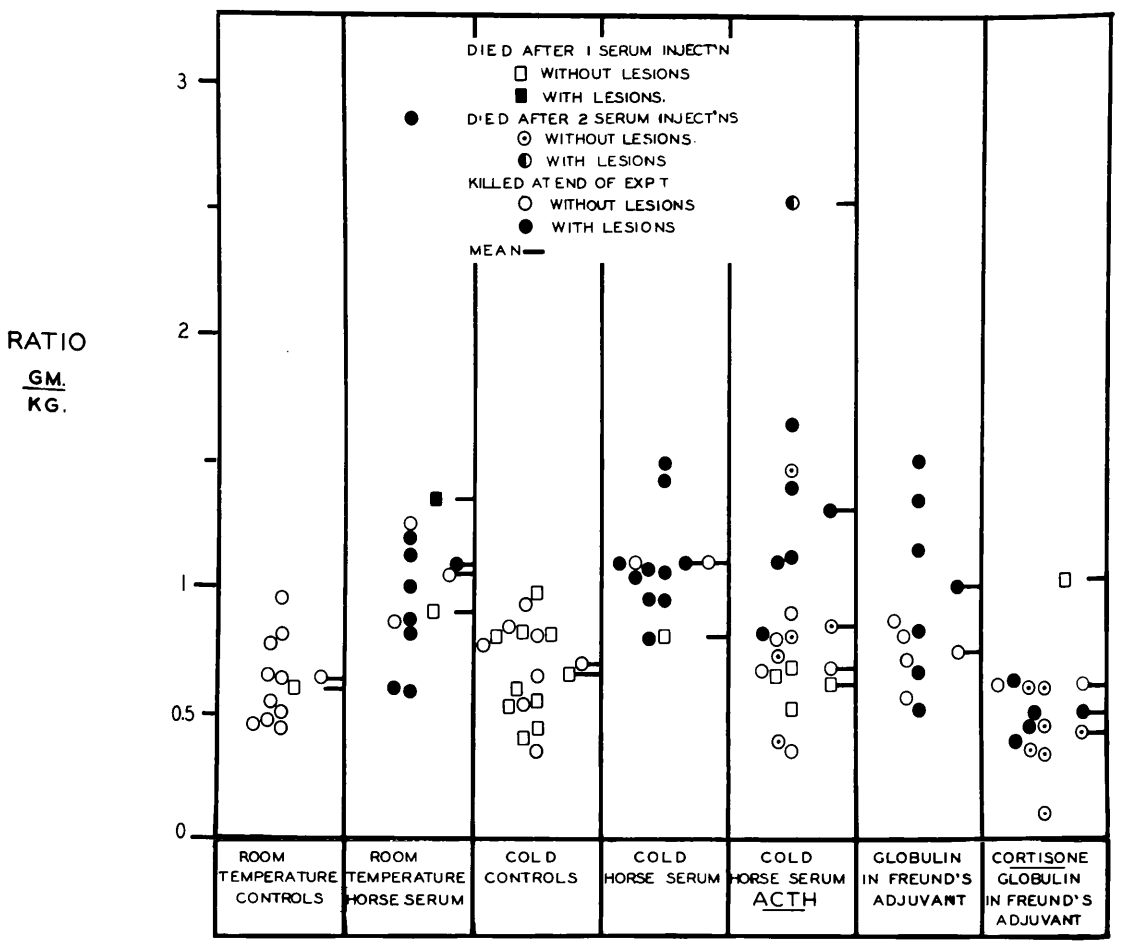


Fig. 40.

in the same direction. This is also shown for individual rabbits in Fig. 41, where the distribution of the actual spleen weight is correlated with lesions and gain or loss of weight. From Fig. 40 it is evident that the animals that lost body weight tended to have lower spleen weights than those that gained weight. Furthermore, the distribution of the ratios (Fig.40) and of the actual weights (Fig.41) is very similar, so that it is possible to refer to the ratios and the actual spleen weights inter-

TABLE 10.

COMPARISON OF AVERAGE ACTUAL SPLEEN WT.
AND AVERAGE SPLEEN WT./CARCASS WT. RATIOS
WITH RESPECT TO GAIN OR LOSS OF BODY WT. DURING THE EXPERIMENT.

Mean of:	Group I		II		III		IV		V		VIII		IX	
	Wt. gm.	Ratio $\frac{gm.}{kg.}$	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio
all animals in group	1.35 (11)	.625	2.25 (13)	1.087	1.26 (16)	.699	1.87 (11)	1.066	1.58 (17)	0.966	1.915 (10)	.890	.726	.503
animals with wt.loss	0.85 (1)	.592	0.95 (1)	0.897	0.92 (7)	.629	1.26 (2)	1.016	1.24 (11)	0.893	0	0	.599	.490
animals with wt.gain	1.4 (10)	.629	2.35 (12)	1.033	1.46 (9)	.700	2.0 (9)	1.077	2.19 (6)	1.17	1.915 (10)	.890	.917	.540
Ref. (209)	0.99	.331												

changeably. Examination of Figs. 40 and 41 indicates that animals treated with horse serum, Groups II and IV, or bovine gamma globulin, Group VIII, show a general increase in spleen weight. Although the highest spleen weights are those of animals showing lesions, it is apparent that there is no correlation between the occurrence of lesions and the weight of the spleen. The exposure to cold does not, by itself, alter the distribution or the mean value of the spleen weights, or spleen to carcass weight ratios, Groups I and III being essentially similar. In Group V, receiving horse serum in the cold while under ACTH treatment, the effect of the horse serum in increasing the spleen weight appears in some of the animals and for the most part the animals with the larger than control spleens appear to have been the ones to develop the lesions. However, in many of the animals in this group, the increase in spleen weight observed to be the effect of horse serum sensitization, seems to have been reversed by the ACTH,and the spleen weights of some

of the animals are equal to or below those of the controls. In Group IX, Cortisone appears to have effected a decrease in the size of the spleen, despite the increase that might have been expected to result from the gamma globulin that the animals also received. This decreased spleen weight was present even in the animals that lost weight.

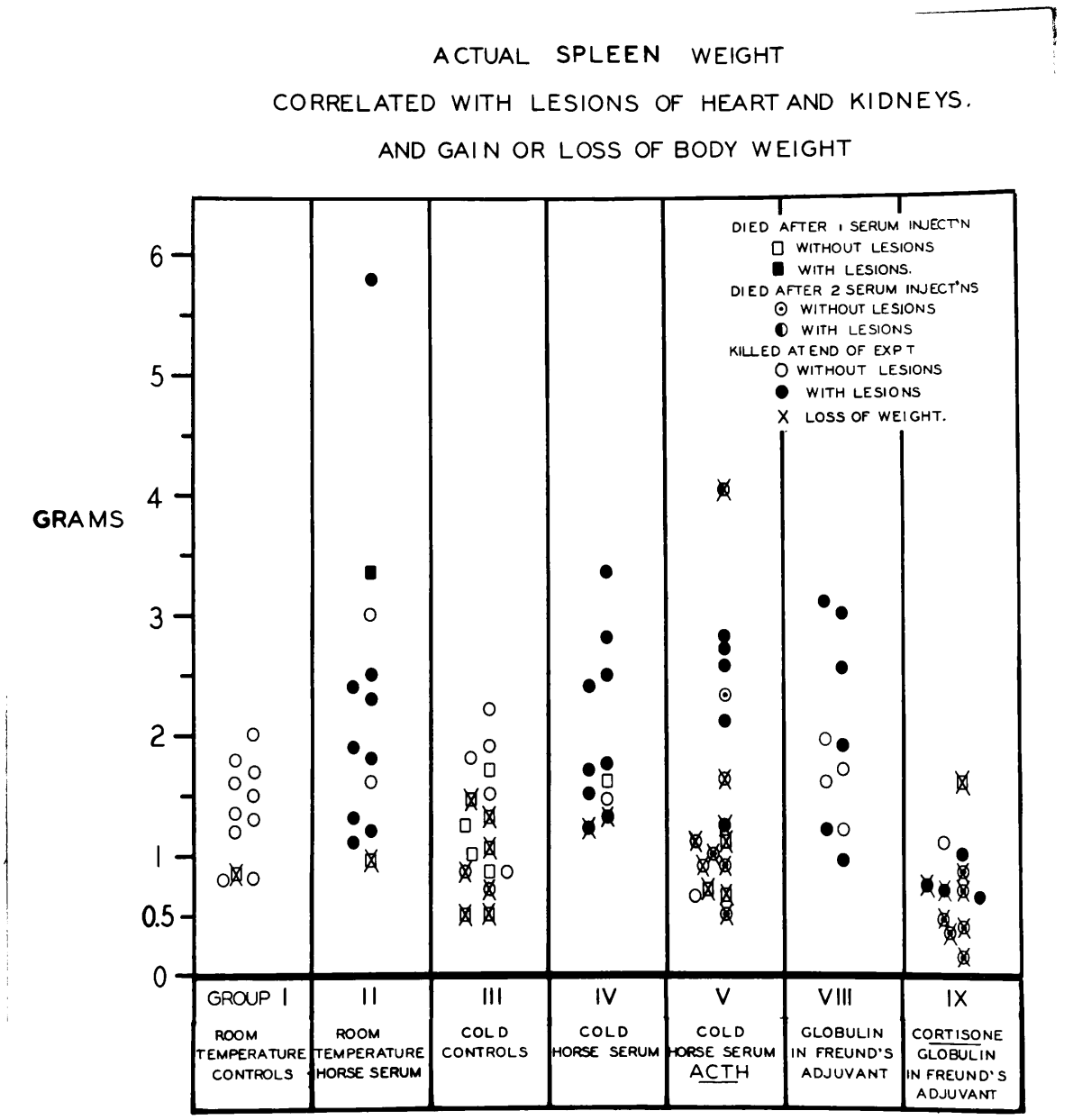


Fig. 41.

It should be noted that the increase or decrease of any individual spleen weight from the normal is not of great magnitude, but it is evident from the charts that these tendencies are real. However, it is unlikely that any statistical significance could be hoped for from the figures themselves, since there were not enough animals forming completely homogeneous groups with respect to spontaneous death, gain or loss of weight etc., to be susceptible of fine statistical analysis.

The increase in spleen weight resulting from sensitization to foreign proteins is in accord with the findings of other investigators(5,10,132). The decrease in spleen weight resulting from Cortisone has also been described(188,212).

Pituitary weight:

The distribution of pituitary to carcass weight ratios correlated with lesions of the heart and kidneys is indicated in Fig. 42. The correlation of the ratios with the lesions of the heart or kidneys, individually, is shown in Text-figs.IX and X of Appendix A, but these do not differ materially from Fig. 42.

The influence of the changes in body weight on the ratios may be seen in Table II, where a comparison is made between the average actual pituitary weight and the average pituitary weight to carcass weight ratios, with respect to gain or loss of body weight. It is evident from the Table that in animals losing weight, the ratios do not reflect the size of the pituitary, but in the animals gaining weight, the magnitude of the ratio

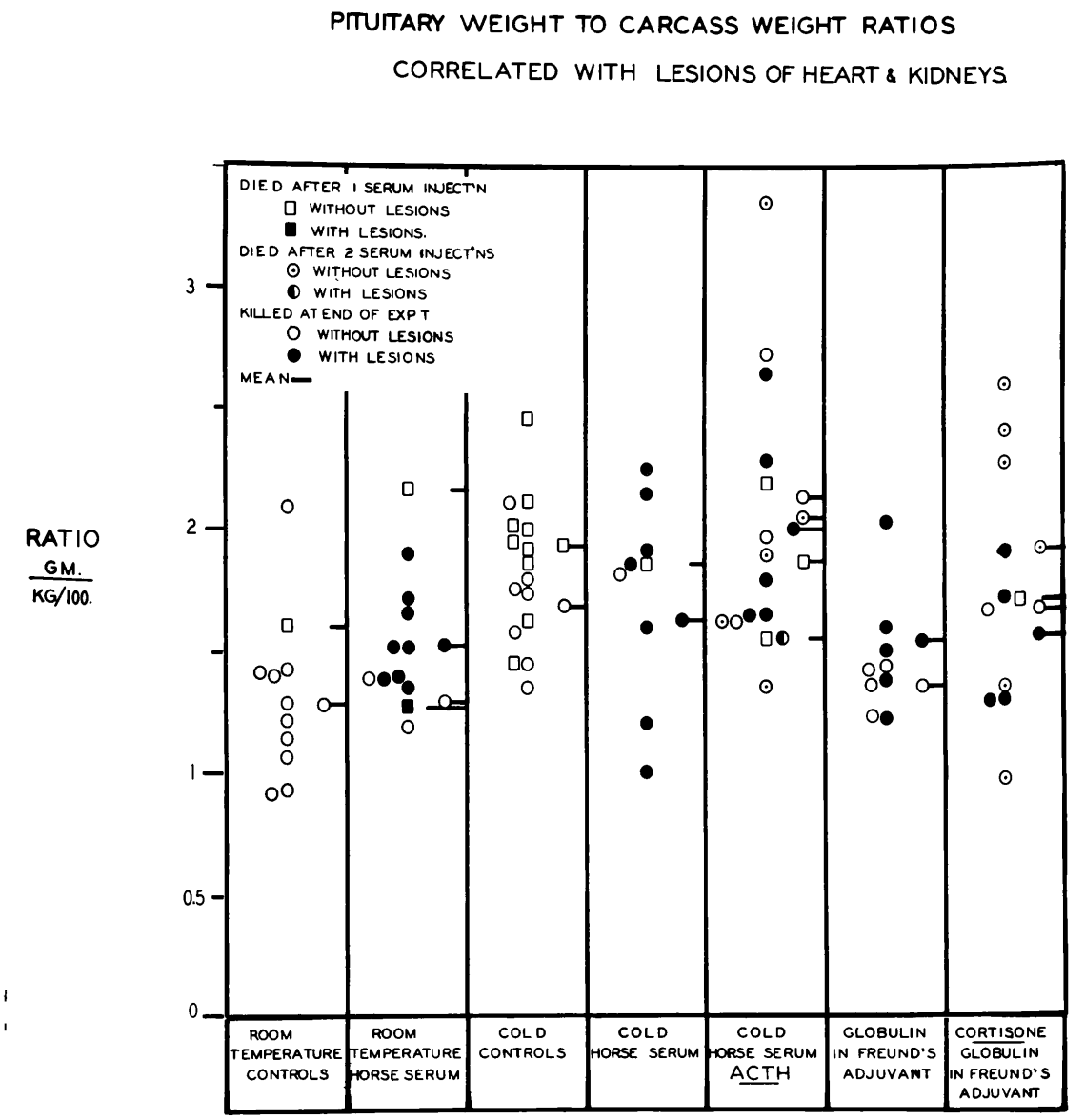


Fig. 42

is directly proportional to the weight of the pituitary in the first six groups. However, in the Cortisone-treated group, the ratio is greater than the weight of the pituitary warrants, which is accounted for by the fact that the animals in this group gained less weight than comparable animals in the previous group. This group will, therefore, be considered separately from the data in Fig. 43, showing the distribution of the actual pituitary weights correlated with the presence or absence of

TABLE 11.

COMPARISON OF AVERAGE ACTUAL PITUITARY WT.
AND AVERAGE PITUITARY WT./CARCASS WT. RATIOS
WITH RESPECT TO GAIN OR LOSS OF WT. DURING EXPERIMENT.

Mean of:	Group I		II		III		IV		V		VIII		IX	
	Wt. gm.	Ratio gm./kg×100	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio
all animals in group	.026 (11)	1.315	.030 (12)	1.539	.032	1.761	.032 (9)	1.744	.030 (15)	1.988	.030 (10)	1.455	.025 (11)	1.734
animals with wt.loss	.023 (1)	1.602	.023 (1)	2.173	.028	1.647	.027 (1)	2.25	.026 (10)	1.995			.024 (8)	1.801
animals with wt.gain	.028 (10)	1.286	.031 (11)	1.481	.035	1.907	.032 (8)	1.609	.037 (5)	1.980	.030 (10)	1.455	.026 (3)	1.558
Ref. (209)	.028	1.54												

Numbers in brackets = Number of animals.

lesions and gain or loss of body weight. Since the animals gaining weight are represented by the survivors of the experiment in most of the groups, the ratios are comparable and represent the state of the organ weight (within 16.23%) in Fig. 41.

Before comparing the data from the different groups, attention may be drawn to the similarity in distribution of the values of the pituitary weight to carcass weight ratios (Fig. 42) and the actual pituitary weights (Fig. 43). This is in accord with the work of Brown et al.(209), where it was pointed out that the pituitary weight remains at a relatively constant weight in spite of body weight changes. Since the variation of the actual weight is not great, and since the body weight may vary greatly under the conditions of the experiments, it is quite logical that the changes in body weight should be reflected in the ratios of the pituitary to carcass weight

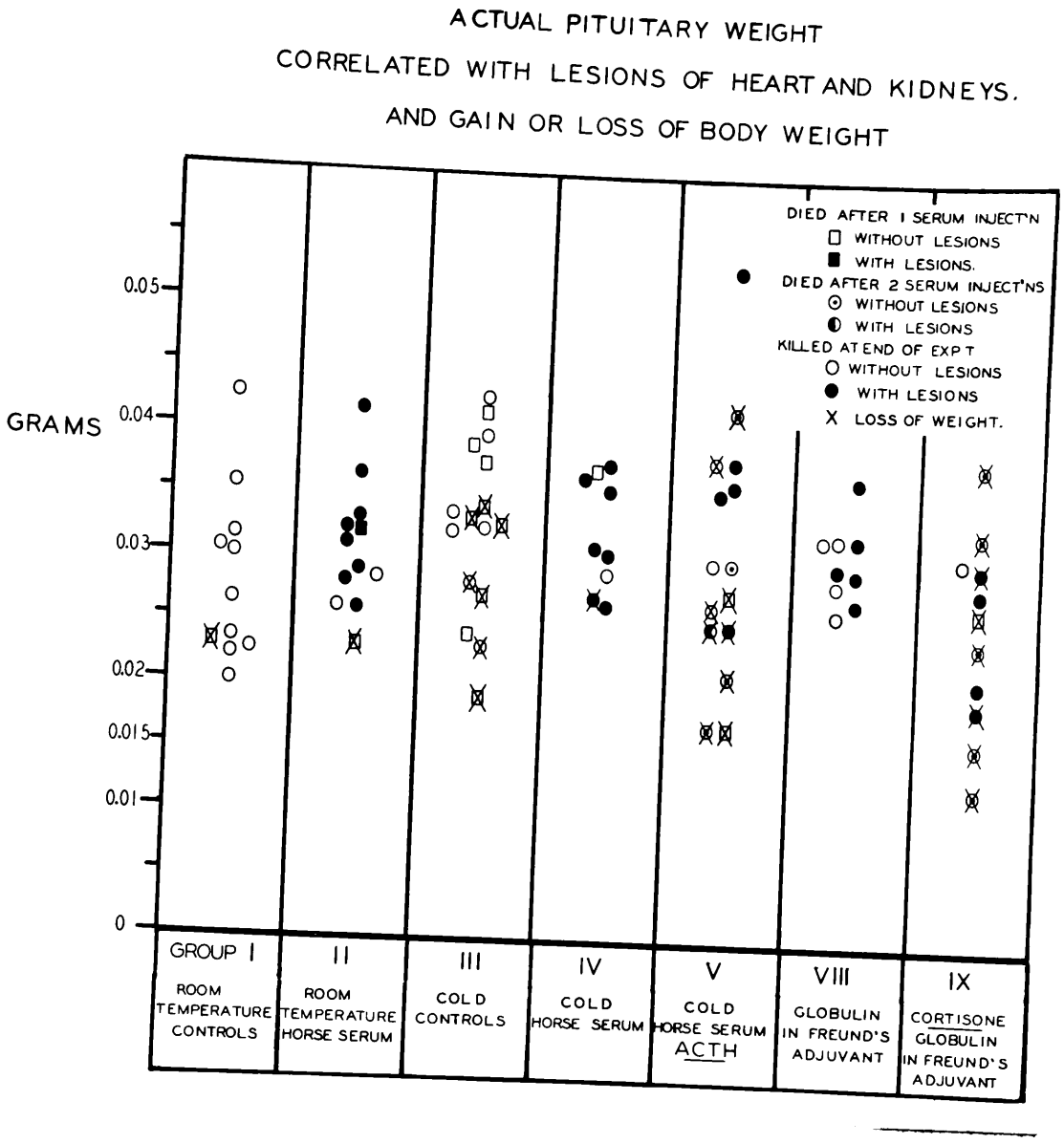


Fig. 43.

much more than in the ratios of the weight of any other organ to the carcass weight. It is therefore necessary to pay more attention to the actual weights of this gland than to the ratios in the evaluation of the results.

Considering the ratios of the surviving animals and the actual organ weights of the animals that gained weight, it appears that there is a slight increase in pituitary weight resulting from treatment with horse serum, bovine gamma globulin in Freund's adjuvant and from exposure to cold.

These increases cannot be regarded as statistically significant in view of the large overlap of the distribution in the various groups. The administration of ACTH appears to produce an increase in pituitary weight, while the pituitary weight under Cortisone treatment appears to remain within the normal range. The animals dying spontaneously had pituitary weights within normal limits. There was no correlation between the weight of the pituitary and the occurrance of lesions of the heart, arteries and kidneys.

The variations in pituitary weights under conditions of acute or chronic disease have been studied(211) and the results indicate a rise of 10 to 20% under these circumstances. This variation is consistent with that observed in our animals. No metion is made by Selye of the variations of pituitary weight associated with "Stress"(156). It is remarkable that ACTH treatment produces enlargement of the pituitary, since the well-known effect of giving a hormone is to cause atrophy of the organ by which it is produced. In the same way, one may ask why the pituitary was not larger when Cortisone was administered. To our knowledge, there is no explanation of these findings that would not be purely sepculative.

Adrenal weight:

The adrenal weight to carcass weight ratios correlated with heart and kidney lesions, are indicated in Fig. 44. The correlation of the ratios with the lesions of the heart or kidneys, separately, may be seen in Text-figs. XI and XII of Appendix A.

The influence on the ratios of loss of body weight is summarized in Table 12, in which a comparison is made between the average actual adrenal

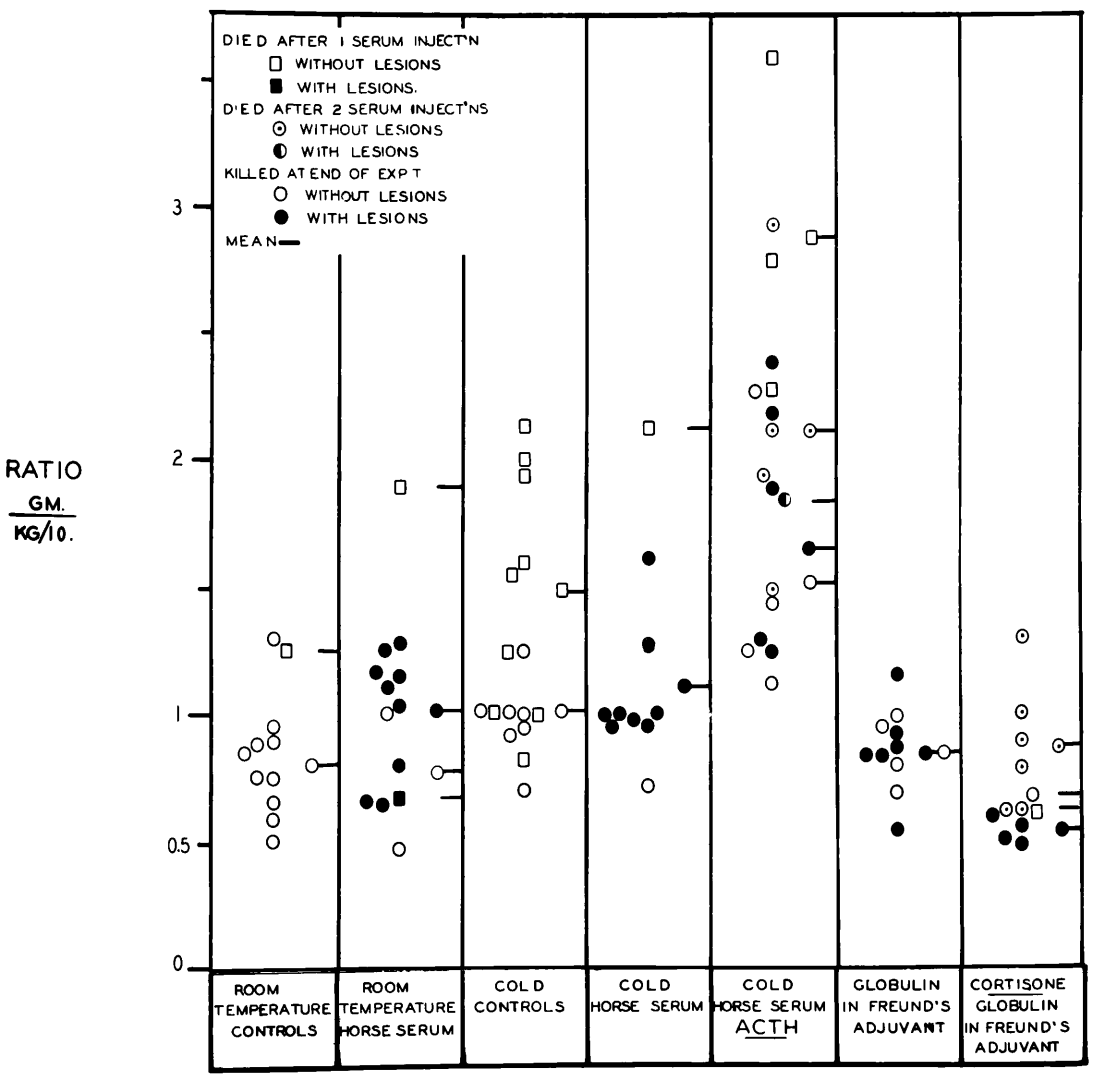


Fig. 44.

weight and the average ratios in relation to gain or loss of body weight. It is evident from Table 12 that a loss of body weight produced a marked elevation of the ratio in spite of a smaller adrenal weight. However, where gain in body weight occurred, the magnitude of the ratios were directly proportional to the weight of the adrenals. Since the gain in body weight occurred, for all practical purposes, in most of the groups,

TABLE 12.

COMPARISON OF AVERAGE ACTUAL ADRENAL WT.
AND AVERAGE ADRENAL WT./CARCASS WT. RATIOS
WITH RESPECT TO GAIN OR LOSS OF BODY WT. DURING THE EXPERIMENT

Mean of:-	Group I Wt. gm.	Ratio gm. kg/10	II Wt.	Ratio	III Wt.	Ratio	IV Wt.	Ratio	V Wt.	Ratio	VIII Wt.	Ratio	IX Wt.	Ratio
all animals in group	.1768 (11)	0.856	.1982 (13)	1.022	.2236 (16)	1.282	.2188 (11)	1.179	.3135	2.011	.1821 (10)	.864	.0818 (12)	.725
animals with wt.loss	.1800 (1)	1.254	.2026 (1)	1.905	.1740 (10)	1.264	.1536 (2)	1.280	.3022	2.122			.0984 (9)	.771
animals with wt.gain	.1765 (10)	0.816	.1979 (12)	0.949	.2622 (6)	1.296	.226 (9)	1.051	.3341	1.805	.1821 (10)	.864	.0996 (3)	.586
Ref. (209)	.380	2.08												

in animals that survived the whole experimental period (c.f. Tables I to VII of Appendix A, and Fig. 45), the variations in the ratios can be considered to represent actual adrenal weight variations. The exceptional groups that should be considered separately are Groups V and IX. In these groups it is necessary to consider the actual adrenal weights more than the ratios. The actual adrenal weights are shown in Fig. 45, correlated with lesions of the heart and kidneys and gain or loss of body weight.

Considering the ratios of the surviving animals and the actual weights of the adrenals, the following general observations can be made:-

It appears that no effect is exerted on the magnitude of the adrenal weights by the administration of horse serum or bovine gamma globulin alone at room temperature. A slight increase in the adrenal weight is manifested by animals exposed to cold. This slight increase is not greater in the animals in the group receiving horse serum in the cold. This confirms the

fact that horse serum does not produce an increase in adrenal weight of its own. There appears to be a quite definite increase in the adrenal weight as a result of ACTH administration. A definite decrease in adrenal weight results from the administration of Cortisone. Animals dying spontaneously, are most often animals that have lost weight. In these animals, the ratios do not represent the adrenal weights and the assessment of the changes in these animals must be made entirely from the actual weights (Fig. 45).

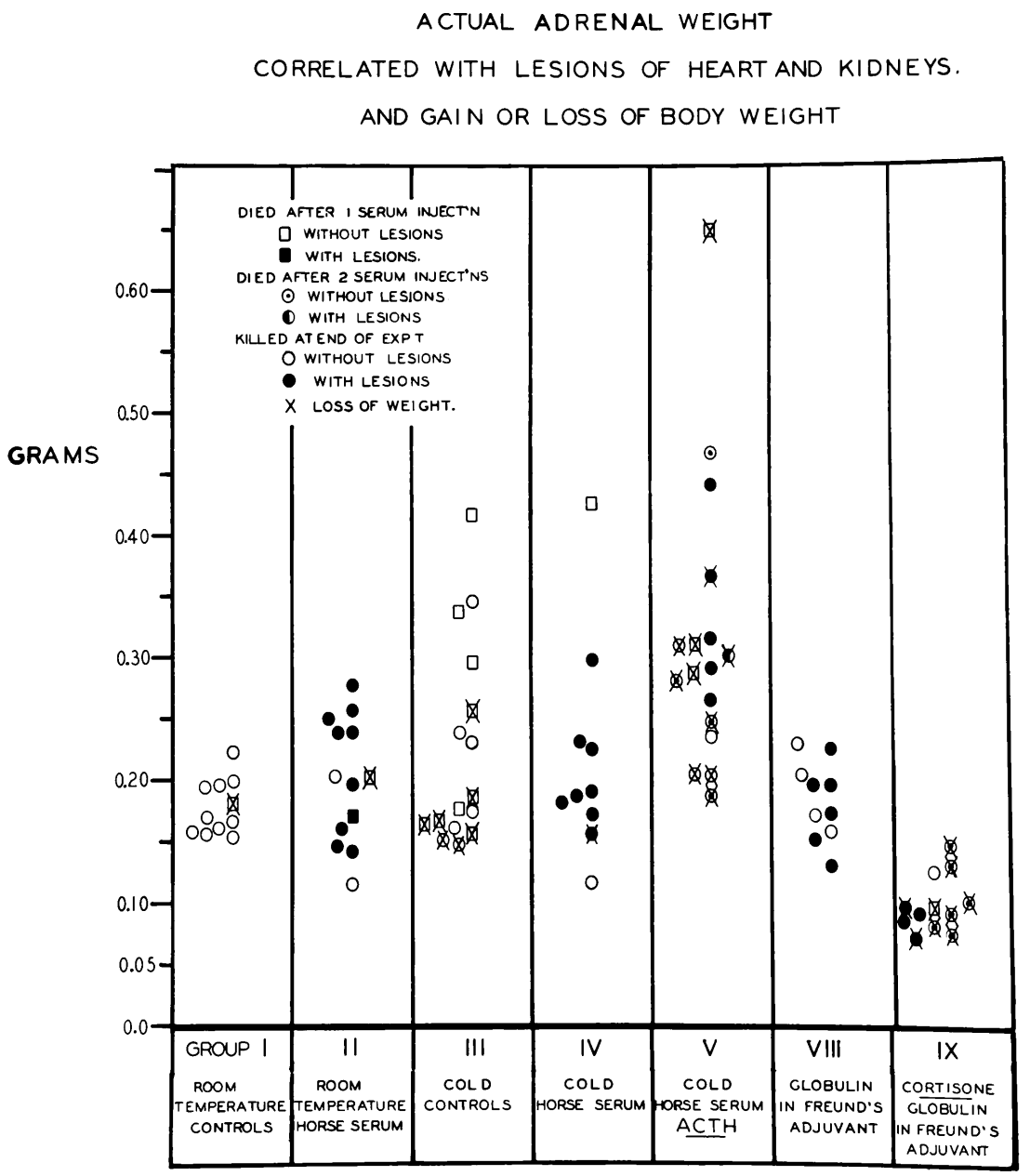


Fig. 45.

The adrenal weights of these animals appear to be well distributed within the groups, but some of the highest values occurred in animals dying spontaneously. No general statement can be made with regard to the variation in the adrenal weight correlated with spontaneous death. There is no correlation between the weight of the adrenal and the occurrence of lesions of the heart, arteries or kidneys.

The increase in size of the adrenals as a result of ACTH administration has been well established(185,186,219-221 and others). The increase in adrenal weight as a consequence of exposure to cold and other agents has also been reported(185,186,222). By this criterion, therefore, the animals exposed to cold in our experiments manifested evidence of the effects of non-specific "stress"(156). However, the animals sensitized to horse serum or bovine gamma globulin did not manifest this expression of the effects of stress. Even within the normal distribution of the adrenal weights there was no evidence of a correlation between the adrenal weight and the occurrence of lesions. It is true that the adrenal size may not be the ultimate criterion of adrenal function, but by the criterion of weight there does not appear to be any correlation with stress and the occurrence or absence of lesions of the heart, arteries or kidneys produced in rabbits by sensitization to foreign serum proteins. These conclusions are further supported by consideration of the groups treated with ACTH and Cortisone. In the former group a definite increase in adrenal weight occurred in many of the animals, but there was no correlation between the weight of the adrenal and the occurrence of lesions. In the latter group the adrenals were within

normal limits, or perhaps somewhat smaller and again, there was a similar lack of correlation with the production of lesions.

Thymus weight:

The thymus weight to carcass weight ratios are plotted in Fig. 46, as correlated with lesions of the heart and kidneys. The correlations with the lesions of the heart or kidneys, individually, were made in Text-figs. XIII and XIV of Appendix A, from which it may be seen that there was no material difference observable from Fig. 46.

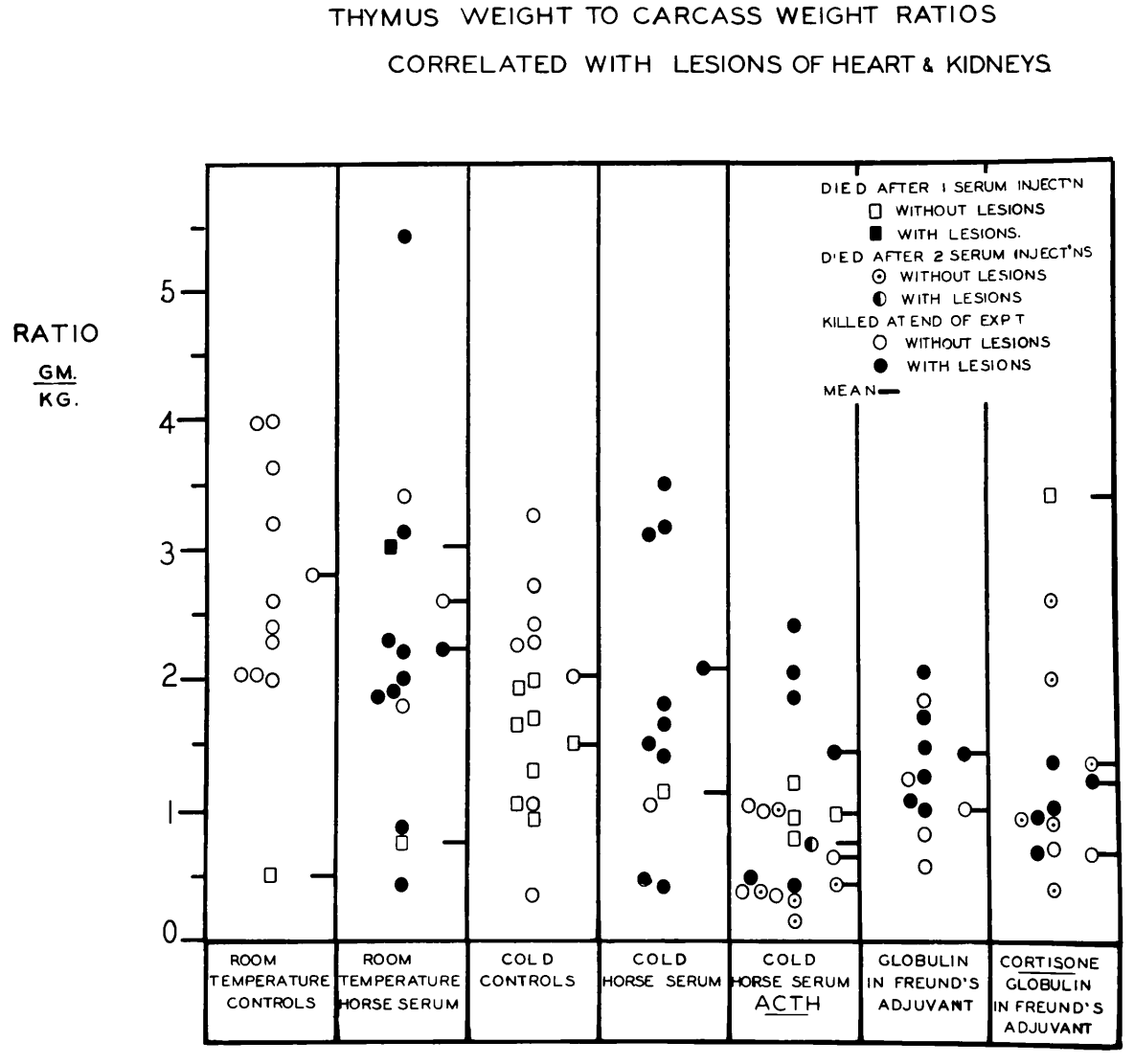


Fig. 46.

The influence of the loss of body weight on the ratios is indicated by the comparison of the average actual thymus weights, and the average thymus weight to carcass weight ratios with respect to gain or loss of body weight (Table 13). It is evident from the Table that the loss of body weight did not alter the ratios appreciably. This lack of effect of the body weight is probably referable to the fact that the decrease in thymus weight was so large, when it occurred, as to obviate the effect of the loss of body weight. However, it is evident that quantitatively the weight changes in the thymus were not accurately expressed by the ratios in animals that lost weight. Therefore, it is still necessary to consider the actual thymus weights in some connections. The actual thymus weight correlated with lesions of the heart and kidneys and gain or loss of body weight is represented graphically in Fig. 47. From

TABLE 13.

COMPARISON OF AVERAGE ACTUAL THYMUS WT.
AND AVERAGE THYMUS WT./CARCASS WT. RATIOS
WITH RESPECT TO GAIN OR LOSS OF BODY WT. DURING THE EXPERIMENT.

Mean of:-	Group I		II		III		IV		V		VIII		IX	
	Wt. gm.	Ratio $\frac{gm.}{kg.}$	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio	Wt.	Ratio
all animals in group	6.15 (11)	2.600	4.99 (13)	2.246	3.62 (14)	1.775	3.58 (11)	1.743	1.541 (17)	.903	2.73 (10)	1.298	2.03 (12)	1.355
animals with wt.loss	0.72 (1)	0.501	0.8 (1)	0.756	2.030 (9)	1.212	0.55 (2)	0.438	0.939 (11)	.954	0	0	2.266 (9)	1.562
animals with wt.gain	6.89 (10)	2.810	5.34 (12)	2.370	4.504 (5)	2.087	4.25 (9)	2.032	2.58 (6)	1.347	2.73 (10)	1.298	1.40 (3)	0.805
Ref. (209)	2.21	1.23												

Numbers in brackets = Number of animals.

Fig. 47 it is evident that a loss of body weight was accompanied by a decrease in thymus weight and that the actual thymus weights are distributed over a wider area than the ratios. However, the general trends are similar in the two figures.

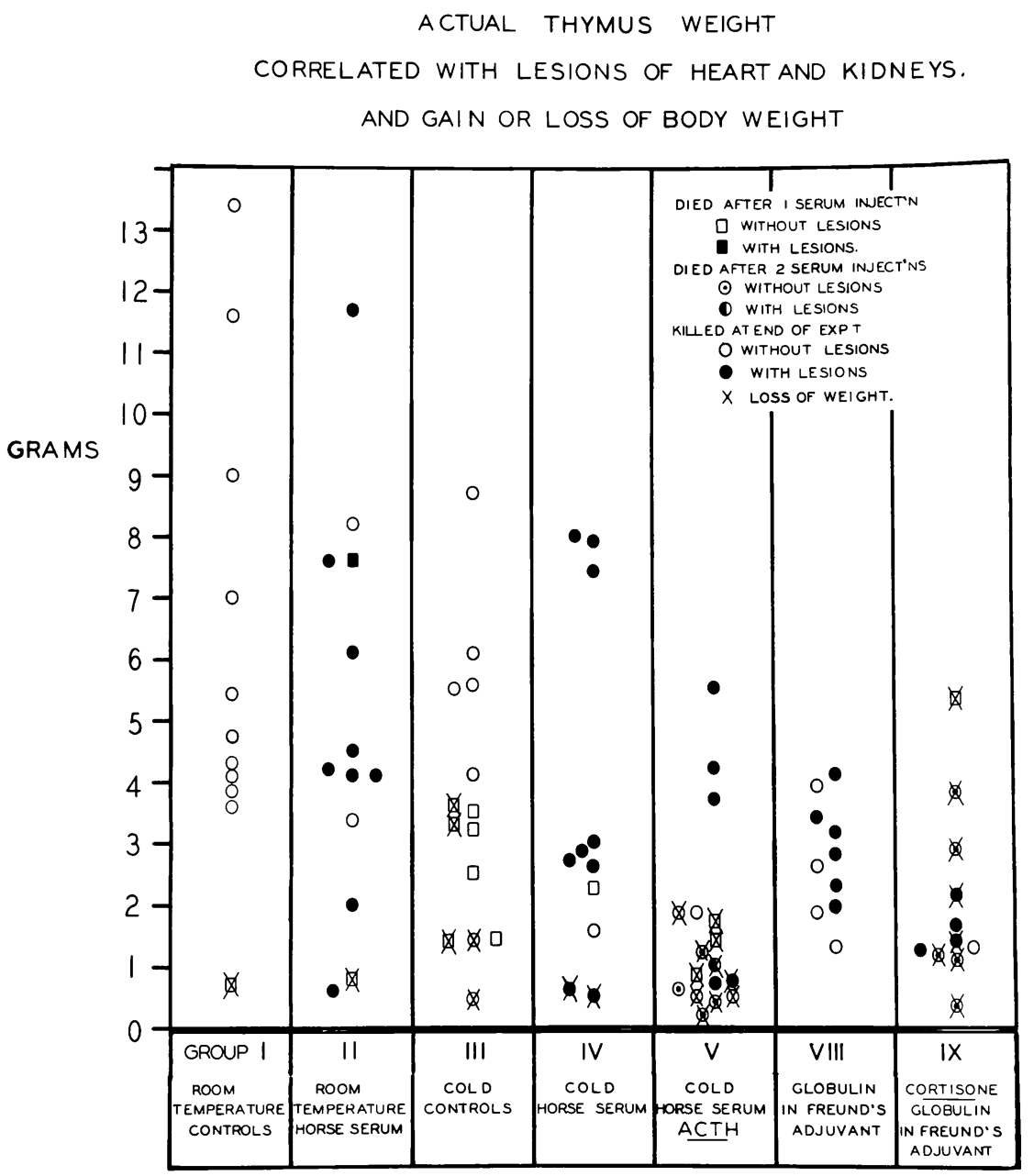


Fig. 47

From the data presented above, it appears that any form of treatment, exposure to cold, injection of horse serum or bovine gamma globulin, Cortisone or ACTH, tends to lower the thymus weight below that of the normal controls. The greatest decrease in thymus occurred in animals dying spontaneously before the end of the experiment, and in animals that lost weight. There is no relation between the size of the thymus and the presence or absence of lesions of the heart, arteries or kidneys, inasmuch as lesions may occur in animals with large or small thymuses.

The involution of the thymus under all kinds of abnormal conditions is well established(209,156,220,223), although Selye points out that this takes 48 hours to occur and under certain circumstances the thymus will fail to involute(223). However, whether one considers that the expression of "stress" or "adaptation" is either a small or a large thymus, one cannot correlate either with the occurrence of lesions induced by sensitization to foreign serum proteins. With the thymus weight as a criterion, there is no evidence to support the thesis that non-specific stress is of major importance in the development of the lesions in our experiments.

Summary of the observations on organ weights:

Loss of body weight was an outstanding characteristic of animals treated with Cortisone. This was also true of animals treated with ACTH, but to a lesser degree. Loss of body weight occurred in other groups of animals when the animals became sick and died before the termination of the experiment. Although the weights of the animals were comparable at the start of the experiment, there was a difference in the terminal weight between

Group I and Group VIII in descending order in the animals that gained weight and survived the experiment, of 16.213%. This differential in weight and the fact that loss of body weight influenced the organ weight to carcass weight ratio led to a consideration of the actual organ weights and the correlation with gain or loss of weight in addition to the analysis of the distribution of the ratios.

Considering the ratios and the actual organ weight, certain observations were made. An increase in heart weight was observed to result from exposure of the animals to cold, but the administration of horse serum, bovine gamma globulin, ACTH, Cortisone or the presence or absence of lesions of the heart, arteries or kidneys were not accompanied by changes in the weight of the heart.

The weight of the kidney increased in relation to the occurrence of severe glomerulonephritis and slightly in association with exposure to cold and treatment with Cortisone. No effect was observed in the kidney weight as a result of ACTH treatment.

The weight of the liver remained in good proportion to the body weight in spite of a loss of body weight under all circumstances except treatment with Cortisone, where the liver showed a marked increase in weight associated with a considerable accumulation of intracellular glycogen. There was no association between the weight of the liver and the occurrence of lesions of the heart, arteries or kidneys resulting from sensitization to horse serum or bovine gamma globulin.

The weight of the spleen was observed to increase in association with sensitization to horse serum or bovine gamma globulin, and to decrease as a result of treatment with Cortisone. There was no correlation between the size of the spleen and the occurrence of lesions.

The pituitary weight was observed to be very constant and not influenced to any great extent by the weight of the animal. A slight increase in the weight of this gland was noted to result from treatment with horse serum, bovine gamma globulin in Freund's adjuvant and from exposure to cold. Treatment with ACTH appeared to increase the pituitary weight, and Cortisone to decrease it. There was no correlation between the weight of the pituitary and the development of lesions of the heart, arteries and kidneys from the sensitization to the foreign serum proteins.

A slight increase in the weight of the adrenals was observed to occur as a result of exposure to cold, but not even this slight increase in adrenal weight was seen to occur in the animals treated with horse serum or bovine gamma globulin. Within the normal distribution, lesions resulting from the administration of foreign serum proteins did not develop in any consistent relationship with the size of these glands. ACTH and Cortisone produced an enlargement and slight reduction respectively in the size of the adrenals.

A reduction of the weight of the thymus was observed in all the treated groups in these experiments. However, there was no relationship between the size of the thymus and the development of lesions of the heart, arteries and kidneys induced by sensitization to foreign serum proteins.

It was concluded, particularly from the data on the adrenal and thymus weights, that there was no evidence from these data that indicated an important pathogenetical relationship between the "General Adaptation Syndrome" of Selye and the cardiac valvulitis, necrotizing arteritis and glomerulonephritis induced in rabbits by two massive intravenous injections of horse serum while exposed to cold or at room temperature; or to glomerulonephritis induced by two intramuscular injections of bovine gamma globulin in Freund's adjuvant.

Food Intake.

In Experiments II, III, IV and V the daily food intake of each animal was recorded. It was evident throughout the experiment that when animals became sick one of the first signs was loss of appetite. Thus, in consideration of the results, it was obviously of no use to consider the animals that were sick and did not survive the whole experiment. Furthermore, if the animals did not survive the experimental period beyond the second serum injection, the prospect for development of lesions was poor, so that in order to consider the influence of food intake on the development of lesions it was also necessary to exclude those animals that did not survive to the point where they could reasonably be expected to develop them.

The final results were drawn up for each individual animal that survived the whole experimental period. The average food intake for each period of the experiment was calculated, tabulated and correlated with the

occurrence of lesions. These data are shown in Tables VIII to XIII of Appendix B. Finally, the mean of all the animals' food intake was calculated for each period of the experiment for each experimental group; and the mean food intake of animals in each group was calculated with reference to the occurrence or absence of lesions of the heart (including arteries) and kidneys. The figure for average normal food intake is of all animals during the periods when they were not under treatment. These data were tabulated and are represented by Table 14.

TABLE 14.

AVERAGE DAILY FOOD INTAKE IN GRAMS OF ANIMALS SURVIVING THE WHOLE EXPERIMENTAL PERIOD OF EXPERIMENTS 2, 3, 4 and 5.

Group	Control Period.	Cortisone or ACTH at Rm. Temp.	Cold Control Period.	After 1st. Serum Inject^{n}	After 2nd. Serum Inject^{n}	Average of whole Exp't.	Av. with heart lesions.	Av. without heart lesions.	Av. with kidney lesions.	Av. without kidney lesions.
I	131.9 S.D. 44.6 (738)									
II				133.5 (149)	119.4 (90)	97.7 (355)	135.75 (8)	111.7 (4)	133.0 (9)	111.8 (3)
III			→	161.5 (160)	←	152.5 (200)				
IV			126.6 (30)	150.1 (125)	138.8 (73)	145.7 (289)	153.5 (6)	133.8 (4)	143.3 (7)	155.0 (3)
V		93.3 (34)	93.9 (36)	123.4 (136)	131.9 (95)	121.1 (331)	143. (4)	109.4 (8)	126.3 (3)	118.8 (9)
VIII			X	113.3 (98)	110.6 (103)	118.0 (261)	129.8 (1)	116.8 (9)	118.7 (6)	117.0 (4)
IX		108.4 (18)	X	160.6 (60)	125.0 (62)	141.1 (158)			137.1 (3)	144.8 (3)

Numbers in brackets = animal days of feeding
X = period not applicable to this group.

Numbers in brackets = number of animals.

It is evident from consideration of Table 14 that there are no periods of food intake that show a statistically difference from the average normal, considering that a statistically valid rise or fall would have to be almost above or below two standard deviations from the mean. Therefore, all the differences to be discussed below are of the order of suggestive variations.

Horse serum given at room temperature appears to be associated with a diminished food intake, and the lowest food intake occurs in the animals that do not have lesions of the heart or kidneys.

Animals exposed to cold appear to eat more than the average normal at room temperature. When horse serum is given to animals in the cold room, they appear to eat somewhat less after the second injection, and their average daily intake for the whole period is smaller than average. Those not developing heart lesions have a lower food intake than those that do show lesions. The correlation with the occurrence of kidney lesions shows that the food intake is greater in those that manifest glomerulonephritis than in those that do not. However, there are only two animals in the latter group.

Under ACTH treatment animals tend to have a smaller food intake than the average normal or than the animals exposed to cold alone. Again, the animals that do not develop lesions of either heart or kidneys have a lower food intake than those that do. A similar trend is apparent in Group VIII, though the reverse is true in Group IX.

In summary, the food intake of animals that develop lesions of heart or kidneys tends to be higher than in those that do not manifest the

lesions. Whether or not this is an important point in the pathogenesis of the lesions cannot be determined from these experiments. Results of an experiment performed in this laboratory by Dr. D. Waugh, in which rabbits were kept on a restricted food intake during sensitization to serum proteins, produced a striking absence of the lesions of the kidneys[132]. However, the number of animals used did not permit final conclusions to be drawn, and the series will be enlarged in the future. It may, then, be that the quantity of food ingested during the experiment is of importance in the pathogenesis of the lesions. Whether the occurrence of lesions is also related to the quality of the food is a subject that would bear study in the future. In this connection, Farr and Smadel[145] have pointed out that restriction of dietary protein inhibits the occurrence of chronic glomerulonephritis in certain strains of rats treated with rabbit anti-kidney serum. The experiments of Selye and associates[155,213-215,220], and Krakower and Goettsch[216] appear to indicate a possible influence of feeding sodium chloride on the development of renal lesions. The influence of qualitative as well as quantitative alterations of the diet in the pathogenesis of the lesions deserves further study.

Blood Sugars.

The blood sugars were determined in all the animals of experiments 2, 3, 4 and 5. The methods of collecting the serum and of making the determinations were described in the section on Materials and Methods - General. The method of representing this data presented a considerable problem, since it is necessary to consider the blood sugar levels throughout the experiment for each animal. It is not sufficient, as with the food intake, to consider a total or terminal level. It was, therefore, decided to include the data on each group of animals in the main portion of the text in order to evaluate changes that might correlate with the presence or absence of lesions. Later, the average values for the whole groups will be considered together.

In the Tables and Figures that follow the bleedings are indicated by the letters A to H. All groups of experiments 2, 3 and 4 were bled at the same time, so that while bleeding "C" represents the sample of blood that was taken after the cold room groups were placed in the cold room, it represented a sample taken before any treatment was administered to the room temperature-horse serum animals. To aid in clarifying this situation, the treatment of each group is indicated in the Text-figs. of the individual groups. However, it is clearly impossible to make a composite chart of all the groups showing the differences between them. Thus, in the chart of "Average Values" it must be remembered that:

Bleeding A represents the sample taken during the untreated control period,

Bleeding B " " " " the day after commencement of Cortisone or ACTH injections.

Bleeding C " " " " the day after the cold room groups were placed in the cold.

Bleeding D " " " " the day after the first massive injection of horse serum.

Bleeding E " " " " mid day between the two massive horse serum injections.

Bleeding F " " " " the day before the second massive horse serum injection.

Bleeding G " " " " the day after the second massive serum injection.

Bleeding H " " " " on the day of autopsy.

TABLE 15

BLOOD SUGARS = Mg.% OF UNTREATED CONTROLS, GROUP I.

Animal No.	Bleeding								Lesions of -		
	A	B	C	D	E	F	G	H	Valves	Arteries	Kidneys
K-38	126		128	120	133	110	139	130	0	0	0
K-40	153	158	148	112	120	149	130	118	0	0	0
L-3	115	108	141	160	125	113		136	0	0	0
L-17	100	140	115	140	128	113		152	0	0	0
L-19	94	123	121	123	135	101		140	0	0	0
L-43	130	126	122	135	126	120	178	148	0	0	0
L-48	167	144	128	140	126	198	198	160	0	0	0
L-55	210	165	170	171	188	168			0	0	0
N-85	140	150	118	140	105	155	140	159	0	0	0
N-91	124	95	140	74	104	143			0	0	0
O-74	103	112	134	120	140	100		114	0	0	0
Mean		131. S.D. 30.916*									

* determined along with pretreatment bleedings of all other groups.

Table 16 of the untreated control animals, indicates the distribution of blood sugars in animals not treated in any way, but bled at the intervals corresponding to the treated groups. The mean normal blood sugar was calculated from the values in the Table, and also the values of all the animals during their untreated control periods, comprising 233 determinations in all. The mean normal random blood sugar was 131 mg.% and the standard deviation was 30.916 mg.%. Thus, the only individual or average values that will be regarded as statistically significant will be those that are lower than 69 mg.%, or higher than 193 mg.%

TABLE 16

BLOOD SUGARS mg.% (GROUP II) ROOM TEMPERATURE HORSE SERUM.

Animal No.	I/V HS I ↓ Bleeding A	B	C	D	I/V HS II ↓ Bleeding E	F	G	H	Valve Lesions	Artery Lesions	Glomerulo-nephritis	Day of Death	
K-27	139	110	105	113	110	113			++	++	0	21	X
K-29	139	117	128	134	98	130	136	128	+	++++	+	20	
L-7	80	70	138	141	123	98	125		+++	++++	+	25	
L-8	73	90	148	140	141	110	115		+	+	+	25	
L-12	98	173	119	146	117	93	108		0	0	+	25	
L-46	235	110	140	135	120	144	120	148	0	0	0	25	
L-47	153	100	130	140	144	120	144	136	+++	++++	++	25	
L-61	150	210	126	148	144	130	140	160	+	++	++	25	
N-83	196	136	174	170	100	159	160	159	+++	++	++	24	
N-89	94	122	105	140	45				0	0	0	13	X
N-95	145	148	125	118	114	118	148	148	++	++++	+	24	
D-55	130	160	128	136	109		113	109	0	0	+	25	
D-58	101	121	125	154	140		125	115	0	0	0	25	
Mean	131.50			138.8	115.8	121.5	130.	133.8					
S.D.	30.916			(13)	(13)	(10)	(11)	(8)					

X = Died spontaneously.

Figures in brackets = Number of animals.

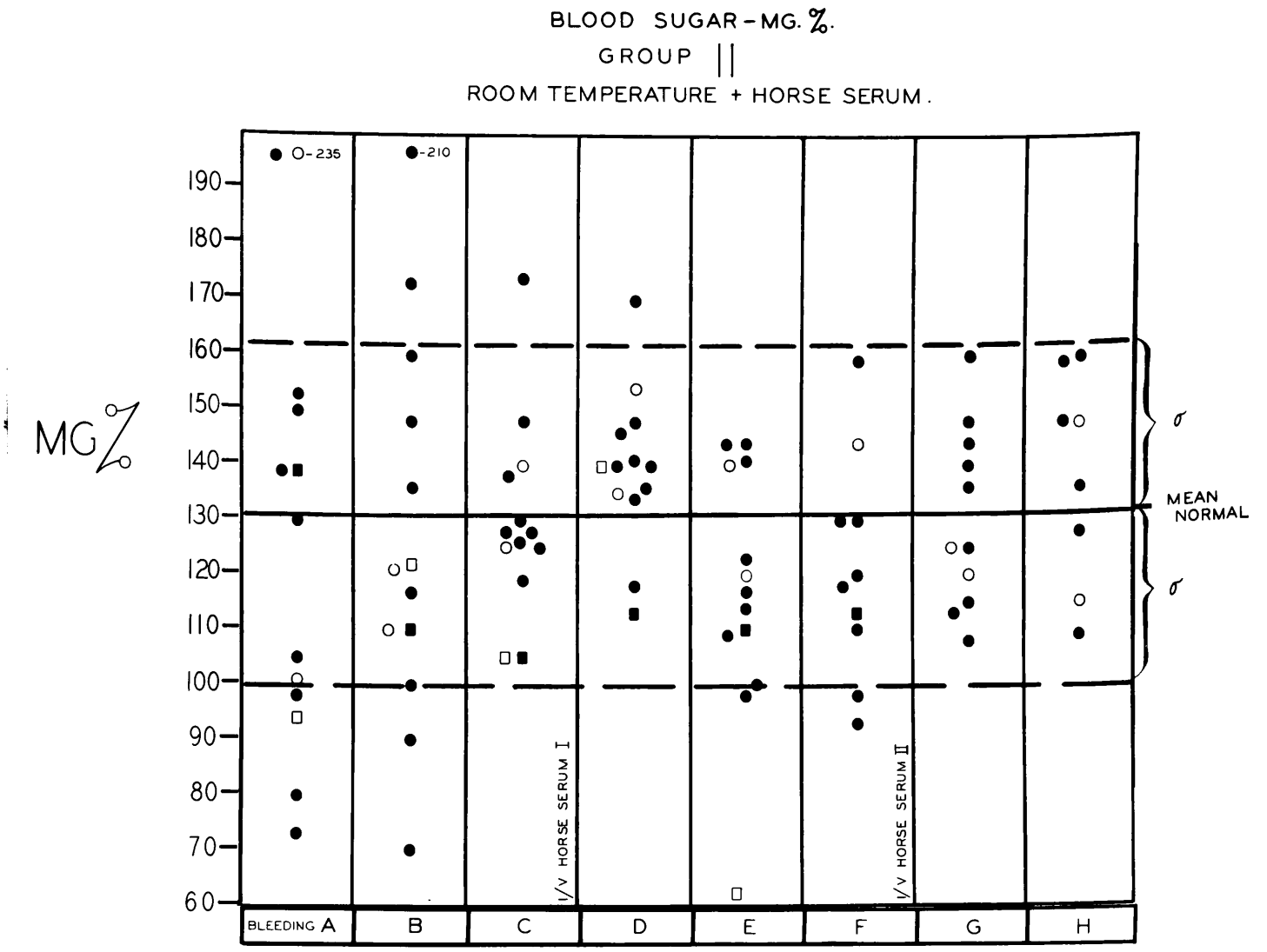


Fig. 48.

It may be seen from Table 16 and Fig. 48 that there is no correlation between the level of the blood sugar at any stage of the experiment, in this group, and the occurrence of lesions of the heart (and arteries) and kidneys.

TABLE 17

BLOOD SUGARS mg.% (GROUP III) UNTREATED COLD CONTROLS

Animal No.	A	B	COLD C	D	E	F	G	H	Valve Lesions	Artery Lesions	Kidney Lesions	Day of Death
K-28	147	147	125	125	140				0	0	0	21 X
K-31	167	92	90	87	108	112	118	125	0	0	0	29
L-2	75	90	145	110					0	0	0	9 X
L-4	88	119	140	143	134	110			0	0	0	24
L-16	98	110	125	164	125	148			0	0	0	22 X
L-52	130	126	110	70	140	118			0	0	0	15 X
L-54	149	134	115	120	160	155	144	188	0	0	0	22
L-63	249	90	140	135	160	288	210	210	0	0	0	21
N-86	180	118	95	140	94	99			0	0	0	16 X
N-90	160	125	100	130	170	105			0	0	0	16 X
N-93	150	188	100	126	137	125			0	0	0	16 X
O-42	114	137	144	148		118	104	111	0	0	0	24
O-48	103	249	125	133		158	196	144	0	0	0	25
O-51	105	114	148	118		158	220		0	0	0	24 X
O-63	148	100	120	130		134			0	0	0	22 X
O-75	118	104							0	0	0	6 X
O-83	130	137	119	128		147	115	151	0	0	0	25
Mean	131		121.3	125.4	136.8	141.	158.1	154.8				
S.D.	30.916		(16)	(16)	(10)	(14)	(7)	(6)				

X = Died spontaneously.
Figures in brackets = Number of animals.

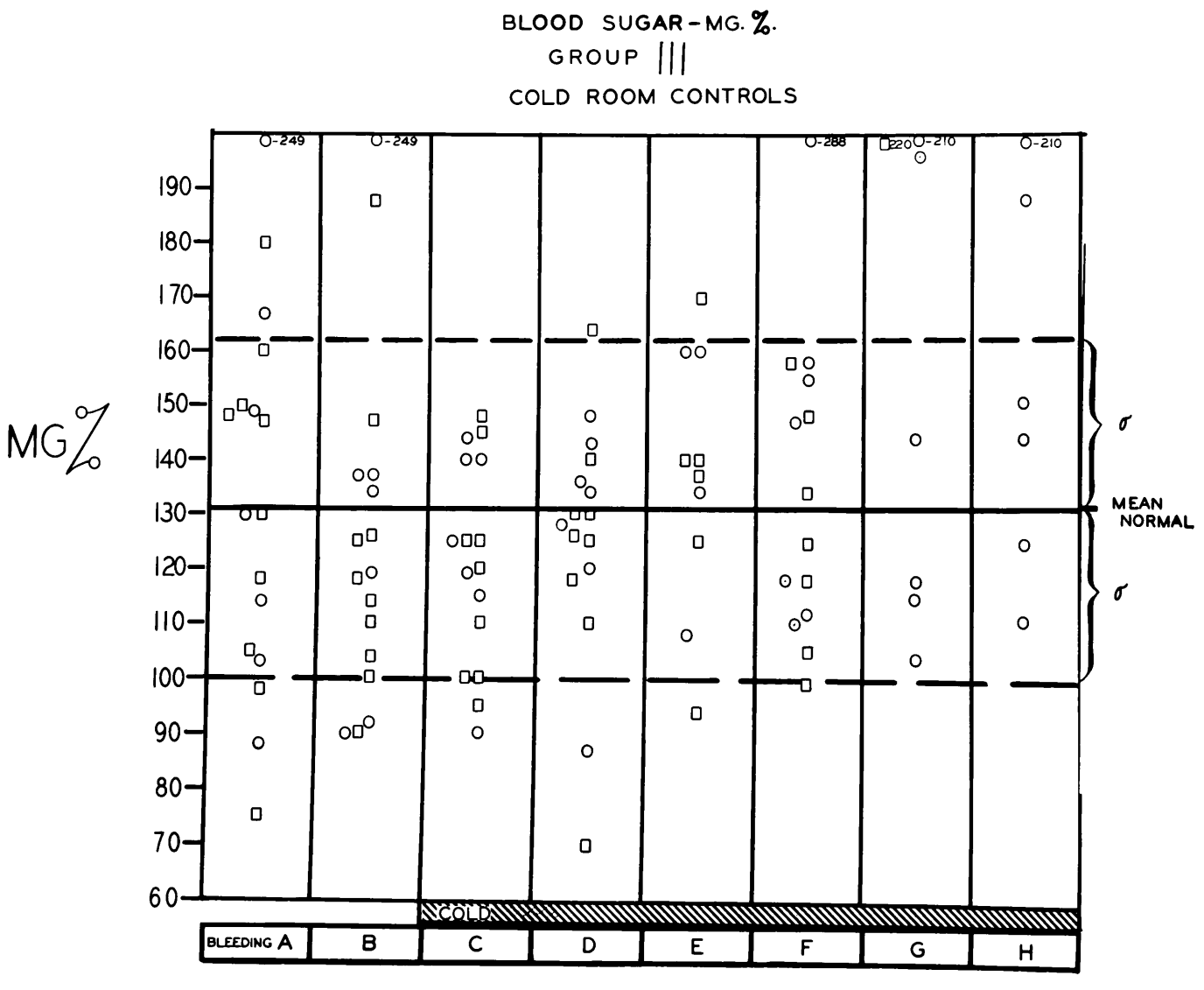


Fig. 49

Consideration of Table 17 and Fig. 49 indicates that during the period of exposure to cold a few animals showed a statistically significant elevation of the blood sugar, although this is not expressed in the average values (Table 22) where the elevations are evident but not statistically significant. There is no correlation between the blood sugar levels in the animals dying spontaneously and those surviving the whole experimental period.

TABLE 18

BLOOD SUGARS mg.% (GROUP IV) COLD - HORSE SERUM

Animal No.	A	B	COLD C	I/V H.S. I D	E	F	I/V H.S. II G	H	Valve Lesions	Artery Lesions	Kidney Lesions	Day of Death
K-26	139	116	128	136	136	128	115	134	?	0	+	29
K-32		148	102	113	184	134	137	143	+	+	0	29
L-5	119	130	117	134	134	138	130		++	+	+	24
L-6	95	132	125	148	240				0	0	0	12 X
L-13	121	134	140	128	128	147	90		+++	+++	++++	24
L-51	142	115	135	98	182	130	168	150	+	+	+	24
L-57	180	140	142	150	160	152	140	144	+++	++++	++	24
L-64	259	70	140	144	152	165	175	362	0	0	0	24
N-79	152	160	120	140	144	160	161	190	0	0	++	25
N-82	220	140	99	154	129	104	178	227	0	0	+	25
N-92	170	150	130	160	99	115	150	151	+	0	+++	25
Mean 131. S.D. 30.916			129.8 (11)	136.8 (11)	153.8 (11)	136.9 (10)	144.4 (10)	187.6 (8)				

X = Died spontaneously.
Figures in brackets = Number of animals.

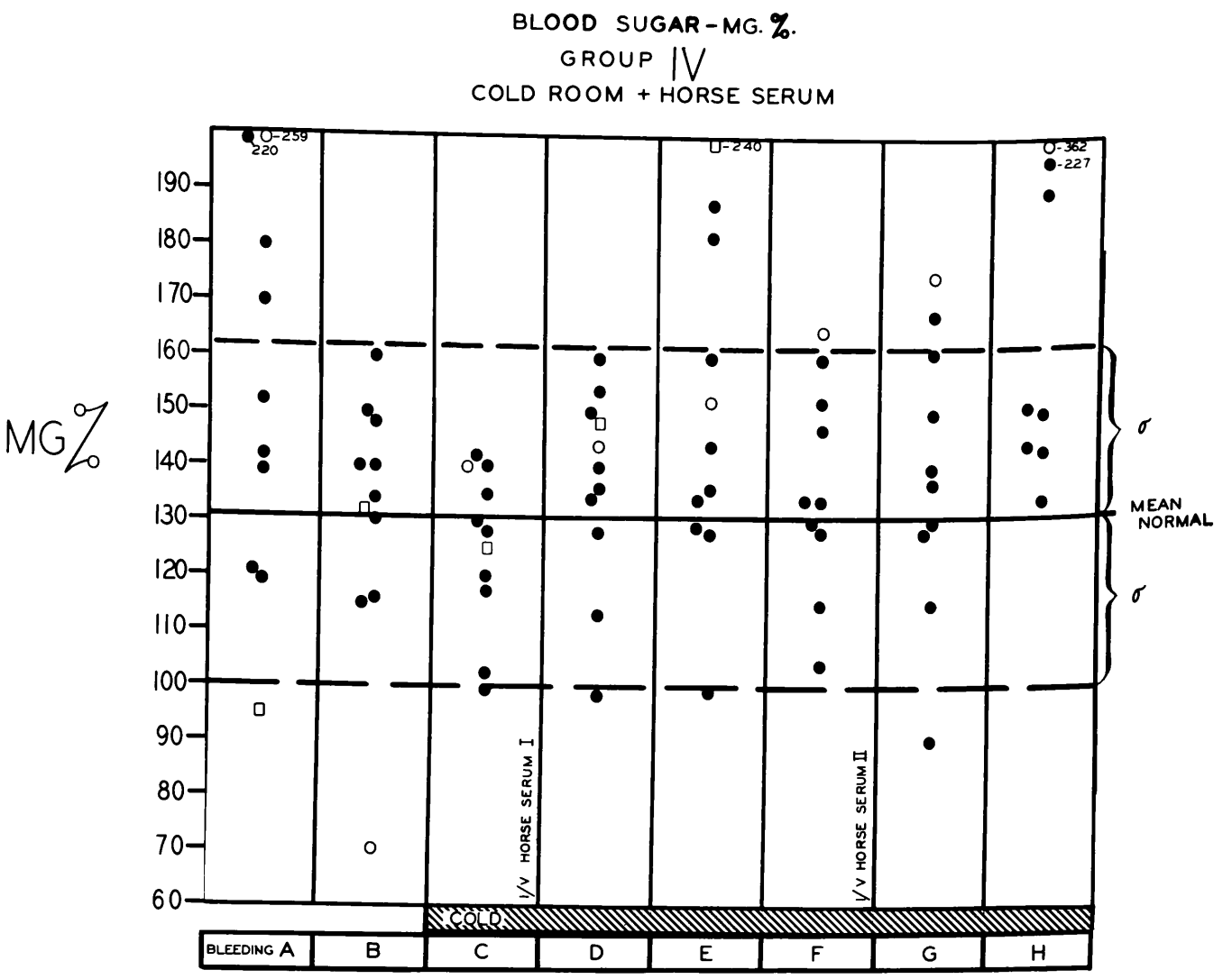


Fig. 50.

Table 18 and Fig. 50 indicate that during the control and pre-horse serum cold periods, there is no correlation between the levels of the blood sugar and the occurrence of lesions of the heart (including arteries) and kidneys. However, in the post-horse serum periods, some of the highest values are those of animals that do not develop lesions. On the other hand, this is by no means a uniform effect. The elevation of the average of the last bleeding is due to a marked elevation of the blood sugar in 2 of the 8 animals studied at this stage.

TABLE 19

BLOOD SUGARS mg.% (GROUP V) COLD - HORSE SERUM - ACTH

Animal No.	A	B	C	D	E	F	G	H	Valve Lesions	Artery Lesions	Kidney Lesions	Day of Death
		ACTH										
		COLD ↓		I/V H.S. I ↓			I/V H.S. II ↓					
K-30	119	93	82	110	188	140	123	130	+	+	0	29
K-36	139	139	136	116	140				0	0	0	15 X
L-1	123	128	140	148	125	113		130	+	+++	++	24
L-18	123	117	110	149	120	140		136	+	+++	+	24
L-20	95	87	130	143	110	116		122	+	+	0	24
L-44		60	148	454	480				0	0	0	14 X
L-49	128	187	175	100	152				0	0	0	13 X
L-67	171	170	83	168	152	140	160		0	0	0	24 X
L-80	128	150	134	160	148	120	170		0	0	0	19 X
N-96	114	134	70	148	230	188	151	178	0	0	0	25
N-97	105	160	110	140	134	123	123		0	0	0	24 X
O-43	110	165	131	136		114	99	120	0	0	+	25
O-53	108	170	140	144		210	179	153	0	0	0	25
O-54	79	180	134	125		147	188	154	0	0	0	25
O-66	108	121	115	119		115			0	0	++	20 X
O-76	118	140	134	130		107	104	109	0	0	0	25
O-80	125	156	144	144		120	104		0	0	0	25
Mean	131	138.6	125.5	153.9	189.	135.2	140.1	136.9				
S.D.	30.916	(17)	(17)	(17)	(11)	(14)	(10)	(9)				

X = Died spontaneously
Figures in brackets = Number of animals.

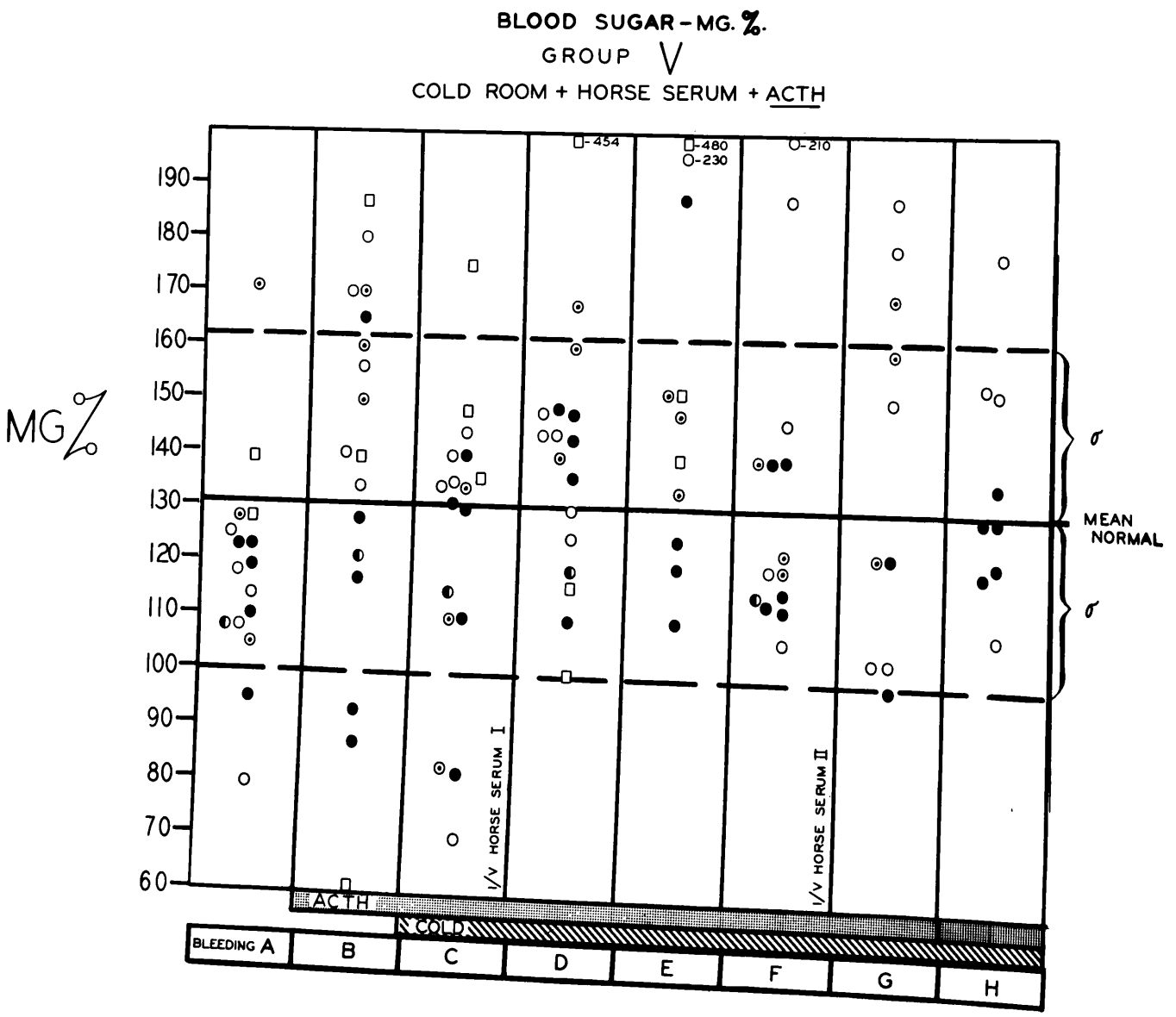


Fig. 51

Table 19 and Fig. 51 indicate that there was a statistically significant elevation of the blood sugar only in a few animals, occurring after the injection of horse serum. Many of the values tend to be in the higher range immediately after the first day of ACTH treatment, and animals that did not develop lesions appear to occupy the higher range at this time. After the injections of horse serum, there again appears to be a tendency for the animals that have a higher blood sugar to coincide with the non-development of lesions. However, this is by no means a uniform trend..

TABLE 20.

BLOOD SUGARS mg.% (GROUP VIII) GLOBULIN - ADJUVANT.

Animal No.	A	B I/M Glob.I ↓	C I/M Glob.II ↓	D	E	Valve Lesions	Artery Lesions	Kidney Lesions	Day of Death
0-46	140	133	146	113	144	0	0	++	22
0-50	105	152	128	100	124	0	+	++	22
0-52	103	160	136	100	107	0	0	+	23
0-64	130	124	131	126	114	0	0	+++	23
0-67	128	122	131	114	113	0	0	0	22
0-69	140	120	134	120	122	0	0	++	23
0-71	140	100	98	100	143	0	0	0	22
0-79	132	126	98	106	140	0	0	0	22
0-81	133	114	128	100	112	0	0	+	28
0-82	105	112	129	122	109	0	0	0	22
Mean 131 S.D. 30.916			125.9 (10)	110.1 (10)	122.8 (10)				

Numbers in brackets = Number of animals.

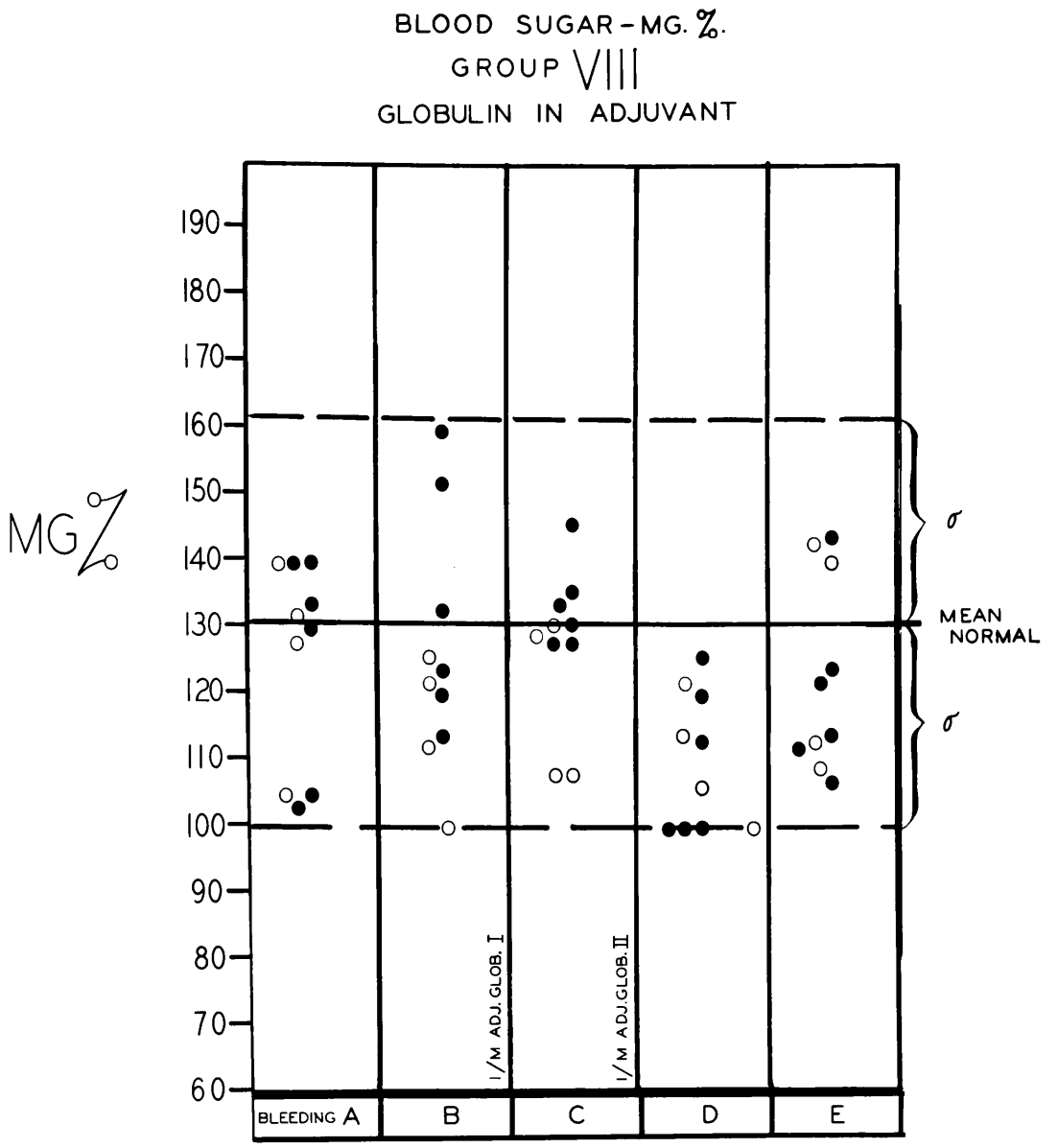


Fig. 52.

It is evident from Table 20 and Fig. 52 that there is no correlation between the levels of the blood sugars and the occurrence of lesions in the heart (including arteries) and kidneys induced in rabbits by two injections of bovine gamma globulin in Freund's adjuvant. In this group, the correlation may be considered as being with the kidney lesions alone, since the occurrence of arteritis and valvulitis was negligible.

TABLE 21

BLOOD SUGARS mg.% (GROUP IX) GLOBULIN - ADJUVANT + CORTISONE

Animal No.	CORTISONE A	B	I/M Glob.I C	I/M Glob.II D	E	Valve Lesions	Artery Lesions	Kidney Lesions	Day of Death
0-44	144	212	165			0	0	0	17 X
0-47	94	218	398	337	840	0	0	0	23
0-49	130	200	159	178		0	0	0	17 X
0-56	118	215	158			0	0	0	18 X
0-59	98	220	187			0	0	0	10 X
0-60	116	215	265	904	1160	0	0	0	18 X
0-61	110	143	169			0	0	0	16 X
0-65	151	159	226	215	134	0	0	+	23
0-68	118	135	220	215	125	0	0	+	23
0-70	110	192	362	365	337	0	0	0	24
0-73	123	159	152	190	352	0	0	0	24
0-78	118	143	243	213	444	0	0	+	24
Mean	131.	184.2	225.3	327.1	484.6				
S.D.	30.916	(12)	(12)	(8)	(2)				

Figures in brackets = Number of animals
X = Died spontaneously.

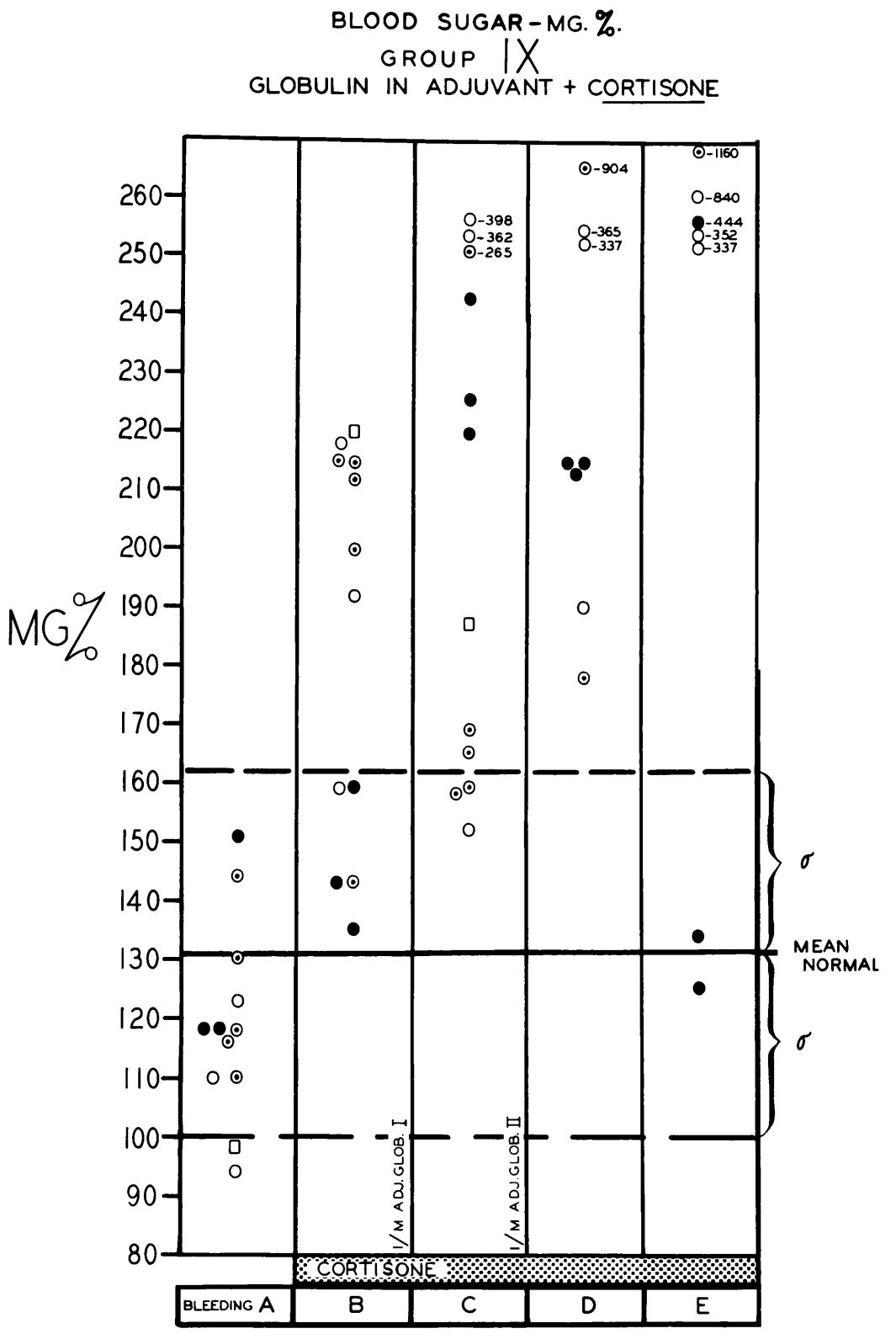


Fig. 53.

From Table 21 and Fig. 53 it is readily evident that the most significant elevations of any of the groups occurred in the blood sugar levels of the animals treated with Cortisone. Most of the animals showed

an elevation of the blood sugar the day after commencement of Cortisone. At this time all the animals showing a good response to Cortisone were those that did not develop lesions. Analysis of the data for the later periods reveals that the animals that did not ultimately show lesions manifested the higher blood sugars. Again, this statement is not without exceptions.

TABLE 22

BLOOD SUGARS mg.%.
AVERAGE VALUES OF ALL GROUPS.

Groups		Bleeding A	B	C	D	E	F	G	H
Rm. Temp. Controls	I								
Rm. Temp. + Horse Serum	II				138.85 (13)	115.85 (13)	121.5 (10)	130.36 (11)	130.57 (8)
Cold room Controls	III			121.31 (16)	125.44 (16)	136.8 (10)	141.0 (14)	158.14 (7)	154.82 (6)
Cold room + Horse Serum	IV			129.82 (11)	136.82 (11)	153.82 (11)	136.9 (10)	144.4 (10)	187.62 (8)
Cold room, Horse Serum + ACTH	V		138.65 (17)	125.47 (17)	153.94 (17)	189.0 (11)	135.21 (14)	140.1 (14)	136.89 (9)
Adj. Glob.	VIII			125.9 (10)	110.1 (10)	122.8 (10)	-	-	-
Adj. Glob. + Cortisone	IX		184.25 (12)	225.33 (12)	327.12 (8)	484.57 (7)	-	-	-

131.5 S.D. (223*) 30.916

Figures in brackets = Number of animals

* Number of normal determinations

From Table 22 it may be seen that only in the Cortisone-treated group does an average increase in blood sugar occur that is statistically

significant. However, in the cold room, Groups III and IV, the animals manifest a slight elevation of the blood sugar toward the end of the experiment. In the mid-portion of the experiment and the day after commencement of ACTH injection, Group V shows a moderate elevation in the average blood sugar.

It only remains necessary to consider the mean variation of blood sugars as correlated with the presence or absence of lesions. This information is contained in Table 23.

TABLE 23

BLOOD SUGARS mg.%
COMPARISON OF AVERAGE VALUES OF ANIMALS WITH AND WITHOUT LESIONS.

Group	Mean of animals	Bleeding A	B	C	D	E	F	G	H
I	with lesions								
	without lesions								
II	with lesions				138.6 (10)	120.1 (10)	122.2 (8)	132.1 (9)	140.0 (6)
	without lesions				143.0 (2)	103.3 (3)	144.0 (1)	122.5 (2)	131.5 (2)
IV	with lesions			112.5 (9)	134.8 (9)	148.4 (9)	133.8 (9)	141.0 (9)	162.7 (7)
	without lesions			132.5 (2)	146.0 (2)	196.0 (2)	165.0 (1)	175.0 (1)	262.0 (1)
V	with lesions	118.5 (8)	118.0 (6)	134.2 (6)	135.7 (4)	122.0 (6)	111.0 (2)	127.6 (5)	
	without lesions	149.6 (11)	128.0 (11)	166.3 (11)	228.8 (5)	144.4 (8)	147.4 (8)	148.5 (4)	
VIII	with lesions		133.8 (6)	109.8 (6)	120.5 (6)				
	without lesions		114.0 (4)	112.0 (3)	126.2 (5)				
IX	with lesions	145.7 (3)	229.7 (3)	214.3 (3)	234.3 (3)				
	without lesions	198.2 (9)	223.9 (9)	394.8 (5)	896.3 (3)				

131.5 S.D. 30.916 (223*)

Figures in brackets = Number of animals

* Number of normal determinations

From Table 23 it is again evident that there was no consistent statistically significant variation of the blood sugar values in any group, except the Cortisone-treated group. However, in one bleeding the animals free from lesions in Group V showed a significant elevation. Both within the limits of the normal distribution and of statistical significance in the instances mentioned above, the animals without lesions appear to have manifested the higher blood sugars. This tendency is most marked in Groups IV, V and IX, not present in Group II, and only minimally in Group VIII.

In summary, no statistically significant variations of the blood sugar level was observed to occur in any of the groups other than the one treated with Cortisone, and one group of animals treated with ACTH that did not develop lesions. However, by considering the distribution of the blood sugars with respect to the occurrence of lesions, there was a tendency detectable in some of the groups (IV, V and IX) toward a correlation between a high blood sugar and an absence of lesions. In this connection, it must be emphasized that these variations were by no means statistically significant. Certainly, no valid curve of blood sugar could be drawn similar to the one presented by Selye[206,207], to which many objections may be made. The distribution of the blood sugars was not indicated in his animals. Furthermore, each point of his curve was drawn from a different group of rabbits, and the basic assumption involved in such calculations was that the changes that he expected were consistent in all animals throughout the course of the treatment. This assumption was never proven.

Furthermore, the curve for fasted animals is similar in form and magnitude to the one representing animals that were allowed to eat. Unless the eating habits of the rats he used were completely different from those of our rabbits, it is difficult to credit such results. It is possible to state from our results that in the animals treated with Cortisone or ACTH a high elevation of blood sugar was associated with inhibition of the lesions. This may be an indication of the efficacy of the treatment and may be correlated with the higher blood sugars present in animals of the other groups that did not develop lesions. However, the magnitude of the changes was so different as to make comparison hazardous. On the basis of the changes in blood it is impossible to ascribe a major role to the adreno-hypophyseal mechanism in the pathogenesis of the lesions produced by foreign serum protein sensitization in rabbits, although it cannot be denied that it may play some more minor role in the processes involved.

Blood Urea Nitrogen

The blood urea nitrogen values were calculated from blood collected during the control period and the last two bleedings of most of the animals in Experiments II, III, IV and V. The determinations were made as detailed in the section on Materials and Methods - General.

The average normal blood urea nitrogen value was calculated from the control bleedings of all the groups and from all the determinations in the room temperature control group. This value was 16.27 mg.%, and the standard deviation 6.02. Thus, in the Tables that follow, any single value

will only be considered to be statistically significant when it exceeds 28.67 mg.%, or is less than 4.27 mg.%

TABLE 24

BLOOD UREA NITROGEN mg.%
ROOM TEMPERATURE - HORSE SERUM. (Group II)

Animal No.	Bleeding A	G	H	Lesions. Valve	Artery	Kidney	Day of death	
L-7	11.4	25.3		++	+	+	25	
L-12	7.4	22.0		0	0	+	25	
L-46	-	10.8	18.9	0	0	0	25	
L-47	16.8	31.1	34.5	+++	++++	++	25	
L-61	17.6	17.3	35.2	+	+	++	25	
N-83	38.1	11.4	25.1	+++	++	++	24	
N-89	25.8	17.8		0	0	0	13	X
N-95	21.5	14.6	16.1	++	++++	+	24	
O-55	15.3	67.6	68.0	0	0	+	25	
O-58	12.6	20.5	22.4	0	0	0	25	
Mean	16.27							
S.D.	6.02*							

*Standard deviation calculated from Bleeding A of this group and the normal control bleeding of all animals in the experimental groups.

In this group it is evident that the terminal blood urea nitrogen value is elevated beyond twice the standard deviation in only three animals. Two of these showed ++ glomerulonephritis and one +. The remaining animals that had glomerulonephritis had blood urea nitrogens higher than the average normal and one standard deviation. It is, therefore, true to say that the blood urea nitrogen is elevated in animals that have glomerulonephritis but normal in those that do not develop the lesions. The elevations are on the borderline of statistical significance when taken all together.

TABLE 25

BLOOD UREA NITROGEN mg.%
COLD ROOM CONTROLS (Group III)

Animal No.	Bleeding A	B	D	E	F	G	H	Lesions. Valve	Artery	Kidney	Day of death.
L-2	8.0		20.9					0	0	0	9 X
L-16	11.8							0	0	0	22
L-52	4.2			6.9	21.7			0	0	0	15 X
L-54					18.3	31.6	30.2	0	0	0	22
L-63	20.2				21.1		32.4	0	0	0	21
N-86	25.8			19.1		24.3		0	0	0	16 X
N-90	22.1			16.4	20.9			0	0	0	16 X
N-93	16.1			24.1	22.9			0	0	0	16 X
O-42	13.3					20.9	19.7	0	0	0	24
O-48	15.2					20.3	26.1	0	0	0	25
O-51	16.2			13.6		35.8		0	0	0	24 X
O-63	13.6		15.0	17.0				0	0	0	22 X
O-75	7.2	17.0						0	0	0	6 X
O-83	12.6					14.2	19.9	0	0	0	25

Mean 16.27 S.D. 6.02

X = Died spontaneously

Although the number of determinations in this group is not great it appears correct to say that an elevation of the blood urea nitrogen to statistically significant levels is possible, even if the animals do not manifest any tissue lesions.

TABLE 26

BLOOD UREA NITROGEN mg.%
COLD ROOM - HORSE SERUM (Group IV)

Animal No.	Bleeding A	F	G	H	Lesions Valve	Artery	Kidney	Day of Death
L-5	11.4		27.3		+	+	+	24
L-13	12.5		61.5		++	+	+	24
L-51	18.9	16.3	21.8	19.9	+	+	+	24
L-57	18.9	19.1	25.9	34.6	+++	++++	++	24
L-64	16.8	18.3	23.3	24.5	0	0	0	24
N-79	27.2		14.6	32.4	0	0	++	25
N-82	23.6		16.7	31.0	0	0	+	25
N-92	18.7		18.7	27.2	+	0	+++	25
Mean	16.27							

In Group IV the terminal blood urea nitrogen levels were elevated in all the animals but one; that is, even in the animals without lesions of the kidneys. In this group there is no correlation between the level of the blood urea nitrogen and the presence or severity of the lesions. It appears that the rise in blood urea nitrogen is an expression of the exposure to cold (c.f.Group III) as well as of the lesions of the kidney, as in Group II.

TABLE 27

BLOOD UREA NITROGEN mg.%
COLD - HORSE SERUM - ACTH (Group V)

Animal No.	Bleeding A	B	D	E	F	G	H	Lesions. Valve	Artery	Kidney	Day of Death
L-1	7.4						18.1	+	+	0	24
L-18	16.3						26.4	+	+++	++	24
L-20	17.9						30.1	+	+++	+	24
L-44				50.6	131.7			0	0	0	14 X
L-49	11.6		23.8	47.0				0	0	0	13 X
L-67	16.8				22.4	25.5		0	0	0	24 X
N-80	24.3				33.2	27.9		0	0	0	19 X
N-96	17.4					21.5	51.9	0	0	0	25
N-97	13.9					27.2		0	0	0	24 X
O-43	9.6					25.2	45.7	0	0	+	25
O-53	9.9					25.9	40.2	0	0	0	25
O-54	27.5	32.8				36.2	33.3	0	0	0	25
O-66	18.4			24.4	27.7			0	0	++	20 X
O-76	12.6				34.6	50.6		0	0	0	25
O-80	11.7				27.0	53.4		0	0	0	25
Mean	16.27										

X = Died spontaneously

In this group (V) the terminal blood urea nitrogen values tended to be high whether or not the animals developed lesions of the kidneys. These values were higher than those seen in exposure to cold alone, or with cold and serum injection. These exceptionally high values can probably by attributed to the ACTH treatment

TABLE 28

BLOOD UREA NITROGEN mg.%
GLOBULIN IN FREUND'S ADJUVANT (Group VIII)

Animal No.	Bleeding A	Bleeding D	Bleeding E	Lesions Valves	Lesions Artery	Lesions Kidney	Day of Death
0-46	12.8	32.0	15.2	0	0	++	22
0-50	12.2	18.6	18.8	0	+	++	22
0-52	13.6	12.6	14.7	0	0	+	23
0-64	19.0	40.6	23.5	0	0	++++	23
0-67	10.2	12.8	16.7	0	0	0	22
0-69	14.3	19.8	18.8	0	0	++	23
0-71	13.7	20.2	12.3	0	0	0	22
0-79	12.3	19.1	19.4	0	0	0	22
0-81	13.4	17.6	18.8	0	0	+	23
0-82	14.7	19.4	33.7	0	0	0	23
Mean	16.27						

In this group there is no correlation between the elevation of the blood urea nitrogen and the occurrence of the lesions. Indeed, the blood urea nitrogen was only elevated in two animals; one with and one without nephritis (the latter had the higher value).

TABLE 29

BLOOD UREA NITROGEN mg.%
GLOBULIN IN FREUND'S ADJUVANT + CORTISONE (Group IX)

Animal No.	Lesions A	B	C	D	E	Lesions. Valve	Artery	Kidney	Day of Death
0-44	9.1		13.7			0	0	0	17 X
0-47	11.4			21.8	32.8	0	0	0	23
0-49	9.9		14.4	12.3		0	0	0	17 X
0-56	13.6		15.1		23.6	0	0	0	18 X
0-59	12.7	18.6	14.0			0	0	0	10 X
0-60	12.6		15.1		26.5	0	0	0	18 X
0-61	12.5	24.5	14.5			0	0	0	16 X
0-65	17.1			17.5	25.1	0	0	+	23
0-68	16.2			13.3	28.1	0	0	+	23
0-70	23.1	28.1		22.4	30.8	+	0	0	24
0-73	15.2			24.3	69.6	0	0	0	24
0-78	19.1			24.4	111.6	0	0	+	24
Mean	16.27								

X = Died spontaneously

In Group IX the most marked elevation of blood urea nitrogen occurred of all the groups studied. There was no relation between the level of the blood urea nitrogen and the occurrence of glomerulonephritis. The elevations observed in these animals may have been due to the renal damage produced by the Cortisone itself (Fig.33).

Summary:

A rough correlation exists in many of the groups between an elevation of the blood urea nitrogen values and the occurrence of glomerulonephritis. Elevations of the blood urea nitrogen appear to occur in some

animals as a result of exposure to cold, ACTH administration and of the renal damage consequent on the administration of Cortisone.

Intradermal Tests.

Intradermal tests to graded dilutions of the antigens used in the treatment of the animals were made in the manner already described in the section on Materials and Methods - General. The average diameters of the oedema, redness, haemorrhage and necrosis were tabulated for each animal in every group. These data are included in Tables XIV to XX of Appendix B.

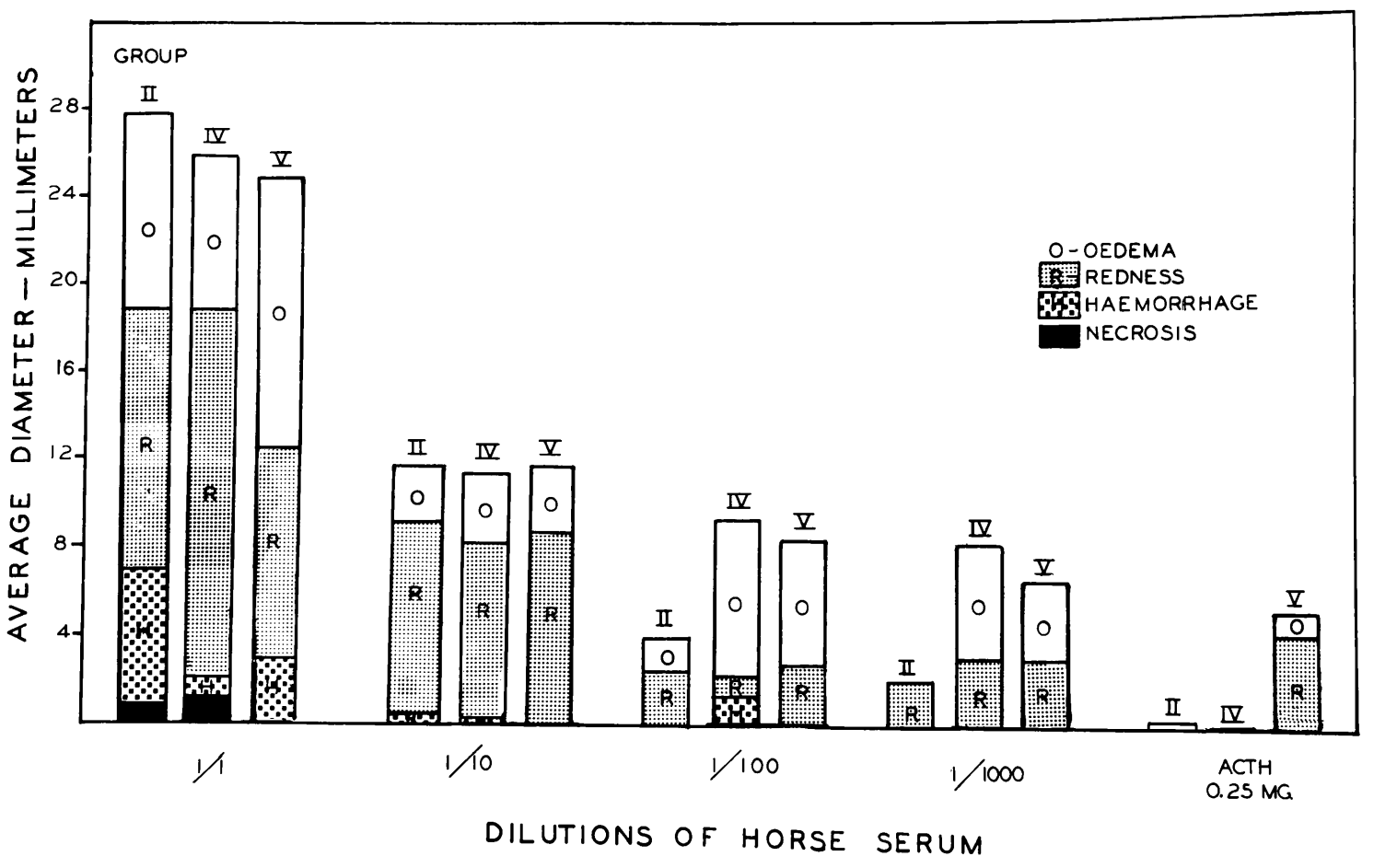


Fig. 54.

Since examining the data compiled for individual animals does not allow of ready analysis, the average diameters of all the animals in the groups was calculated and represented graphically in Text-figs. 54 and 55. The results were broken down further into the average diameters of the skin lesions, correlated with the presence or absence of lesions of the heart and kidneys and included in Fig. 56.

Fig. 54 represents the data of the groups treated with two massive intravenous injections of horse serum, Groups III, IV and V. Groups I and II are not included, since the results were negative in all but two of the animals in each that received three separate intradermal tests at about ten day intervals which served to sensitize them to horse serum so that their skin tests were positive. However, these animals did not have lesions of the heart or kidneys, and animals similarly treated are included in the other groups, so that this factor of sensitization may be ignored in consideration of the results. These animals will be referred to again in the discussion.

It may be seen from Fig. 54 that the difference in degree of skin reactivity in the different groups is very slight. A graded response proportional to the dilution of the test dose is evident in all the groups. With the higher concentrations (1/1 and 1/10), the magnitude of the average response in the animals treated with horse serum at room temperature appears to be somewhat greater than in the animals treated in the cold room (Group III), and the response of both these groups is somewhat more intense than that seen in the group also receiving ACTH (Group V). This applies to all the phases

of the reaction from the severest necrosis to the mildest oedema. However, with the less concentrated doses (1/100 and 1/1000), there appears to be an alteration in the order of intensity in the different groups, Group III being the greatest, the ACTH group (V) the second and the room temperature, horse serum group (II) the least intense. It should be noted that a minor degree of skin sensitivity developed in several animals to ACTH in Group V that was much greater than the non-specific reaction seen in the other groups. It is of particular interest, and will be considered again later, that the administration of ACTH did not exert a significant effect on the state of sensitivity of the group considered as a whole.

The skin sensitivity of the groups receiving bovine gamma globulin in Freund's adjuvant (VIII) and this mixture during Cortisone treatment (IX) is shown in Fig. 55.

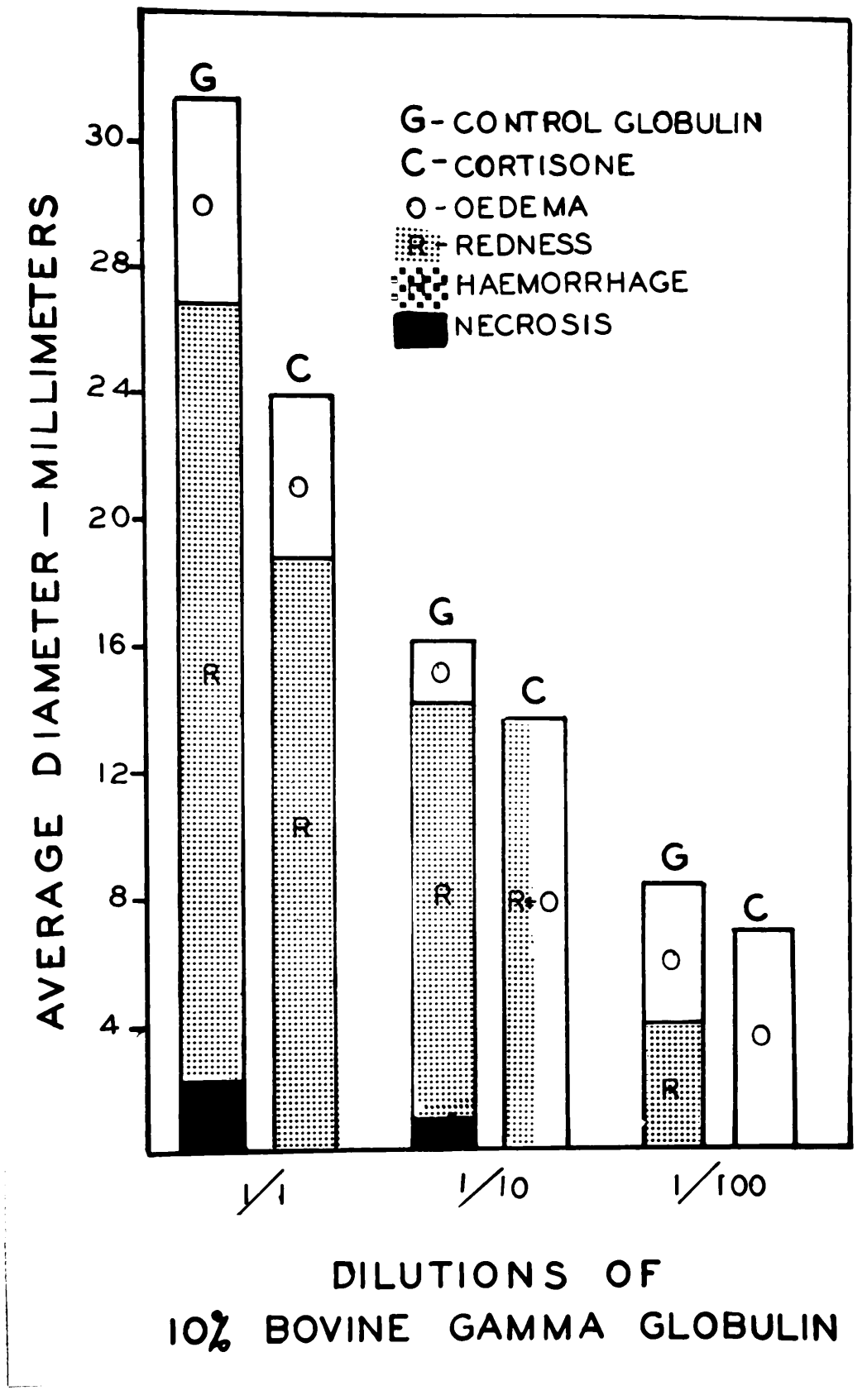

G - CONTROL GLOBULIN
C - CORTISONE
O - OEDEMA
R - REDNESS
H - HAEMORRHAGE
NECROSIS
AVERAGE DIAMETER — MILLIMETERS
30
28
24
20
16
12
8
4
G
C
O
R
R+O
H
1/1
1/10
1/100
DILUTIONS OF
10% BOVINE GAMMA GLOBULIN

Fig. 55.

In Fig. 55 it is apparent that there is a diminution of skin sensitivity in the Cortisone-treated group considering all the dilutions of the test antigens. This diminution is evident in all the intensities of the skin reaction. For example, the oedema and redness were always greater in the group not receiving Cortisone, and necrosis was never seen in the Cortisone-treated group. However, it is interesting to note that this effect is only a quantitative diminution of reactivity and by no means complete inhibition.

One further way of considering the reactivity of the whole groups to the test antigen injections was to consider the number of animals that reacted to each dilution of the antigen. This data is contained in Table 30.

TABLE 30

	Number of animals positive to:-					
Group	Saline	1/1	1/10	1/100	1/1000	ACTH	Cortisone
I	0/8	2/8 (25)	0	0	0	0	0
II	0/9	7/7 (100)	7/9 (77.8)	1/9 (11.1)	1/9 (11.1)	0/9 (0)	0/8 (0)
III	0/8	2/8 (35)	0	0	0	0	0
IV	0/8	8/8 (100)	9/9 (100)	3/9 (33.3)	3/10 (30)	0/8 (0)	0/8 (0)
V	0/12	11/11 (100)	11/11 (100)	3/12 (25)	1/11 (9.1)	4/11 (36.4)	0/5 (0)
VIII	0/9	9/9 (100)	9/9 (100)	2/9 (22)	0/9 (0)	0	0
IX	0/6	6/6 (100)	4/5 (80)	1/6 (16.5)	0/6 (0)		

Numbers in brackets = %

Considering Groups II, IV and V, it may be seen that all the animals reacted to whole horse serum (1/1). All the animals of Groups IV and V reacted to 1/10 but at room temperature (II) only 7 of the 9 animals tested were positive. At a dilution of 1/100 the greatest percentage of the animals that reacted belonged to Group IV, the smallest to Group II,and the ACTH Group (V) occupied a position intermediate between the two. At a dilution of 1/1000, three of Group III reacted, while only one animal of each of the other two groups were positive. The gradation of response considered from the point of view of the number of animals in each group reacting to the graded dilutions of the antigens exactly parallels the intensity of the reactions as represented by the average diameters.

The number of animals reacting to the graded dilutions of 10% bovine gamma globulin was somewhat greater in the group not receiving Cortisone than in the Cortisone-treated group (IX). This is in complete agreement with the results obtained by calculating the average diameters.

The correlation of the intensity of the skin reactions with the presence or absence of lesions of the heart and kidneys is represented graphically in Fig. 56.

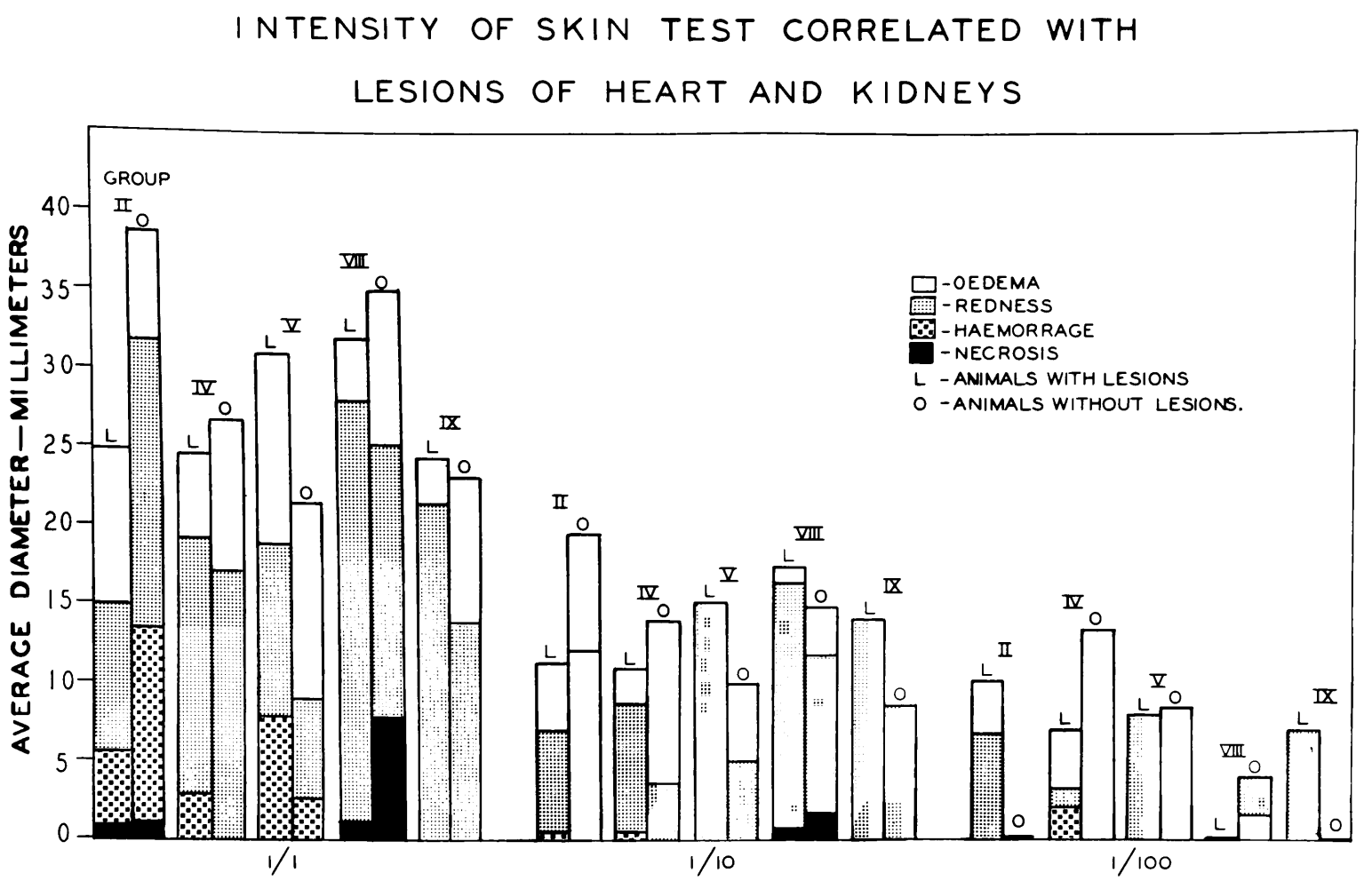


Fig. 56.

When tested with whole horse serum or 10% bovine gamma globulin (1/1), the average diameters of the various phases of the reactions appears to be greater in the animals that do not develop lesions in Groups II and VIII. In Group IV, while the redness and oedema are greater in the animals that do not develop lesions, haemorrhage was observed only in the animals with lesions. In the groups treated with ACTH (V) or Cortisone (IX), the intensity of the reaction appears to be greater in the animals with lesions. At a dilution of 1/10 the situation is similar to that seen at 1/1, for

though the redness and oedema are less in animals without lesions in Group VIII, the necrosis is greater in these animals. At a dilution of 1/100, the animals in Groups II, IV, V and IX show a greater reaction in the presence of lesions - all aspects of the reaction considered - while in Group VIII the animals without lesions continued to show a greater reactivity than those having lesions.

In summary, it is evident that under a variety of experimental conditions, there is no constant correlation between the state of hypersensitivity - as manifested by the skin reactivity - and the presence or absence of lesions of the heart and kidneys. ACTH does not exert an appreciable diminution of the skin reactivity of horse serum-treated animals. Cortisone causes a quantitative diminution of the state of skin hypersensitivity, but not complete suppression of the reaction. Exposure of animals to cold does not enhance the state of hypersensitivity to horse serum under the conditions of the present experiment.

It is evident from the review of the literature that an absence of correlation of intensity of skin tests with the state of hypersentivity is not an unusual finding. The hope that was held at the outset of the experiment that the refinement of graded doses would demonstrate some relationship, was not realized. The failure of ACTH to inhibit the Arthus phenomenon is also not exceptional[180,181,185,187], so that the result cannot be accounted for by any inadequacy of administration of ACTH. Furthermore the dosage administered was at the limit of tolerance. The quantitative diminution, but not complete inhibition, of the skin reactivity

to the injected proteins by Cortisone is in accord with published reports (180,188,227). In this group, no question of inadequacy of dosage can be raised, since the animals were maintained at the limit of tolerance, and since it is well recognized that the level of Cortisone is well maintained on one dose per day, and our animals received it twice daily. However, it may be reasoned that the Cortisone was more effective in suppressing the skin reaction because of a more adequate mode of administration. On the other hand, the reports in the literature in which the ACTH was given four times daily, showed that ACTH had a less pronounced effect on the skin reactions than did Cortisone when given in equal doses(180,181,185,187). One must, therefore, conclude that our results are in no way discordant with those in the literature, and that the mode of administration cannot be held accountable by any fancied(203) differences.

Ring Tests

Ring tests were done on the sera indicated in the Tables by the methods detailed in the section on Materials and Methods - General. Since this is a qualitative precipitin test, no estimate can be made of the amount of antibody present in the serum. However, as a qualitative test, it is a very sensitive index of the presence or absence of antibodies. Thus, it was possible by this method to observe only the pattern of antibody production. For each animal this is indicated in Tables XXII to XXIX of Appendix B, where the correlation with lesions may also be seen. From perusal of these Tables it becomes evident that there is no correlation

between the results of the ring tests and the development of lesions.

The results were analyzed in terms of the ratio of the number of animals in each group with positive ring tests to the number of animals observed on any given day of the experiment. These results are shown in Table 31.

From Table 31 it is evident that first of all the animals not treated with horse serum (Groups I and III) do not develop antibodies, as would naturally be expected. Two unexplained positive reactions occurred which may have been errors in reading, or non-specific reactions. Even the animals that received repeated intradermal injections and developed positive skin reactions did not manifest antibodies in the blood.

In all horse serum treated groups (II, IV and V), antibodies were observed to develop as early as the fourth day after the first antigen injection. These were exceptional instances, and in most cases, significant numbers of animals did not show antibodies until the fifth day. All the animals of Group II had positive ring tests on the eighth day, of Group IV on the ninth day, and of Group V on the tenth day. On the twelfth day, the animals in all three groups showed a variable response, and some that were positive previously became negative. The twelfth day was the day after the second massive injection of horse serum, and this effect may be regarded as a "desensitization" phenomenon[224-226]. The animals tested on the fourteenth to nineteenth days were all positive. The difference in the time of development of circulating antibodies in the different groups, as expressed in the Table, are slight, and cannot be

TABLE 31

RING TESTS - Ratio of the number of animals positive to the number of animals observed correlated with time after the first antigen injection

Group	Day after 1st antigen injection.																			
	-7	-2	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	19
I	0/5						0/5	0/4	0/5	0/5				0/5			2/3*		0/2	0/4
II	0/6	0/3	0/2	0/3	0/2	0/2	4/11	4/5	5/7	7/7	5/5		1/4		2/2	2/2		2/2	3/3	4/4
III	0/5		0/1				0/11	0/3	0/11	0/11	0/11		0/2		0/5				0/6	
IV	0/5	0/3	0/5				1/9	1/3	6/6	6/7	6/6	2/2	4/6			2/2	5/5		5/5	
V	0/5	0/1	0/5		0/6	1/6	5/15	2/8	7/11	8/9	3/4	3/3	2/4			5/5	4/4		6/6	
VIII		0/10	0/10	8/10	10/10		10/10				10/10			10/10	10/10					10/10
IX		0/12	0/12	3/9	3/8	3/5	9/11				6/8									5/6

*Unexplained positive reaction.

interpreted as clearly representing a delay in antibody formation due to cold (Group III) or to ACTH (Group V), since the majority of the animals behaved alike in all three groups. As might be expected, the slight delay in antibody formation manifested by individual animals did not correlate with either the presence or absence of lesions (see Tables XXII to XXIX of Appendix B).

In Group VIII most of the animals developed antibodies in the serum on the fifth day, and all had positive ring tests on the sixth day. In the Cortisone-treated group (IX) there was, however, no time when all the animals manifested antibodies. The majority of the animals had positive ring tests on the seventh and eighth days. It would appear, then, that the Cortisone-treated group evidenced a delay in antibody formation when considered as a whole. However, there was no relation between the delay in antibody formation of the surviving animals and the development of lesions (see Table XXIX, Appendix B).

In considering the antibody formation in the different groups, it would be amiss not to examine the occurrence of antibodies in animals that survived to the point where antibodies could reasonably be expected to be present. Such data is presented in Table 32.

TABLE 32

RING TESTS

Results in animals surviving beyond 5 days after 1st antigen injection

Treatment	No. Rabbits treated	Positive	Negative	Doubtful
Cold + Horse Serum	11	11	0	0
Cold + Horse Serum + ACTH	16	16	0	0
Bovine gamma globulin	10	10	0	0
Bovine gamma globulin + Cortisone	11	9	1	1

In summary, there is no evidence from these experiments that would indicate a significant delay in antibody formation due to exposure to cold or to treatment with ACTH. Antibodies to horse serum develop between the fourth and sixth days after the first serum injection, the majority of the animals show positive ring tests on the seventh day and antibodies were present in all the animals on the eighth day. In the group treated with bovine gamma globulin, antibodies appeared in most of the animals on the fifth day, and all the animals had positive ring tests on the sixth day. When the animals received Cortisone as well as the globulin, their antibody development was more irregular, and generally tended to be delayed. There was no time when all the animals in the Cortisone group manifested antibodies. There was no correlation between

the time of development of antibodies and the occurrence of lesions of the heart, arteries and/or kidneys in any of the groups.

The presence of antibodies in the animals without lesions is the rule in serum-treated rabbits, and it has been pointed out, in the discussion of the role of hypersensitivity, that a correlation between the antibody titers and the occurrence of lesions has only been possible by the use of very refined techniques[128-130], and in association with efforts to prevent the lesions. In our groups where prevention was attempted with Cortisone and ACTH, it was only with Cortisone that there was an appreciable difference in the way in which antibodies and a diminution of titer can be inferred from the fact that there was no point in the experiment when all the animals demonstrated antibodies. Under ACTH treatment, the antibody formation was not as regular as in the controls, but all the animals manifested antibodies at some stage of the experiment. These observations can only be inferred from the results of ring tests, and no firm conclusions can be attempted on the basis of such inferences. However, further analysis of the sera of these animals by estimation of the antibody nitrogen is being carried out by Dr. Joan de Vries of the Department of Bacteriology, in order to study the exact titers of antibody present in the animals treated in these experiments. With these results, it will be possible to make a more accurate estimation of the relationship between antibody formation and the occurrence of the lesions and of the effect of ACTH and Cortisone on antibody formation. However, such results as are available to us at this time are consistent with the results in the literature, where it has been reported that a

diminution of antibody response occurs as a result of ACTH treatment, but that this effect is much more marked with Cortisone[180,188]. These results of the ring tests and the observations on the effect of ACTH and Cortisone on skin sensitivity presented in the previous section, correlate well with the effect of the hormones on the lesions. Thus, the lesions appear to have been somewhat decreased in severity, more by Cortisone than by ACTH, and the decrease in skin sensitivity and serological activity appears to have been parallel to these changes in the lesions. Although the evidence presented is far from conclusive, it appears that there is some correlation between the intensity of the state of hypersensitivity in the ACTH and Cortisone treated animals and the development of lesions.

Albuminuria

The albumin content of the urine was measured daily by the method outlined in the section on Materials and Methods - General. At the end of the experiment, the values were compiled as indicated in the following tables and correlated with lesions. The only method of correlation used in this portion of the experiment that appeared to have any validity was that in which individual animals were considered. Hence all the values are presented in Tables 33 to 39.

TABLE 33.

ALBUMINURIA mg.%
GROUP I. UNTREATED WARM ROOM CONTROLS

Animal No.	Day of Experiment.																											
	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
K-38										12			12					8							tr	18	9	
K-40																		10									0	0
L-3		0.5			7						40																30	
L-17																											50	
L-19											10																30	
L-43							10						15	15														
L-48								25				50						10										
L-55														10				15						15	15			
N-85			tr		10						10																	
N-91			5		20		10	10							50		20	X										
O-74	10		5		10				10	10						15			15	45	10	0						

X = Died spontaneously
tr= trace.

Table 33 shows that albuminuria appears sporadically in normal animals kept under the conditions of the experiment (Group I). Scattered values, most of which are 10 to 15 mg.% but which occasionally reach 50 mg.%, may occur on one, two or three successive days, but seldom more regularly. This may be accounted for by some physiological mechanism. However, there are other possibilities. Occasionally, the rabbits spill food onto the grill-bottom of the cage, and it is possible to conceive that urine washing over some of this food or over the feces that accumulates in the bottom of the cage might dissolve some protein material from these sources. Unnoticed tearing of the grill, or incomplete cleaning of the funnels, or bottles, might also account for this albuminuria.

In Group II (horse serum at room temperature) (Table 34), there is a good correspondence between the occurrence of marked albuminuria on successive days and the presence of glomerulonephritis in 4 animals (L-47, L-61, N-83 and O-55); between the absence of albuminuria and the absence of lesions in two of the three animals without renal lesions (N-95, K-27). In 3 animals with renal lesions (L-7, L-8, L-12) a single moderately high value was obtained immediately prior to death. In these animals it was possible that allowing the animals to live for a few days, further albuminuria might have manifested itself. One animal (K-27) showed a moderately high albuminuria on only one day between two days of slight albuminuria, and had minimal kidney lesions. One animal (O-58) showed an early high value and then moderately high and slight albuminuria on successive days without manifesting kidney lesions at autopsy. It is also evident that a greater amount of

TABLE 34

ALBUMINURIA mg.%
GROUP II. HORSE SERUM AT ROOM TEMPERATURE

Animal No.	Day of Experiment 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	18	19	20	21	22	23	24	25	26	Lesions Valves	Arteries	Kidneys
						I/V H.S. I										I/V 1 cc.H.S. Desens.												
																I/V H.S. II												
K-27																									0	++	++	0
K-29																10	80	18						5		+	+++	+
L-7													10											30		+++	++++	+
L-8									?				10					10						15		+	+	+
L-12						10						10	30											40		0	0	+
L-46															25										0	0	0	0
L-47								10	15						25	10	10	15	10		120	340	400	300	120	+++	++++	++
L-61	10					30		10								20	25		10		300	400	360	90	130	+	++	+++
N-83																							75	60		+++	++	++
N-89							10	10		10	50	50	50	X												0	0	0
N-95				25	8																			0		++	++++	+
O-55					8	10			60		30							240	300	100		75	60	60		0	0	+
O-58	5	180	5								140	30	25		30		10	40	50	20	10		15			0	0	0

albumin was present in the urine of the animals whose kidneys were more severely affected than in those less severely diseased.

In view of the sporadic occurrence of albuminuria in normal animals and the similarity of these values to those occurring in Group II during the period prior to the second injection, it is impossible to attribute the latter to the administration of the first injection of horse serum. The consistent appearance of albuminuria in rabbits L-7, L-8 and L-12 on day 12 gives rise to some question as to their significance with respect to the serum injection and the subsequent development of glomerulonephritis, but this situation does not obtain in any of the other animals with lesions.

In the cold room control animals (Group III), shown in Table 35, sporadic occurrence of mild albuminuria occurs, but is not related to the exposure to cold, since the pattern is similar to that seen in Group I. It is evident too, that when an animal dies spontaneously it usually develops albuminuria which may reach high levels for several days prior to death. This may be correlated with the development of tubular degeneration (Fig.31).

TABLE 35.

ALBUMINURIA mg.%

GROUP III. UNTREATED COLD ROOM CONTROLS

Animal No.	Day of Experiment																							
	-2	2	3	4	5	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
				COLD ROOM →																					
K-28																			70	30X					
K-31																10		11	25	15		5		9	10
L-2							5	40X																	
L-4							10																		0
L-16																									0
L-52								10					95	40	100X										
L-54														40			35								0
L-63			10					30	10	15		15	10									24			0
N-86	tr	10			2				X																
N-90					tr	15			10	15	10	15	10	25	X										
N-93									X																
O-42																								0	0
O-48		5	5																		10		0	0	
O-51			40																200 X						
O-63					10								25	50	10	300	240	X							
O-83										10													10		0

X = Died spontaneously.
tr= trace.

TABLE 36.

ALBUMINURIA mg.%
GROUP IV. HORSE SERUM + COLD.

Animal No.	Day of Experiment 7	8	9	10	12	13	14	16	17	18	19	20	21	22	23	24	25	26	Lesions		
	I/V H.S. I ↓								COLD ROOM FROM DAY 4 ↓												
														I/V 1 cc. Desens. →							
															I/V H.S. II →						
K-28														8	10	7.5	0	15	0	++	+
K-32										8	5	5						0	+	+	0
L-5				0.5						10		8			15	15	100		++	+	+
L-6					200	200	X												0	0	0
L-13						10	20		30	120	600	720	720	480	480	60	100		+++	++++	++++
L-51		40						40			320	400	50?	45	25	80	100		+	+	+
L-57																0	0		+++	++++	++
L-64		30	10							10						0	0		0	0	0
N-79		45	10							15	20	25	40	200	480	400			0	0	++
N-82		35								30		15				10			0	0	+
N-92		30										10	25	800	560	300			+	0	+++

X = Died spontaneously

TABLE 36.

ALBUMINURIA mg.%
GROUP IV. HORSE SERUM + COLD.

Animal No.	Day of Experiment 7	8	9	10	12	13	14	16	17	18	19	20	21	22	23	24	25	26	Lesions		
	I/V H.S. I ↓								COLD ROOM FROM DAY 4 →									I/V 1 cc. Desens. → I/V H.S. II →				
K-28														8	10	7.5	0	15	0	++	+
K-32										8	5	5						0	+	+	0
L-5				0.5						10		8			15	15	100		++	+	+
L-6					200	200	X												0	0	0
L-13						10	20		30	120	600	720	720	480	480	60	100		+++	++++	++++
L-51	40							40		320	400	50?	45	25	80	100			+	+	+
L-57																0	0		+++	++++	++
L-64	30	10								10						0	0		0	0	0
N-79	45	10								15	20	25	40	200	480	400			0	0	++
N-82	35									30		15				10			0	0	+
N-92	30											10	25	800	560	300			+	0	+++

X = Died spontaneously

The correlation between the occurrence of consistent, high albuminuria and the occurrence of glomerulonephritis is even more striking in Group III than it is in Group I, as shown in Table 36. However, there are still exceptions to be noted, e.g. the albuminuria in N-82 cannot be regarded as being outstandingly consistent, though it is moderately high on one occasion. The early albuminuria occurring on days 8 and 9 in the last three animals is difficult to consider as being caused by treatment, though it may represent a loss of protein following the first massive injection.

It is evident from Table 37 that most animals receiving ACTH develop a consistent low grade albuminuria more than any of the previous groups. However, this only reaches high levels in the animals that develop glomerulonephritis or just prior to death in the animals dying spontaneously.

TABLE 37

ALBUMINURIA mg.%

GROUP V. COLD + HORSE SERUM + ACTH

Animal No.	-2	-1	0	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Lesions: Valves	Arteries	Kidneys
	ACTH + COLD ROOM (4)								I/V H.S. I (7)			Day of Experiment														I/V 1 cc. H.S. Desens + I/V H.S. II (17)					
K-30												0.5														8		+	+++	0
K-36															5	8	9	15X										0	0	0
L-1																									320	45		+	+++	++
L-18																		10								25	50	+	+++	+
L-20																					25	75	15	40	45	40	60	+	+	0
L-44				10										10	35	25X												0	0	0
L-49				20										60	30X													0	0	0
L-67				40										10		15					20	20		50	15	30		0	0	0
N-80				60						40			20	30								X						0	0	0
N-96			10	60	10											10										10	0	0	0	0
N-97	30	15			10	15	10	10		30	30	20		10			15								20		40X	0	0	0
O-43	10			45			10		25	20	15	10				15					60	10	20	10	0	20	20	0	0	+
O-53				10																							0	0	0	0
O-54				20		20			15					15	10	10	10	10						10		20	10	0	0	0
O-66				5	25			5		30	10	10	10	120	150	10	tr	40	20	125	60	10X						0	0	++
O-76					10	15	10	10	0	15	tr	30	8	60	10	20	15	10	10			15	20	45	10	15	15	0	0	0
O-80					10		8	5		20	8			10	10		10	10	10	10			75			10	10X	0	0	0

X = Died spontaneously
tr= trace

TABLE 38

ALBUMINURIA mg.%
GROUP VIII. ADJUVANT GLOBULIN

Animal No.	2	3	I/M Adj.Glob. I ↓ 4	5	6	7	8	10	11	12	13	I/M Adj.Glob. II ↓ 14	15	16	17	18	19	20	21	22	23	24	Lesions Valve	Artery	Kidney
										Day of Experiment															
0-46									120	2860	1280	110	0	25	15					120	50	0	0	0	++
0-50			5						15	400	360	10	0	10	0	10	10	15	40	400	180		0	+	++
0-52		10	5										10	10						15	0	0	0	0	+
0-64		8							300	400	380	15	15				50	40	200	30	5	10	+	+	+++
0-67			8	8	10																10		0	0	0
0-69										800	650	480	140	60	15	0	0	0	25	10	10	10	0	0	++
0-71	15	10	10	8				8		200	240					15	25	20	50	55	tr		0	0	0
0-79			5			20	10	320		tr			30					10		25	18		0	0	0
0-81			7	10	10								tr										0	0	+
0-82										30	10									200	135	10	ϕ	0	0

tr = trace

In Group VIII (Table 38) the correlation between high consistent albuminuria and the occurrence of nephritis is present in most instances, though exceptions are N-81, N-82 and O-52. It appears that very high values may occur before the second injection, which is a distinct difference between this group that received bovine gamma globulin in Freund's adjuvant, and those that received horse serum. In the latter groups high albuminuria only occurred after the second injection. The early appearance of albuminuria is similar to that seen when bovine gamma globulin alone is given intravenously[107,132], and may correspond to the tendency to develop antibodies earlier in the globulin-treated animals than in the horse serum treated.

TABLE 39

ALBUMINURIA mg.%
GROUP IX. ADJUVANT GLOBULIN + CORTISONE

Animal No.	Cortisone from day 1 — Adj.Glob. I — Day of Experiment 4	5	6	7	8	9	10	11	12	13	Adj. Glob. II — Day of Experiment 14	15	16	17	18	19	20	21	22	23	24	Lesions: Valves	Arteries	Kidneys	Cortisone
0-44													10	15X								0	0	0	+
0-47															10		10	20	10	5X		0	0	0	++
0-49												tr	10	10X								0	0	0	0
0-56		8	5	10		tr	10				10		20	0	0	X						0	0	0	+
0-59						30	96	20X														0	0	0	++
0-60					20	5	tr	15	10	5	32		75	30	50	200X						0	0	0	+
0-61	158	15	20			240	200	160	25	15	10	200	100X									0	0	0	+
0-65							18	45	30	40	40	8	85	75	120	100	120	200	300			0	0	+	++
0-68							5	10		10	10	10	10	20	10	25	20	20	35	15		0	0	+	+
0-70				30	60	30	60	30	15		20	25	10	30	25	30	25	55	200	240	240	0	0	0	+++
0-73									10						10			10		10	50	0	0	0	0
0-78				30					tr		20	20	45	30	20	50	50	80	200	240	300	0	0	+	

X = Died spontaneously
tr = trace

In Group IX it appears that animals receiving Cortisone in the dosage employed, develop albuminuria related to damage of the kidneys. This kidney damage may be glomerulonephritis of the type recognized to be produced by the bovine gamma globulin; or of that considered at the present time to be due to the effect of Cortisone, subject to the qualifications stated in the section on the morphological aspects of the results (see also Figs. 32 and 33).

In summary, it may be stated that the occurrence of albuminuria in animals treated with horse serum or bovine gamma globulin is correlated with the occurrence of glomerulonephritis,and the amount of the albuminuria is proportional to the degree of involvement of the kidney. Cortisone damage to the kidney is also expressed clinically by the presence of albuminuria.

It was noted that the albuminuria occured in the animals receiving horse serum developed predominantly after the second injection of horse serum, while the protein appeared in the urine of animals treated with bovine gamma globulin at least three days earlier. This is correlatable with the later development of antibodies to horse serum than to bovine gamma globulin. While the occurrence of the albuminuria does not correspond to the day of appearance of antibodies, it is probable that the peak of the antibody titers is related to the day of appearance, and that the highest concentration of the antibodies to horse serum occurs later than that to the bovine globulin. These results are in accord with previous experiments (109,132), and provide some evidence for a correlation between the develop-

ment of the state of hypersensitivity in the animals and the development of lesions of experimental glomerulonephritis.

In the past failures to find evidence of a relationship between the circulating antibodies and the development of lesions of the heart and kidneys has led to the hypothesis that the state of hypersensitivity in these experimental conditions resided in the tissues themselves, and it was claimed that the animals developed antibodies to their own heart or kidneys (autoantibodies) after damage to the organs had occurred as a result of the administration of the foreign proteins[228,229]. However, these results failed to find confirmation in this laboratory[22], or in the hands of others[230].

It may, therefore, be concluded from the results of the skin tests and serological reactions; the effect of Cortisone or ACTH on the skin tests; the development of circulating antibodies and the time of development of albuminuria that the state of anaphylactic hypersensitivity is probably related to the development of lesions of the heart and kidneys of animals sensitized to foreign serum proteins.

Experiment 6.

The effect of two massive intravenous injections of bovine albumin on rabbits when given at an interval of twenty-three days.

Experiment 7.

The effect of two massive injections of bovine albumin on rabbits when given at an interval of nine days.

Experiment 8.

The effect of two massive intravenous injections of bovine albumin given at an interval of nine days to unilaterally nephrectomized rabbits.

Introduction:

These three experiments were undertaken as an additional test of the hypothesis that the different forms of lesions, cardiac valvulitis, necrotizing arteritis and glomerulonephritis each develop in response to a different component of the sensitizing serum(17,21). Although our previous experiments(13,46,47) have shown that this was not a tenable hypothesis, it was thought desirable to complete the series of investigations using bovine albumin, since the bovine gamma globulin had already been investigated(13). The investigation of the effects of bovine albumin was also prompted by the fact that quantitative antibody determinations are most readily determined on pure antibody-antigen systems(231), and bovine albumin would be susceptible to purification by recrystallization, and would thus allow accurate immunological studies to be done in connection

with the pathogenesis of the lesions. Furthermore, the original investigations utilizing bovine albumin[17] had included experiments in which only one injection was given, and since increasing the injections to two had effectively produced more florid lesions in the case of bovine globulin[13], it was decided to attempt these experiments with two injections. It has also been shown[132] that the dose of the antigen was important in the production of the lesions. Accordingly, the present experiments employed a dose of 2 grams of albumin per kilo of body weight. The time interval between injections had also been shown to be of importance in the development of glomerulonephritis[118], so that it was planned to vary the interval between the injections. Similarly, the reported effect of nephrectomy on increasing the incidence and severity of the lesions [19,118] led us to attempt an evaluation of the effect of nephrectomy on the lesions produced by bovine albumin. The three experiments in which bovine albumin was injected are considered together, since the results will be evaluated by comparison with one another.

Experimental procedures in Experiment 6:

On the first day of the experiment 16 rabbits (E-13, E-14, E-15, E-16, E-17, E-18, E-19, E-20, E-21, E-22, E-24, E-25, E-27, E-28, E-29, E-30) were given an injection of 10 c.c. of a 20% bovine albumin solution (=2 gm. of albumin) per kilo of body weight. Repeated intradermal tests were done on days 8, 14, 18 and 22, and repeated samples of 1 c.c. of blood were taken and ring tests performed on days 7, 15, 17 and 22. When all the skin and ring tests were positive on the 23rd day, a second massive intravenous

injection of similar quantity to the first was given. The animals were killed on the 36th day, and a complete autopsy performed. The results of the histological examinations of the hearts and kidneys only will be presented in this report.

Experimental procedures in Experiment 7:

Six albino rabbits (M-26, M-27, M-28, M-29, M-30, M-31) were given two massive intravenous injections of 10 c.c. of a 10% albumin solution per kilo of body weight on day 1 and day 10 of the experiment, and killed on day 19. A complete autopsy was performed, but only the results of the histological examination of the heart and kidneys will be presented. The small number of animals in this experiment and the small dose of albumin were determined by the limited supply of the bovine albumin available at the time.

Experimental procedures in Experiment 8:

Fifteen rabbits were unilaterally nephrectomized. Four of these died during or after nephrectomy. Two weeks later the remaining eleven were given an injection of 20 c.c. of a 10% bovine albumin solution per kilo of body weight. This injection was repeated on the 10th day of the experiment. The animals were killed and autopsied on the 16th and 17th days. Only the results of the histological examination of the hearts and kidneys will be presented.

Results of Experiments 6, 7 and 8:

The hearts and kidneys were sectioned and studied in the manner described in the section on Materials and Methods - General. The incidence of the lesions in these experiments is tabulated in Table 40.

TABLE 40

INCIDENCE OF TISSUE LESIONS IN EXPERIMENTS 6, 7 and 8.

Exp't No.	No. of Animals	Valve lesions	Valve ring lesions	Necrotizing arteritis	Glomerulonephritis + ++	Glomerulonephritis +++ ++++	Total
6	16	1 (6.2%)	2 (12.5%)	4 (25%)	1 (6.2%)	2 (12.5%)	3 (18.7%)
7	6	1(16.7%)	1 (16.7%)	1 (16.7%)	1 (16.7%)	0	1 (16.7%)
8	11	2(18.1%)	1 (9.0%)	3 (27.2%)	5 (45.4%)	1 (9.0%)	6 (54.5%)

The structure of the lesions that appeared in these experiments is similar to that described in the section on morphology. The cardiac valvulitis and the arteritis tended to appear in the healed stages, as opposed to the acute stages observed with horse serum. This is in accord with the observations of Hawn and Janeway[21]. The glomerulonephritis that appeared in these experiments was of the extracapillary type, and the lesions from an animal in Experiment 1 are illustrated in Figs. 24 and 25.

The incidence of all the lesions was low in these experiments and appeared to be the reverse of the results of Hawn and Janeway[21], who observed an incidence of 40% in the case of necrotizing arteritis, 30% in the case of valvulitis and only 5% of glomerulonephritis in a group of 20 rabbits treated with only one injection of 10 c.c. of albumin per kilo of

body weight. In our experiments two doses were given and in Experiments 6 and 8 twice the amount of bovine albumin was given at each injection. It appears hardly likely that the incidence of arteritis would be masked or reduced by either a second dose or a greater amount of antigen. Furthermore, the method of giving one injection of antigen is generally productive of a low incidence of lesions. It is, therefore, difficult to explain why the incidence of lesions was reversed in their series, when compared to ours. The only explanation readily at hand is the notorious variability of the incidence of lesions in any experiments involving sensitization to foreign proteins. The glomerulonephritis that appeared in these experiments was low in incidence, but in Experiments 6 and 8, the lesions were of severe grade (see Table 37 and Fig. 29). No nephritis was present in Experiment 7 of severe grade and only one of the six showed a + lesion. The highest incidence was seen in Experiment 8. Due to the unfortunately low dose in Experiment 7, it is not possible to reason accurately as to the factors behind the incidence and severity of the kidney lesions. However, it is evident that in the dosage given by the previous investigators[21], but injected twice instead of only once, the lesions of the kidney appeared as often as the lesions of arteries. In the other experiments (6 and 8) where a double dose was used each time, whether at the 23rd day interval (Experiment 6) or at the 9th day interval in unilaterally-nephrectomized animals (Experiment 8), the incidence of glomerulonephritis exceeded that of the arteritis or the valvulitis. It is apparent from these results that the specificity of the antigen cannot account for individual lesions, but that the antigen, be it horse serum, bovine serum, bovine globulin,

bovine albumin, or the numerous other antigens that have been used, produces an effect on the cardiac valves, arteries and kidneys together. Whether there are other factors involved in the pathogenesis of each lesion that are specific to the individual locations is a question that cannot be answered at this time from our results, or from those of others in the literature.

Although the low incidence of lesions at first sight would seem to preclude the use of this method to study the pathogenesis of the lesions in the future, it may be particularly adaptable to the study of the variations in individual animals, if the animals are first unilaterally nephrectomized. In this instance, one might expect an incidence of glomerulonephritis in about half of the animals, so that in a group of 20 animals some statistically valid results might be obtained from comparison of the findings in animals with and without glomerulonephritis. Such a study would be of particular value in association with quantitative antibody determinations. On the other hand, the use of bovine albumin would not be suitable for experiments designed to test an inhibiting agent for reasons already discussed.

Experiment 9.

The effect of two massive intravenous injections of bovine gamma globulin on intact rabbits.

Introduction:

When the induction of glomerulonephritis was first reported by Hawn and Janeway[21] to result from a single injection of bovine gamma globulin, a modification of the mode of administration of the globulin was

introduced in this laboratory by Drs. More and Waugh[22], whereby more florid lesions could be produced. This modification consisted of giving two injections of globulin instead of the single injection used by Hawn and Janeway[21], and also of unilaterally-nephrectomizing the animals prior to the injections. Since all of the experiments subsequently performed by Drs. More and Waugh[107,132,134] utilized this technique, it appeared to the present writer that it was not clear whether the florid lesions were the result of the unilateral nephrectomy or the use of the two doses of globulin. It was therefore decided to give two injections of globulin to intact, non-nephrectomized animals.

Experimental procedure:

Twelve rabbits were given injections of 10 c.c. of a 10% solution of bovine gamma globulin per kilo of body weight on day 1 and day 11 of the experiment. They were sacrificed and autopsied on the 17th day. Only the histological studies of the heart and kidneys will be presented.

Results:

The structure of the lesions appearing in this experiment was similar to that described in the section on morphology. The incidence of the lesions is indicated in Table 41, and is compared with the incidence in other experiments in Figs. 26, 27, 28, and 29. From the Figures, it is apparent that except for the fact that there was no arteritis in this experiment, the incidence of lesions is essentially similar to that obtained when the bovine globulin was given to unilaterally nephrectomized animals. However, no lesions of grade ++++ were seen in this experiment, so that it

TABLE 41

INCIDENCE OF LESIONS IN EXPERIMENT 9

No. of animals	Valve lesions	Valve ring lesions	Necrotizing Arteritis	Glomerulonephritis + ++	Glomerulonephritis +++ ++++	Total
12	3 (25%)	1 (8.7%)	0	6 (50%)	3 (25%)	9 (75%)

may be considered that the lesions were somewhat more severe in the nephrectomized group. The absence of necrotizing arteritis in the present experiment can only by attributed to the general variation in incidence that occurs in this type of experiment, for it is difficult to see why unilateral nephrectomy should influence the incidence of arteritis, since in Experiments 7 and 8 arteritis appeared whether or not the animals were nephrectomized.

In summary, it may be stated that the treatment of intact animals with two injections of bovine gamma globulin with two injections of 1 gram per kilo of body weight, results in cardiac valvulitis and in glomerulonephritis. However, the lesions of the kidneys were somewhat less severe than in unilaterally nephrectomized animals, but the difference in intensity cannot be regarded as statistically significant in view of the variability experienced in the incidence and intensity of lesions in this type of experiment.

Experiment 10

The effect of a double dose of horse serum (20 c.c. per kilo of body weight) given on two occasions intravenously to rabbits.

Introduction:

In the review of the literature, it has been pointed out that the occurrence of lesions of the heart, arteries and kidneys in animals sensitized to foreign serum proteins was more frequent in animals treated with large doses of the serum, or with small doses given at frequent intervals over long periods of time. It was thus of interest to us in attempting to obtain a high incidence of lesions in order to study the effects of inhibiting agents, to attempt to use larger doses of the antigens.

Experimental procedure:

Twelve rabbits (B-76, B-80, C-15, C-16, C-18, M-32, M-33, M-34, M-36, M-37, M-39, M-40) were given an intravenous injection of 20 c.c. of horse serum per kilo of body weight on day 1 and day 18 of the experiment. The animals were killed and autopsied on the 25th day. Only the results of histological examination of the hearts and kidneys will be presented.

Results:

In this experiment a considerable mortality was experienced for no apparent reason. The animals merely died the day after the first or second injections, manifesting no pathological processes that could satisfactorily explain the resons for death. It is presumed that the large volumes of fluid injected may have accounted for the death of these animals. The animals that died after either injection did not show lesions of the

heart, arteries or kidneys. The incidence of lesions in the remaining eight animals is shown in Table 42.

TABLE 42

INCIDENCE OF LESIONS IN EXPERIMENT 10

No.of animals	Valve lesions	Valve ring lesions	Necrotizing arteritis	Glomerulonephritis + ++	Glomerulonephritis +++ ++++	Glomerulonephritis Total
8	3 (37.5%)	2 (25%)	3 (37.5%)	5 (62.5%)	0	5 (62.5%)

The incidence of the valvulitis, valve ring lesions and necrotizing arteritis is not as high in this experiment as has been seen after injection of 10 c.c. per kilo at the 17-day interval. The appearance of a mild glomerulonephritis is no more striking than that shown in column one of Fig.29. The incidence of the glomerulonephritis is lower that that seen with a smaller dose given at an 11-day interval (see Experiment 2, and Fig. 29, column 9). It is thus possible to conclude that the interval of injection is more important in determining the occurrence of glomerulonephritis than the amount of antigen given in each injection.

GENERAL DISCUSSION OF EXPERIMENTAL RESULTS

The nature of the material to be reviewed from the literature and the diversity of the experiments, has made it necessary to include considerable discussion with each section of this treatise. It therefore only remains necessary to attempt to integrate the experimental results with one another, and to evaluate them in the light of the pathogenetical factors under investigation in the experiments. Repetition of previous discussions will be avoided but, where necessary for the continuity of the arguments, material already included may be repeated.

The first question to be discussed is how our results contribute to an understanding of the importance of the state of hypersensitivity in the development of the experimental lesions produced by two injections of foreign serum proteins. In this connection, it is pertinent to consider the results of the intradermal tests, of the ring tests and the correlation between the positive serological reactions and the occurrence of albuminuria in conjunction with the developing glomerulonephritis.

As was stated in connection with the results of the intradermal tests in Experiments 2, 3, 4 and 5, there was no clear correlation between the intensity of the reaction to the intradermal injections of antigen and the development of lesions of the heart or kidneys in many of the groups.

However, in the group treated with Cortisone, where the lowest incidence of lesions was observed, the intensity of the skin tests was less in the animals that did not develop lesions than in those manifesting the lesions. It is difficult to decide what emphasis to place on the results obtained in this group in the absence of similar results in the other groups. If, however, it is assumed that Cortisone so reduced the state of hypersensitivity (as measured by the intensity of the skin reactions) in all the animals in the group to the point where the differences between individual animals developing and not developing lesions could become evident, the results are explainable. While the justifiability of such an assumption may be questioned at this time, there is evidence of a quantitative diminution of the intensity of the state of hypersensitivity (as observed in the intensity of the skin tests) in animals treated with Cortisone if one considers the results of the skin tests of the group as a whole in comparison with its control group (Fig. 55, page 159). Furthermore, using the ring tests as a measure of the response to sensitization, there is an irregularity in the development of the antibodies and a difficulty of reading the rings that indicates a reduction in the amount of precipitin produced by these animals and by this criterion, a diminution in intensity of the state of hypersensitivity. The incidence and severity of the lesions was also less in the Cortisone than in the control group. It is, of course, an urgent necessity to consider these results along with more accurate quantitative tests of the antibody levels in the blood, and this will be done as soon as such results become available.

One further factor that should be considered is the association between the development of clinical evidence of albuminuria in the animals developing nephritis and the development of antibodies. First, it should be mentioned at this point that a fairly regular pattern of high albuminuria developed in association with the presence of the more severe grades of glomerulonephritis. In the horse serum-treated animals, this appeared after the second injection of horse serum. In the animals treated with bovine gamma globulin, the proteinuria occurred earlier - two or three days prior to the second injection. Correlated with these findings is the fact that antibodies to horse serum tend to develop positive ring tests later in the animals sensitized to horse serum than in those receiving gamma globulin. It is easy to assume that the peak antibody titers follow the development of the antibodies in sequence, and that the peak is later with horse serum than with gamma globulin, and that it is at the peak of antibody formation that the lesions develop to the point where they are evident clinically. This assumption would require the proof of quantitative estimations. However, at this stage, it appears that this association may be held to be one between the developing state of hypersensitivity and the developing lesions but subject to confirmation.

Although, at the present stage, it does not appear justifiable to conclude from our results that there is a definite relationship between the intensity of the state of hypersensitivity induced in rabbits by means of two injections of foreign serum proteins and the development of lesions

of the heart, arteries and kidneys, the further studies using refined techniques are required, since other investigators have utilized such techniques[128-130,135], and have reported a parallel between the intensity of hypersensitivity and the development of the tissue lesions. The reports of the other investigators also require confirmation.

Could such a correlation between the intensity of the state of hypersensitivity and the development of the lesions be established, it would still be necessary to give some consideration to the mechanism whereby this state produces the lesions. It has been considered by nearly all the experimental investigators in this field that the lesions are caused by a union of antibody in the tissues of the animals producing the lesions at the site of this reaction. Although attempts have been made to localize the site of antigen-antibody reactions by such methods as attaching radioactive iodine[232] or dye to proteins[233,234] and attempting to observe the localization of the tracer material. However, these studies were done on anaphylactic shock[232] or on animals sensitized but not developing lesions comparable to those seen in our experiments[234]. The association between the localization of such a labelled antigen has not been demonstrated in experiments similar to ours. It is therefore not possible at this time to find positive evidence of antigen antibody reaction in the tissues in relation to our lesions, although it has been stated that with certain dyes and proteins the localization tends to be in the endothelium of the heart valves, in Anitschkow cells of the myocardium and in the renal glomerulus. From this it is inferred that the localization takes place in

many sites commonly the seat of lesions in experimentally sensitized rabbits[234]. However, it should be pointed out that the dyes deposited themselves in locations remote from the sites where lesions commonly develop and that, furthermore, no lesions were observed in the animals treated, partly because the animals were not observed beyong ten days after injection and partly because they received only one injection of the protein. Hawn and Janeway[21] have opposed the idea that the free antigen unites with the antibody being formed in the cells on rather dubious grounds, since no one can really say where the antibodies are formed. The present theories concerning the site of antibody formation centre about an unsettled controversy as to whether antibodies are produced by lymphocytes[235-237] or in plasma cells[238], so it is futile to attempt, without positive evidence, to ascribe the site of formation of the antibodies to any particular cell and then to reason toward the pathogenesis of the lesions induced by foreign proteins in animals. Alternatively, Hawn and Janeway[21] have proposed that the physico-chemical properties of the injected antigens are the determinants of the occurrence and localization of the lesions.

In support of this hypothesis, they presented experiments utilizing purified bovine gamma globulin and purified bovine albumin, in which the results were supposed to support their reasoning. However, critical examination of their experiments[21] indicates that they failed to demonstrate a true difference in the types of lesions induced by the different antigens employed. Similar results were reported with rather

impure fractions of horse serum by Wissler, Smull and Lesh(12) where it is evident that there was no constant association between the type of antigen injected and the localization of the lesions. In other experiments performed in this laboratory (13,22) it was pointed out that a high incidence of cardiac valvulitis, glomerulonephritis and a low incidence of arteritis could occur in animals treated with bovine gamma globulin. Thus three types of lesions can be produced with gamma globulin alone. Experiments 6,7,8 utilizing bovine albumin have demonstrated that the three types of lesions may also develop as a result of injection of this material. It is apparent therefore that the hypothesis that ascribes the localization and occurrence of the lesions to physico-chemical factors related to be antigen cannot be upheld.

Although it appears to be true that tissue lesions occur in animals sensitized to foreign proteins in direct proportion to the dose of antigen (118,132), this appears to be so only where the range of dose varies from the lowest doses (1-2 cc.) to the level of 10 cc. per kilo of body weight in the case of horse serum. However, Experiment 10 indicates that once the level of 10 cc. per kilo there is no apparent effect of increasing the dose on the incidence and severity of the lesions of the heart arteries and kidneys encountered. On the other hand shortening the time interval between injections of horse serum to 11 days (Experiment 2) seems to produce lesions of the kidney more regularly than if the longer interval of 17 days is used (see Figures 25, 27,28,29, pages 65-68).

Comparison of the effect of giving equivalent doses of bovine

gamma globulin to intact animals as in Experiment 9 and to unilaterally-nephrectomized animals (see Reference 22 and Figures 26-29) indicates that the incidence of glomerulonephritis is not significantly altered but that the lesions appear to be somewhat more severe in the nephrectomized animals. Although the picture is clouded by the alteration of the dose in Experiment 7, it appears from Experiments 7 and 8 that nephrectomy is associated with a slightly higher incidence and severity of lesions produced by bovine albumin given at an interval of 9 days. In attempting to assess the effects of dosage, time interval and nephrectomy, the ever-recurring question of the variability of incidence and severity of lesions in different experimental groups must again be considered in order to assess these factors properly. Further experiments utilizing large groups of animals with particular attention being paid to the constancy of the dose of the antigens, are clearly necessary. At the present stage, one cannot but conclude that nephrectomy appears to be capable of producing either an increase in severity or of incidence of the experimental glomerulonephritis.

It should be stressed at this time that from consideration of the somewhat equivocal type of evidence invoked to support the presence of a relationship between quantitative alterations in the state of hypersensitivity, dosage of the antigens, time interval between injection or unilateral nephrectomy and the production of tissue lesions, it is impossible to consider that any one of these factors is solely responsible for the development of the lesions. Furthermore, the very

difficulty in obtaining unequivocal evidence to support the above contentions, as indicated both from our own experiments and those in the literature, indicates the probability that there are still other factors involved in the pathogenesis of the lesions whose nature and importance are at present not known.

Of the other factors involved in the pathogenesis of the lesions of the heart, arteries and kidneys produced by massive injections of foreign serum proteins, our studies have concerned themselves with the possible role of the adreno-hypophyseal mechanism or the "General Adaptation Syndrome" of Selye. In this connection we have studied variations in adrenal and thymus weight and the levels of the blood sugars. These aspects have already been extensively discussed with the appropriate results so that a brief summary will suffice at this time.

The changes in adrenal weight of animals exposed to cold indicates that "stress" was produced by Selye's standards but administration of horse serum or bovine gamma globulin in the cold room or at room temperature failed to produce a similar increase in adrenal weight that could be attributed to the effecf of the serum antigens. Furthermore, within the normal range of adrenal weight, it was evident that there was no relation of the adrenal weight with the occurrence of the lesions nor was any relationship evident when the adrenals were increased under ACTH or decreased under Cortisone treatment.

Treatment of the animals by exposure to cold, with horse serum, bovine gamma globulin, ACTH or Cortisone produced a decrease in thymus weight however, there was no correlation between the weight of the thymus

and the occurrence of tissue lesions in the animals.

With regard to the blood sugars, it was evident that the variation in the normal blood sugars, under the conditions of the experiment where all the samples were random with respect to the food intake (no animal being fasted at any time), was great. Within the framework of a standard deviation equal to 25% of the average normal random blood sugar, a statistically significant increase was not observed in any group but that treated with Cortisone. Within the normal range, a moderate increase in blood sugar was observed at the end of the period of exposure to cold. No measureable decrease in the blood sugar levels was observed. When the blood sugars of individual animals were considered, a tendency was observed for the animals with the higher blood sugars to have failed to develop lesions. This was by no means uniform nor was the elevation in these instances statistically significant with respect to either the individual readings or to the averages of animals with or without lesions. No relationship between blood sugar changes and albuminuria development was observed in the animals in which nephritis occurred. It was also pointed out in previous discussion that no smooth curve of variation in blood sugars could be constructed from our results that was comparable to those presented by Selye (206) nor could his statement,that the curves were similar in animals that were not fasted prior to the taking of the blood sample, be credited on theoretical grounds, unless a wide variation was ignored. The data that would permit evaluation of his statements are not present in his published work (206). Objection was also made to the manner in which the

curves were constructed in his experiments since it appeared that basic assumptions that were not justified both in the performance of the experiments and in the interpretation of the results.

The fact that ACTH or Cortisone exert some effects on the lesions albeit not statistically significant, brings up the question as to whether these effects imply a pathogenetical relationship between the adreno-hypephyseal mechanism and the lesions. It would appear logical that if the relationship between the effect on the lesions were a pathogenetical one, some other effect of the hormones would also be related to the development of the lesions. No significant relation of this kind was present other than a slight association between an elevation of the blood sugar and the absence of the lesions. The effect of these hormones may therefore be a pharmacological one and not related to the pathogenesis at all. Further work is in progress to elucidate a possible relationship by study of the effects on blood electrolytes but, even here, no relationship has been demonstrated by other studies in this laboratory (217).

While it is admitted that the weights of the adrenals and thymuses and the levels of the blood sugar and the effects of ACTH and Cortisone are not the only criteria that might be used in evaluating the role of the adreno-hypophyseal mechanism in the pathogenesis, it is evident that by these criteria, our investigations have shown that this mechanism cannot be regarded as playing any significant role in the development of the lesions. Certainly no major role, such as is suggested by the writings of Selye (156,157), can be ascribed to it on the basis of the findings that we have thus far recorded.

One other finding in our experiments that appears to bear on the pathogenesis of the lesions is a positive correlation that was observed between a low food intake during the course of the experiment and the failure to develop glomerulonephritis. This correlation cannot be regarded as based on statistically significant results but represents a trend that might be further investigated. The influence of dietary restriction on the development has been studied in this laboratory by Dr. Douglas Waugh (132) in a preliminary experiment where he observed that glomerulonephritis did not occur in unilaterally-nephrectomized rabbits treated with bovine gamma globulin in the cold room. Farr and Smadel have also reported the inhibition of experimental nephritis in some strains of rats (145) and the feeding of sodium chloride has also been shown to have an effect on the development of renal lesions (155,213,216). Thus it appears that the dietary aspect of the pathogenesis of the glomerulonephritis induced in rabbits by foreign serum proteins should be investigated further.

Thus it appears that the pathogenesis of lesions produced in rabbits by means of two massive injections is not due to any one of the factors investigated. The only positive correlations of a suggestive nature between pathogenetical factors and the occurrence of lesions were in the realms of quantitative diminution of the intensity of the state of hypersensitivity, a decreased food intake, and an elevation of the blood sugars in association with the absence of lesions. The most significant of these appeared to be with the alteration and development

of the state of hypersensitivity. It is probable, therefore, that there are multiple factors that interact to produce lesions of which those discussed may be part, but of which some have probably not been considered. Considerable work remains to be done to eludicate the pathogenesis of the lesions.

SUMMARY AND CONCLUSIONS.

The morphological aspects of the lesions produced by foreign serum proteins in animals has been reviewed and considered along with the results of previous and present experiments in comparison with certain diseases of man. After due consideration, it was concluded that the lesions seen in the heart valves and myocardium of animals treated with serum proteins do not resemble the cardiac lesions of human rheumatic fever but rather the lesions in the hearts of patients dying of serum sickness or sulphonamide allergy. The necrotizing arteritis of rabbits closely resembles the lesions reported in poly-arteritis nodosa and those of the kidneys are identical to acute and subacute glomerulonephritis (Type 1 of Ellis) as seen in man.

The etiological and pathogenetical factors concerned in the production of tissue lesions by foreign proteins have been discussed with reference to the literature and with emphasis on the factors investigated in the original experiments namely, the role of the state of hypersensitivity and of the adreno-hypophyseal mechanism.

In Experiment 1 the effect of cold on the lesions produced by two massive injections of horse serum was investigated. A very high incidence of severe glomerulonephritis and cardiac valvulitis

resulted. It was determined that cold had no effect on the body temperature of rabbits.

In Experiments 2, 3, 4, the effects of two massive injections of horse serum was investigated in animals treated at ordinary room temperatures, in the cold, under treatment with ACTH while in the cold, and of cold itself on normal animals. The following observations and conclusions were made:-

Cardiac valvulitis, lesions of the valve rings, necrotizing arteritis and glomerulonephritis can be produced under all these three types of serum or hormone treatment while untreated control animals at room temperatures or in the cold do not show these lesions. The incidence of the lesions in the group treated with horse serum in the cold did not exceed that of the group treated while at room temperature. The lesions in the group receiving ACTH were not statistically different from those of the approptiate controls however, the renal lesions that developed under this treatment were of milder intensity.

In these experiments an increase in the heart weight was observed to occur as a result of exposure to cold alone, horse serum and/or ACTH treatment having no effect.

The kidney weight was observed to increase slightly as a result of exposure to cold and markedly as a result of severe glomerulonephritis but could remain normal in the face of mild glomerulonephritis.

No effect was observed on the weight of the liver from cold, horse serum or ACTH. An increase in the weight of the spleen occurred

as a result of the horse serum injections but under no other circumstances. There was no association between the weight of the spleen and the occurrence of lesions of the heart arteries or kidneys.

A slight increase in pituitary weight occurred as a result of exposure to cold, treatment with horse serum or with ACTH.

The adrenal weights increased slightly as a result of exposure to cold and to ACTH but no similar elevation resulted from treatment with horse serum. It was concluded from this that stress was produced in the animals exposed to cold, by this criterion, but not in the animals treated with horse serum. There was no correlation between the weight of the adrenal and the occurrence of lesions resulting from the foreign protein injections. As indicated by the weight of the adrenal, there was no evidence that the adreno-hypophyseal mechanism played a part in the pathogenesis of the lesions.

The thymus weight was decreased as a result of exposure to cold, injection of horse serum and treatment with ACTH. In no instance was there any correlation between the size of the thymus and the occurrence of the lesions resulting from horse serum injection. It was concluded that if either enlargement or diminution of the thymus gland were regarded as an expression of "stress" that the occurrence of the lesions was correlated with neither and that, by this criterion, the adreno-hypophyseal mechanism did not appear to play a part in the production of the lesions. It was emphasized that the increases or decreases in organ weights was not statistically significant but represented consistent

trends.

The food intake, of the animals in these experiments, was observed to be somewhat higher in the animals exposed to cold than in the normal room temperature controls. There was a suggestive association between a decreased food intake and a failure to develop lesions of the kidneys. However, these differences were not statistically significant.

There was no constant effect on the blood sugar attributable to the effects of horse serum but ACTH and cold appeared to induce a slight increase in the blood sugar. The animals that were free from lesions tended to have blood sugars in higher ranges but this was neither a statistically significant finding nor was the generalization applicable to all the animals. The results of the blood sugar determinations did not follow the pattern described to result from "stress" by Selye after exposure to cold, horse serum or ACTH injection. It was concluded that the investigation of the blood sugar did not support the contention that the adreno-hypophyseal mechanism played an important part in the pathogenesis of the lesions induced by sensitization to foreign proteins.

Elevation of the blood urea nitrogen value was correlated, in many of the groups, with the occurrence of glomerulonephritis and appeared to occur as a result of exposure to cold and ACTH.

Intradermal tests to graded dilutions of the antigens failed to demonstrate and consistent correlation between the intensity of the

skin reactions and the development of lesions in the animals exposed to cold, at room temperature or under treatment with ACTH.

Qualitative precipitin tests (ring tests) performed on the sera of all the animals indicated that antibodies to horse serum can develop as early as the 4th and 5th day after the first injection of horse serum but the majority of animals did not show positive ring tests until the 8th, 9th and 10th days. The animals treated with horse serum at room temperature had all developed positive ring tests on the 8th day; in the cold on the 9th day and under ACTH treatment on the 10th day. There was no correlation of the day of development of antibodies in individual animals and the occurrence of the lesions. The slight nature of the differences between the three groups was not accepted as being a significant effect.

Albuminuria occurred in the cold-exposed rabbits, that died before the end of the experimant, which was probably related to the kidney damage observed in some of the animals. Consistent albuminuria was observed to occur in association with the development of glomerulonephritis on the day after the second injection of horse serum. ACTH-treated animals also developed albuminuria.

In Experiment 5, a group of animals was treated with twice-daily injections of Cortisone during a period of sensitization with two injections of bovine gamma globulin in Freund's adjuvant and compared with a group similarly sensitized but not treated with Cortisone. The sensitization to bovine gamma globulin resulted in the development of

mild to moderate glomerulonephritis in the majority of the animals. The Cortisone-treated group developed a smaller but not statistically different incidence. However, the lesions were milder in intensity in the latter group. It was therefore concluded that a mild but not complete inhibitory effect was present due to the Cortisone treatment. Animals under Cortisone treatment developed lesions of the kidney that were described in the text. The relationship of the lesions produced by Cortisone was discussed and it was concluded that further study of control groups treated with Cortisone alone was necessary to evaluate the possible relationship.

Studies of organ weight, blood sugars, blood urea nitrogen, intradermal tests and serology were made on these groups in parallel with those of Experiments 2, 3, and 4. The findings, in the group treated with bovine gamma globulin in Freund's adjuvant alone, were similar to those seen in the group treated with horse serum at room temperature except for the instances to be summarized below. The exceptional findings were that the antibodies tended to appear earlier than to horse serum and the albuminuria also occurred earlier, in relation to the first injection instead of to the second as was the case with the horse serum treated groups. Thus it was concluded that there was some evidence for a relationship between the development of the state of hypersensitivity and the development of the kidney lesions. In the Cortisone-treated group a diminution of the intensity of the skin tests and an irregularity of development of the antibodies was

Observed. These were taken to indicate a possible diminution in the production of the antibody levels but it was emphasized that any quantitative interpretation of serology should be reserved for the evaluation of finer methods of quantitative antibody determinations that will be made in the future. However, it appeared that there was a suggestive association between the diminution of the state of hypersensitivity as measured by the intradermal reactions and the serological tests that could be correlated with the decreased incidence and severity of the lesions in this group. This conclusion was in accord with observations reported in the literature.

Rabbits treated with Cortisone tended to show a marked loss of body weight during the experiment, a decrease in adrenal weight and pituitary weight; and a marked increase of liver weight associated with a marked increase in intracellular glycogen; and an increase in kidney weight associated with the damage inflicted on the kidney by the Cortisone.

In Experiments 6, 7, and 8, animals were treated with two intravenous injections of bovine albumin at different intervals and with and without prior unilateral nephrectomy. It was concluded that there was no evidence that the physico-chemical composition of the antigen determined the localization of the lesions since all three types of lesions, valvular, arterial and renal were observed to occur in these groups. Nephrectomy appeared to increase the incidence and severity of the the lesions of the kidney although difficulties of interpretation of the results in this regard were discussed and considered to require

confirmation by further experiments.

Experiment 9, consisted of treating a group of rabbits with two injections of bovine gamma globulin while intact (that is, not unilaterally nephrectomized) in order to compare the results with experiments previously performed in this laboratory. It was concluded that the lesions were less intense but not significantly decreased in incidence when compared with the nephrectomized groups.

In Experiment 10, the effect of doubling the dose of horse serum was investigated, and it was concluded that, at the dosage levels of all the experiments, this increase in dose did not produce a significant difference in the incidence and severity of the lesions.

It was concluded that no single factor investigated accounted for the pathogenesis of the lesions in any location. The most suggestive evidence of a pathogenetical association was between intensity of the state of hypersensitivity and the lesions, although this was not firmly established by the present experiments. The relation between a low food intake and between high blood sugars and the absence of lesions were considered to be suggestive leads that should be followed by further investigations. No evidence was found to indicate a significant role in the pathogenesis of the lesions of the adreno-hypophyseal mechanism.

CLAIM OF ORIGINALITY

The attempt to assess the effect of cold on the lesions produced by two injections of horse serum is the only experiment of its kind known to exist. The observations made in the experiments utilizing horse serum in the cold room are also original contributions, including those on the use of ACTH on animals in the cold. The idea of using ACTH and Cortisone as a means of influencing the production of the lesions was also original in its inception, although studies of a similar nature have since appeared in the literature. The mode of administering foreign serum proteins in large doses in Freund's adjuvant, and the production of tissue lesions by this means, also originated in the writer's work. The observations on the variations in blood sugar in association with the lesions produced by foreign serum proteins is new. The observations of the variations in the organ weights related to the carcass weights in association with these experiments, is original. The whole attempt to evaluate the results in terms of the adreno-hypophyseal mechanism is new to this field.

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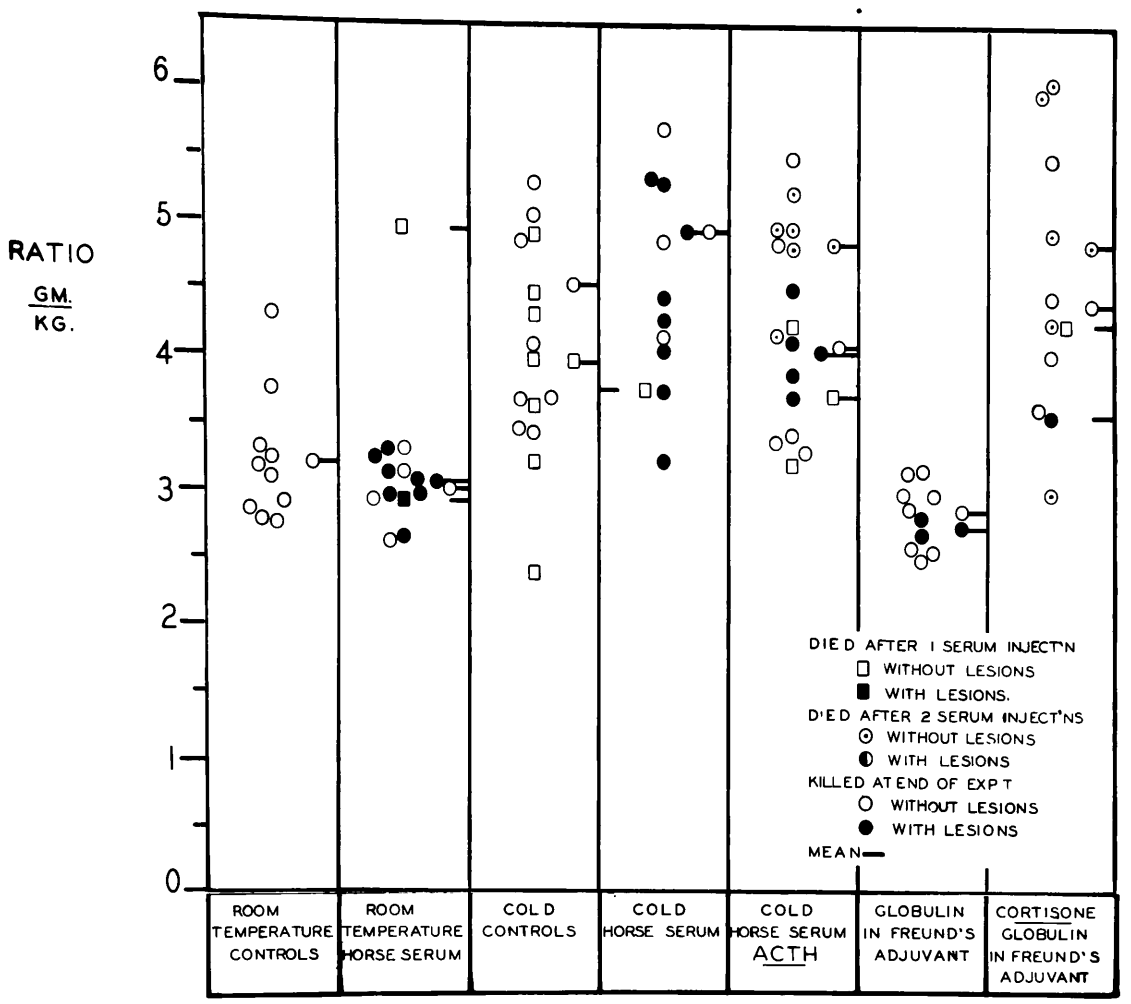
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APPENDIX "A".

Text-figures I - XIV.

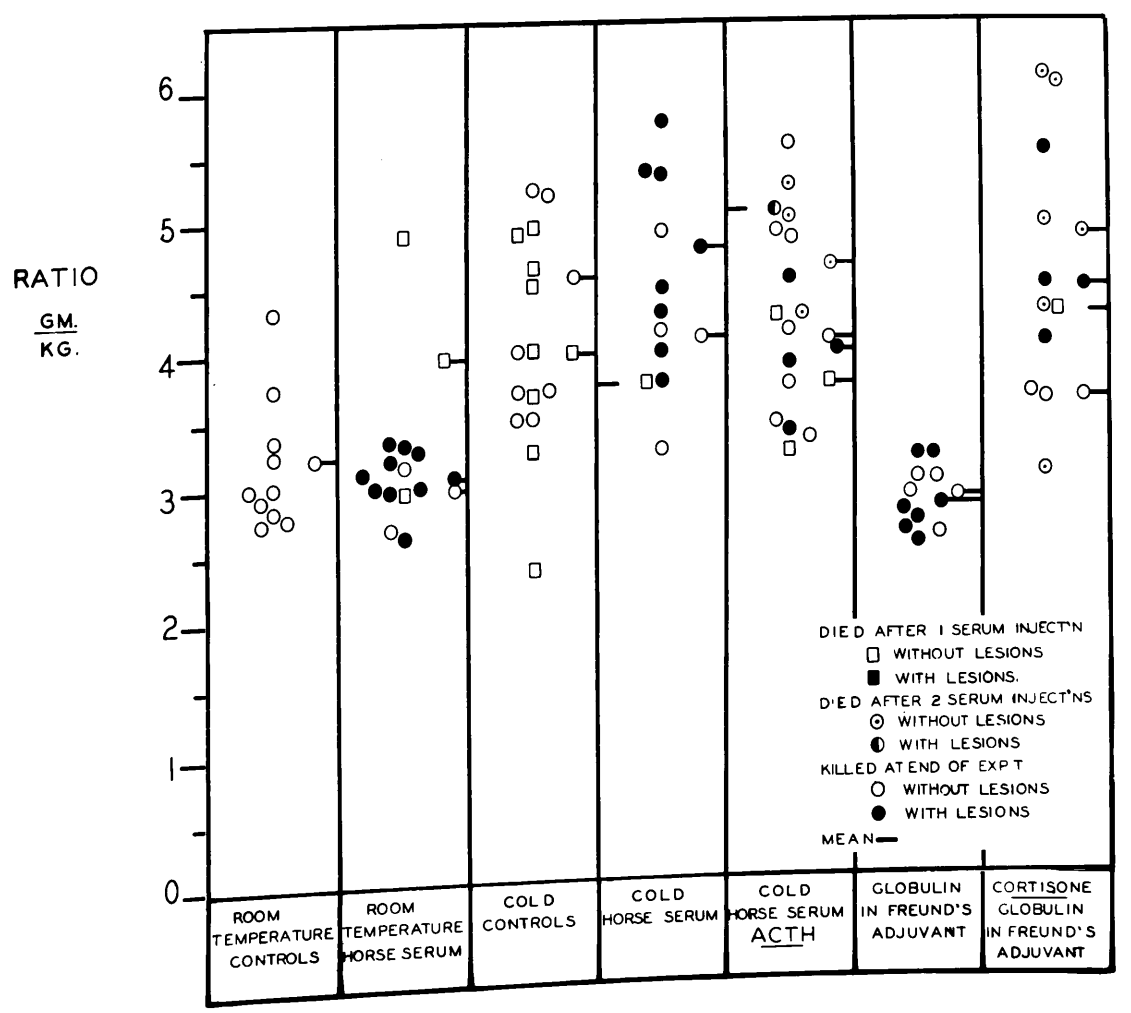
Appendix A.

HEART WEIGHT TO CARCASS WEIGHT RATIOS
CORRELATED WITH HEART LESIONS.



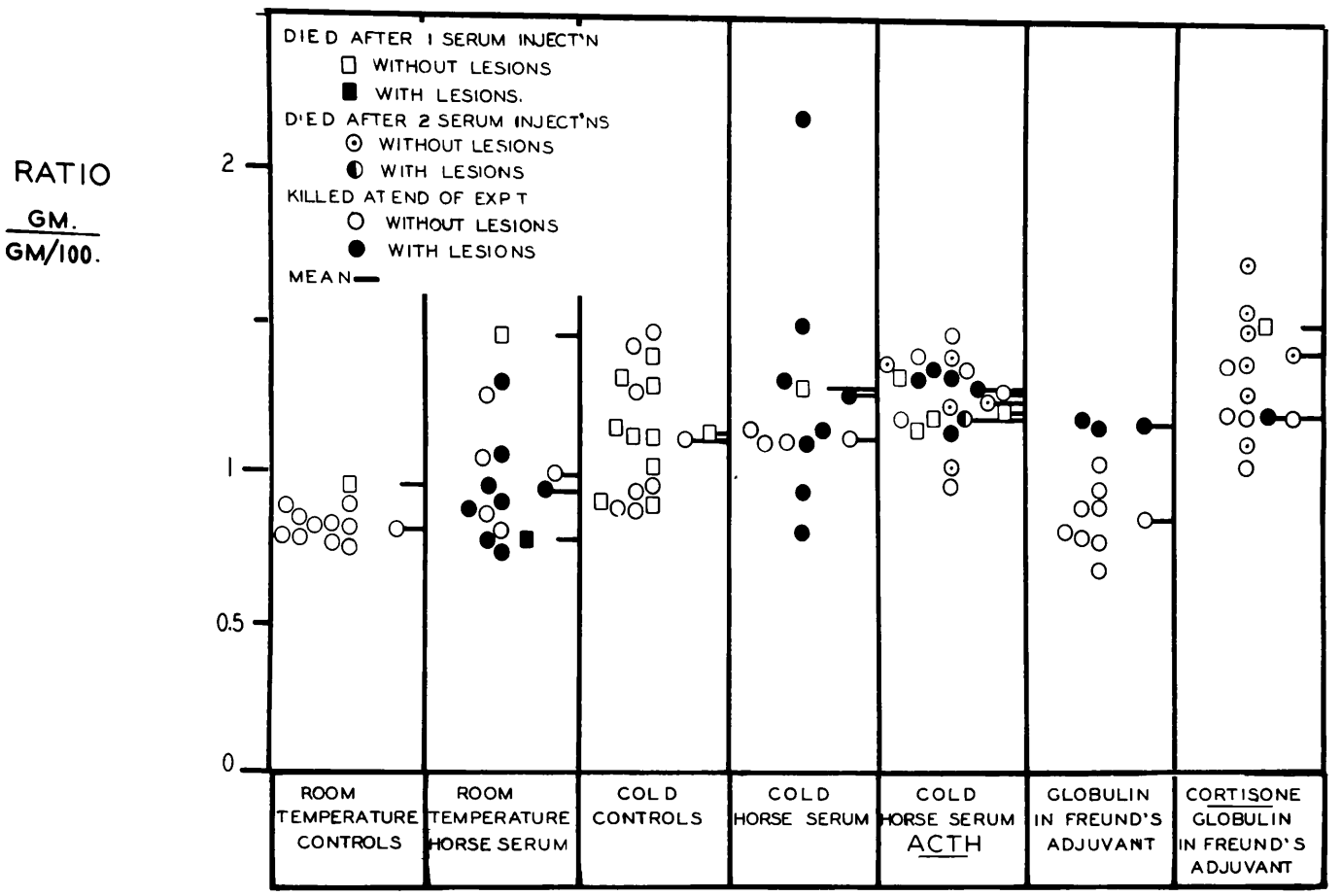
Text-Fig.I

HEART WEIGHT TO CARCASS WEIGHT RATIOS
CORRELATED WITH KIDNEY LESIONS.



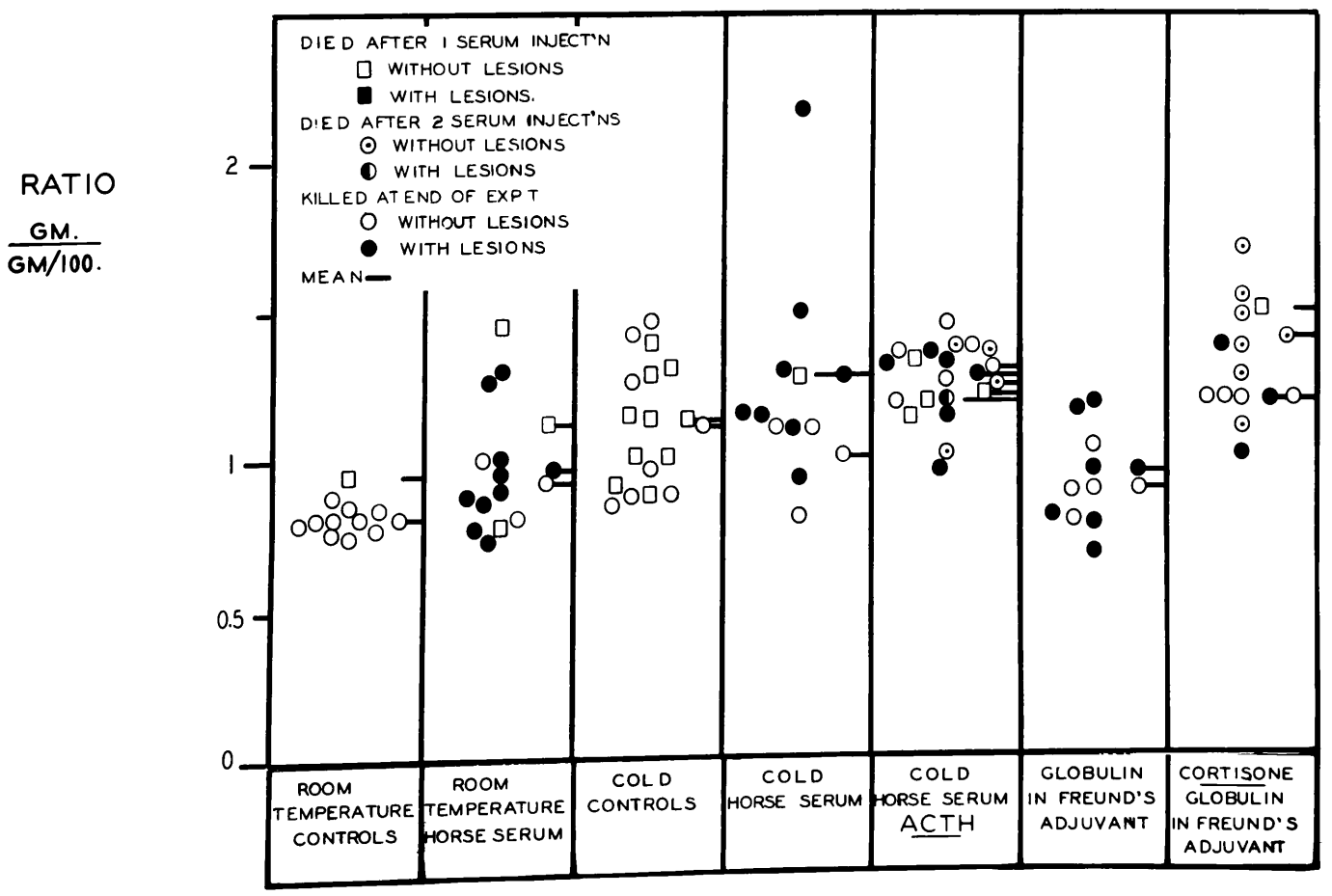
Text-Fig.II

KIDNEY WEIGHT TO CARCASS WEIGHT RATIOS
CORRELATED WITH HEART LESIONS.



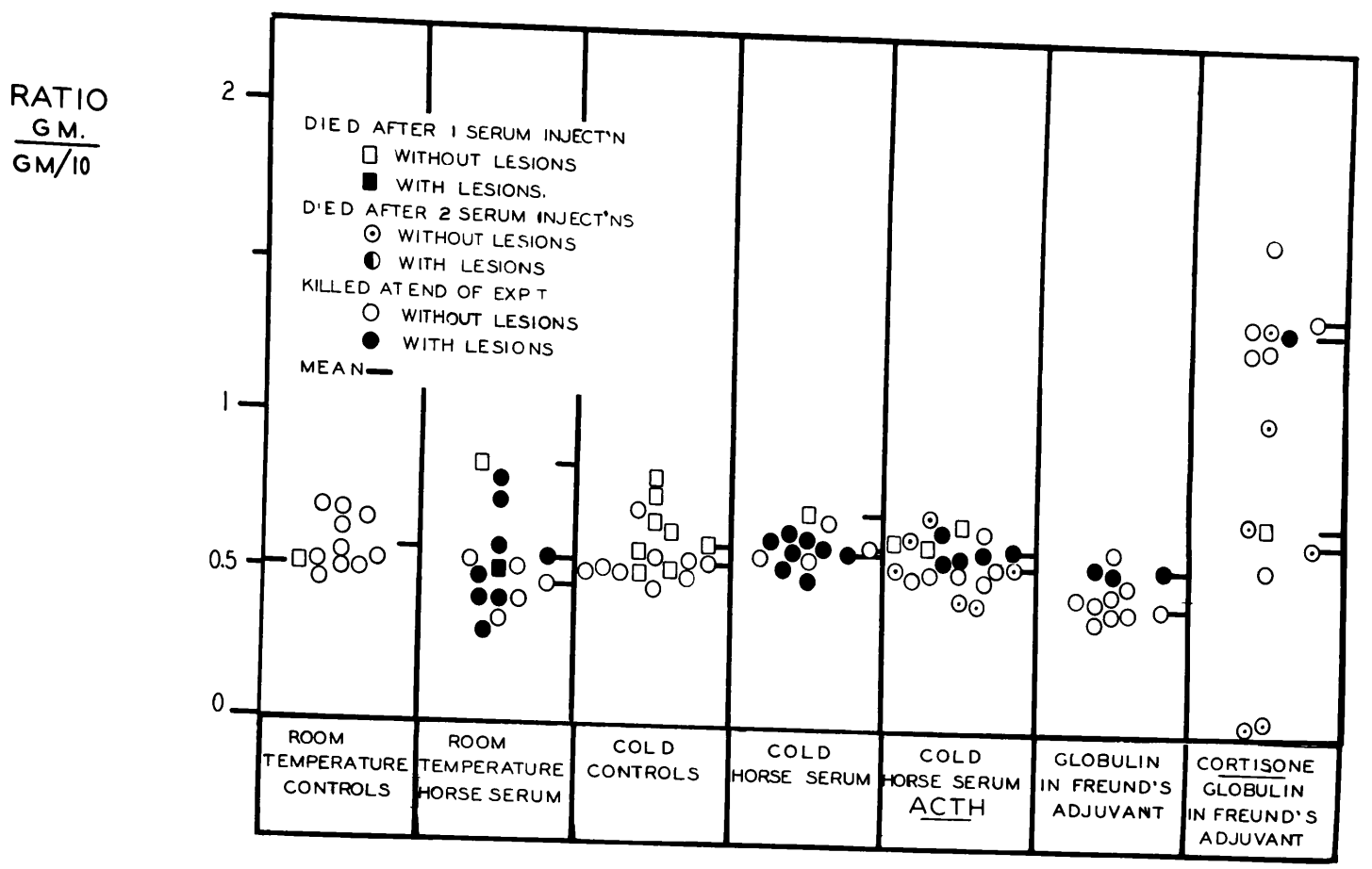
Text-Fig. III.

KIDNEY WEIGHT TO CARCASS WEIGHT RATIOS
CORRELATED WITH KIDNEY LESIONS.



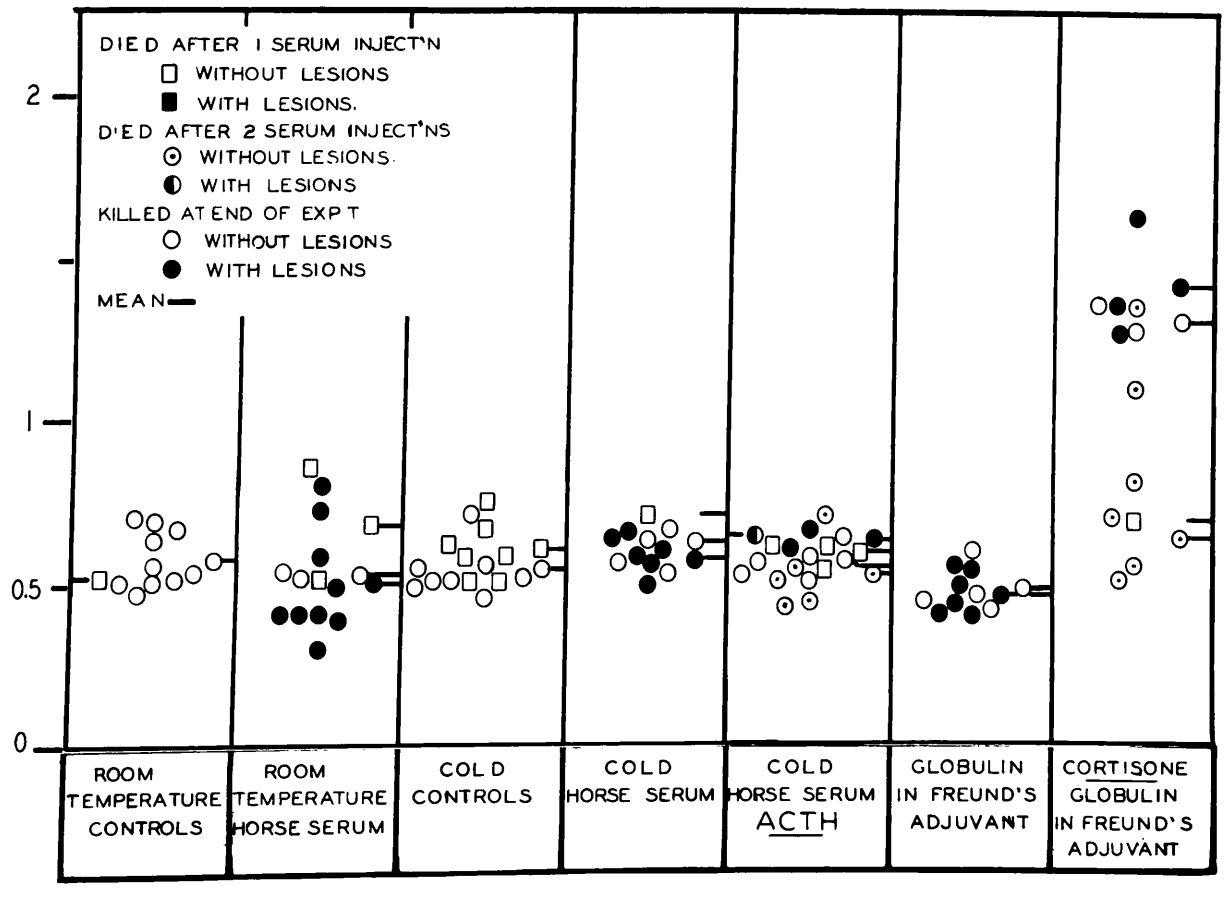
Text-Fig. IV.

LIVER WEIGHT TO CARCASS WEIGHT RATIOS
CORRELATED WITH HEART LESIONS.



Text-Fig. V.

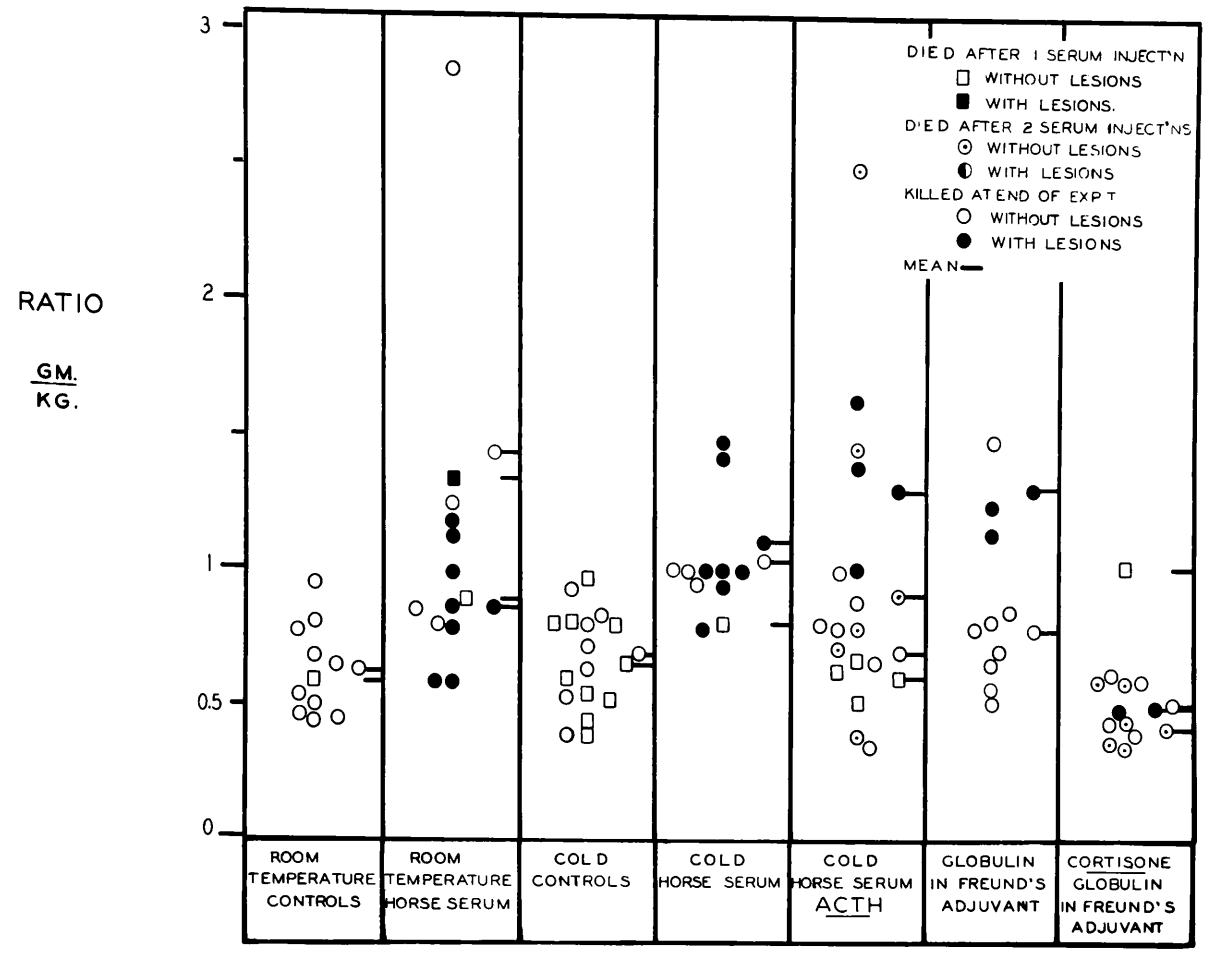
LIVER WEIGHT TO CARCASS WEIGHT RATIOS
CORRELATED WITH KIDNEY LESIONS.



Text-Fig. VI.

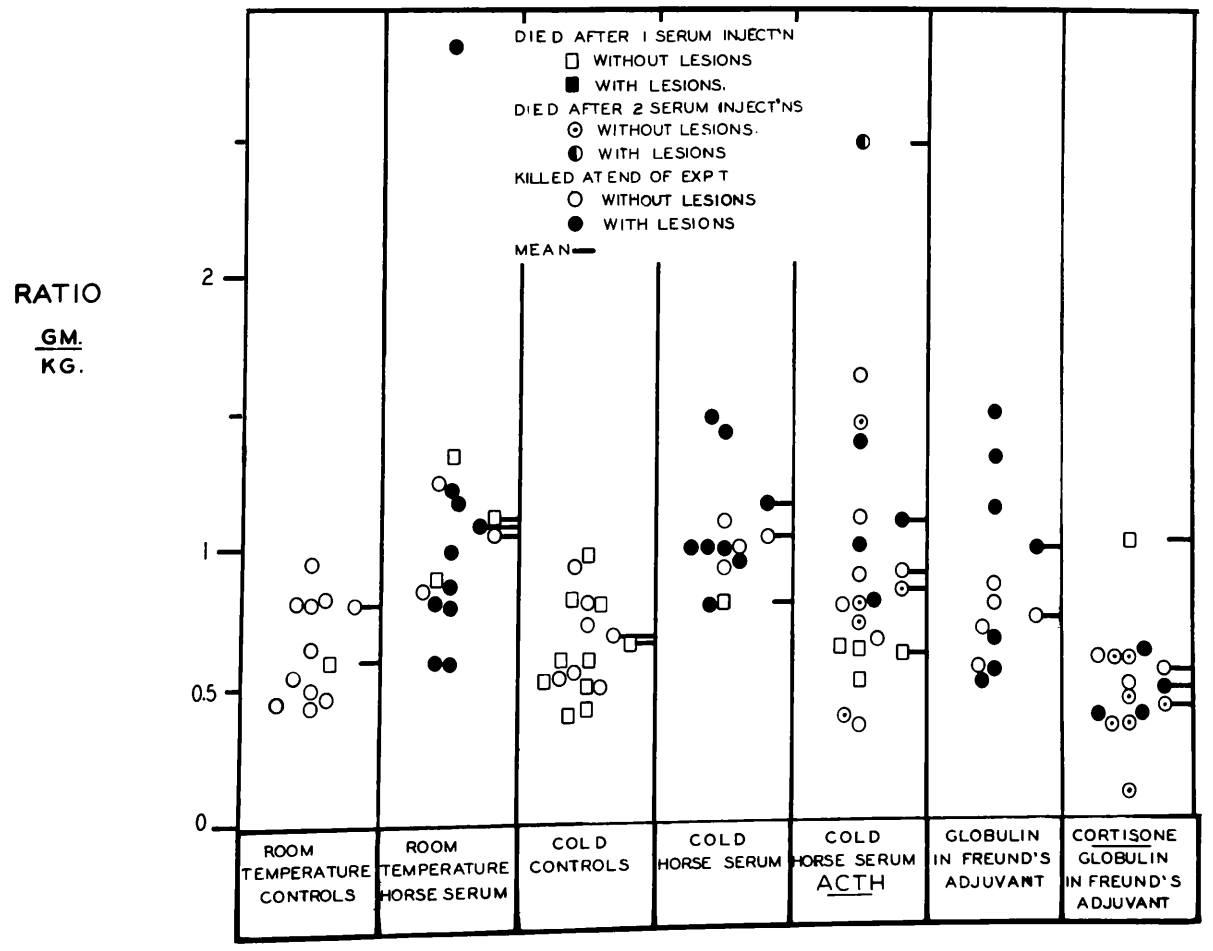
Appendix A.

SPLEEN WEIGHT TO CARCASS WEIGHT RATIOS
CORRELATED WITH HEART LESIONS.



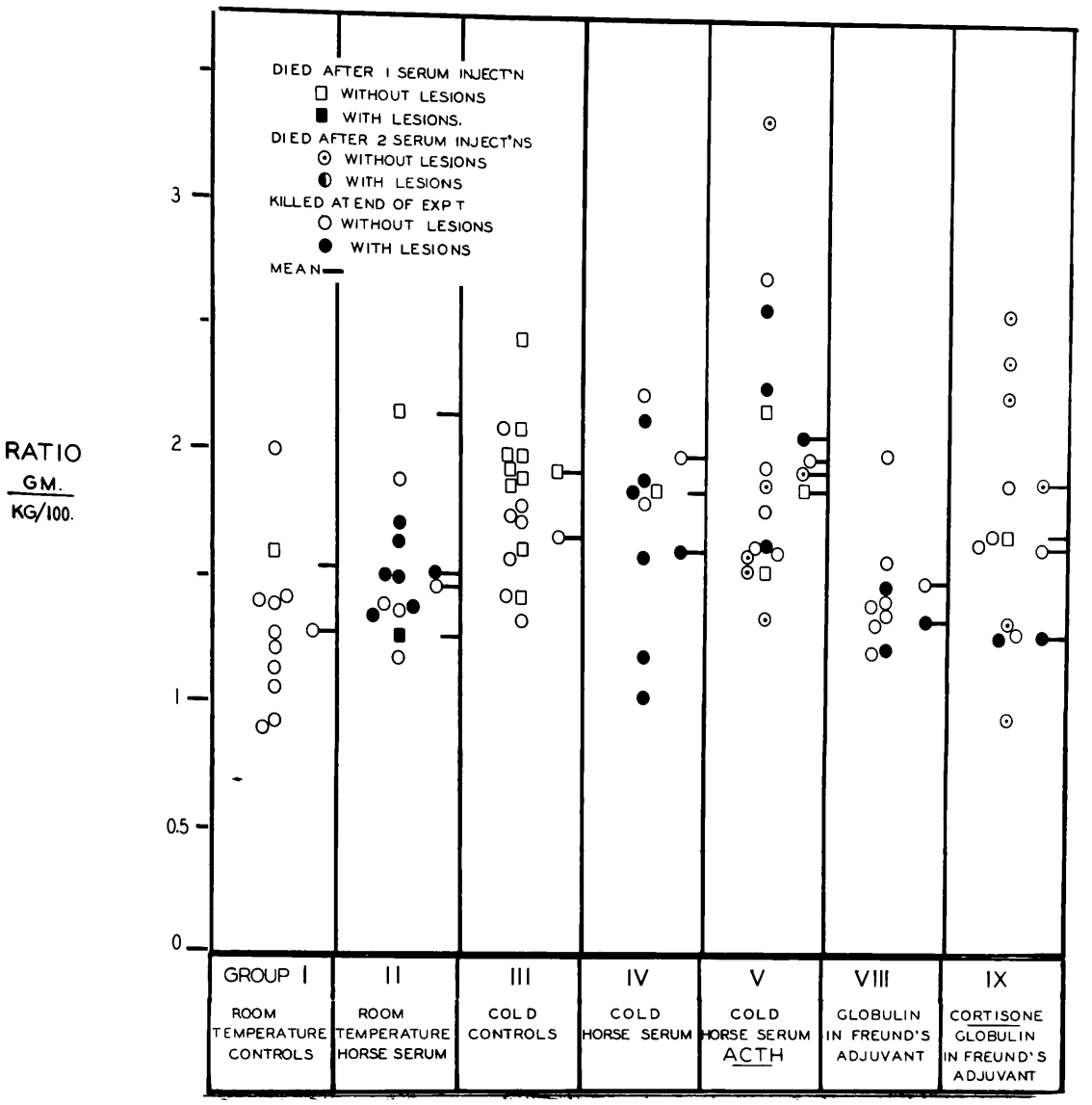
Text-Fig.VII.

SPLEEN WEIGHT TO CARCASS WEIGHT RATIOS
CORRELATED WITH KIDNEY LESIONS.



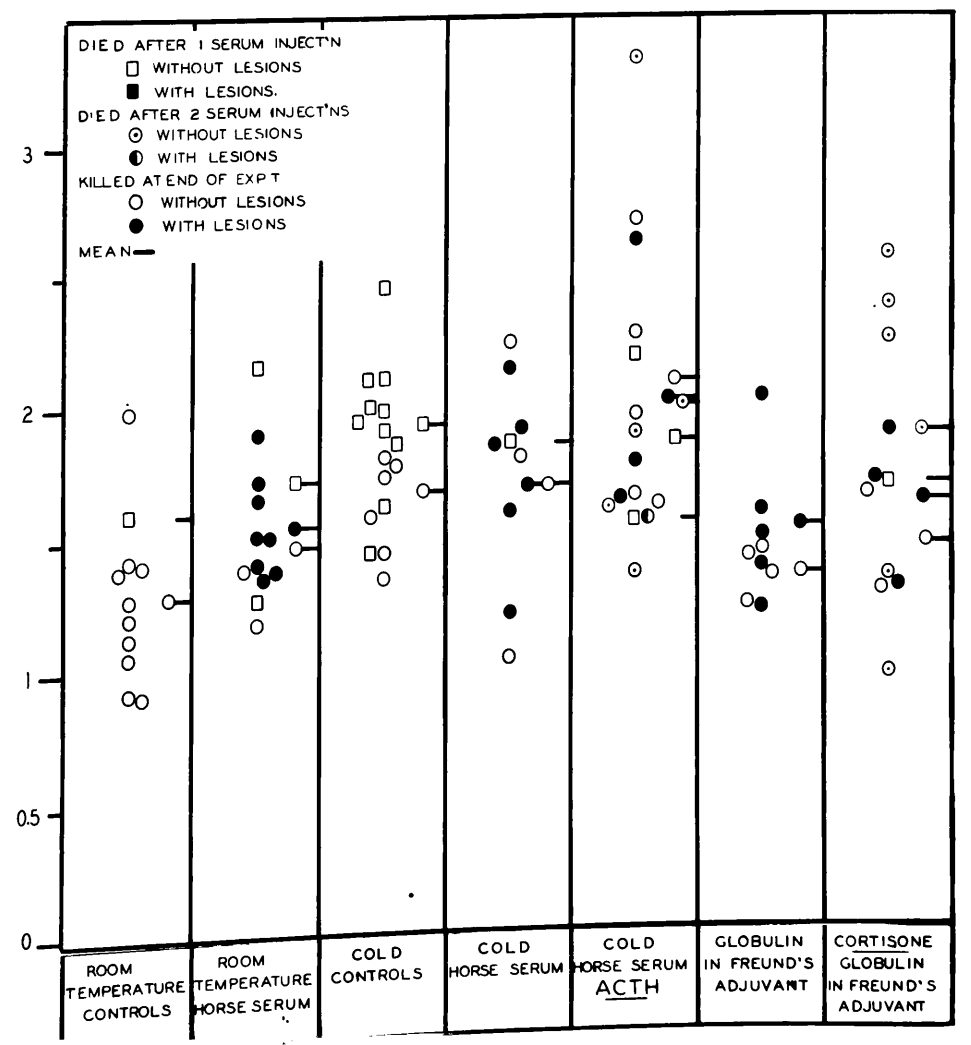
Text-Fig.VIII.

PITUITARY WEIGHT TO CARCASS WEIGHT RATIOS
CORRELATED WITH HEART LESIONS.



Text-Fig.IX.

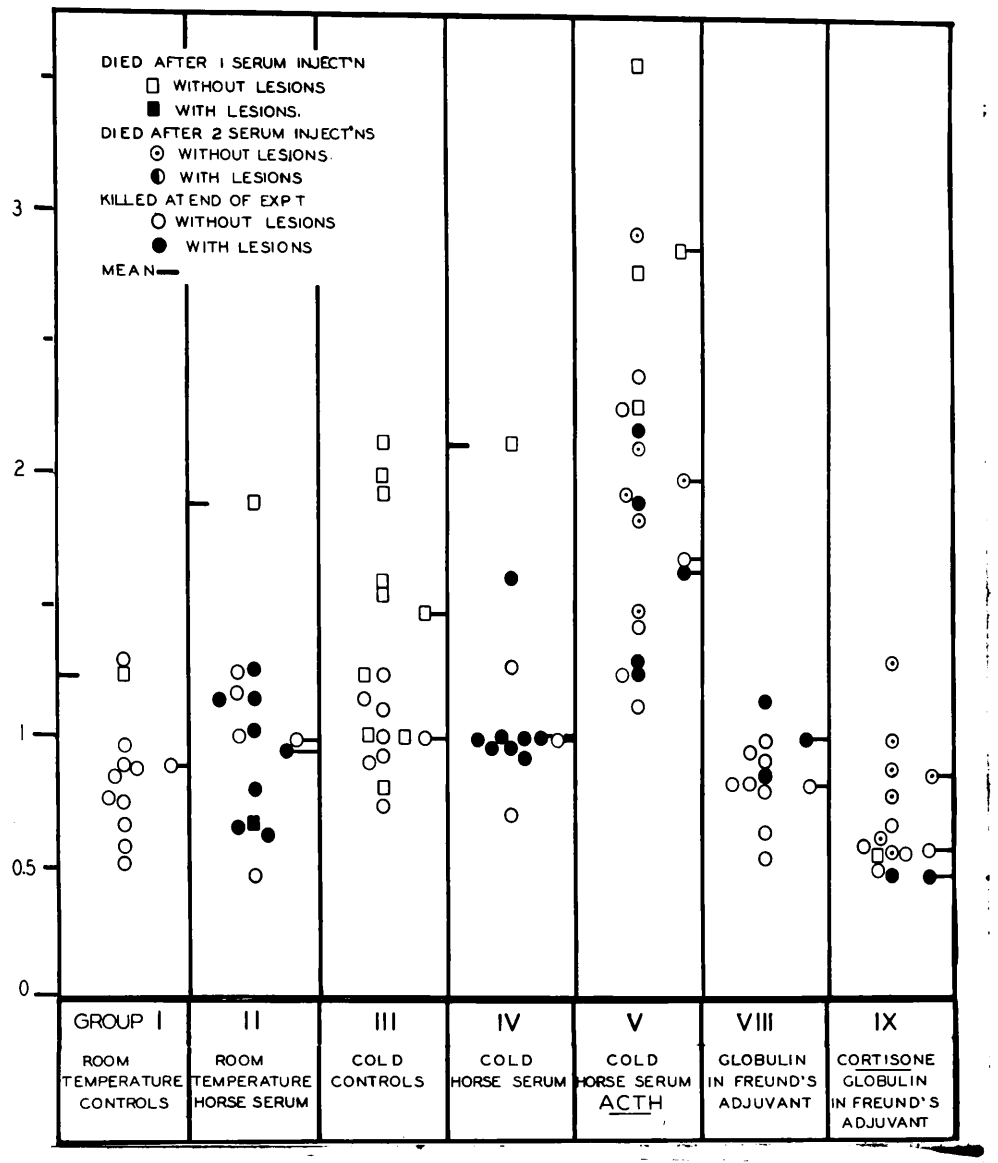
PITUITARY WEIGHT TO CARCASS WEIGHT RATIOS
CORRELATED WITH KIDNEY LESIONS.



Text-Fig.X.

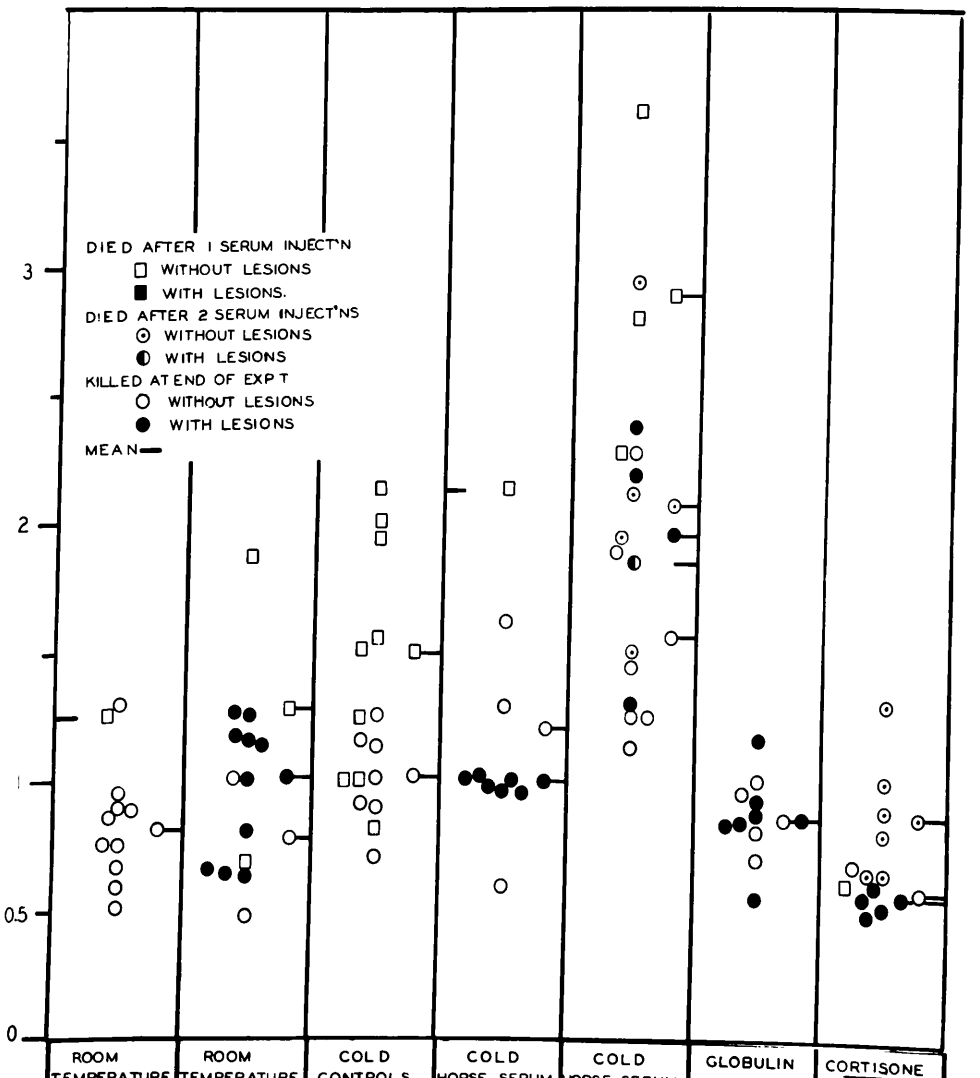
Appendix A.

ADRENAL WEIGHT TO CARCASS WEIGHT RATIOS
CORRELATED WITH HEART LESIONS.



Text-Fig.XI.

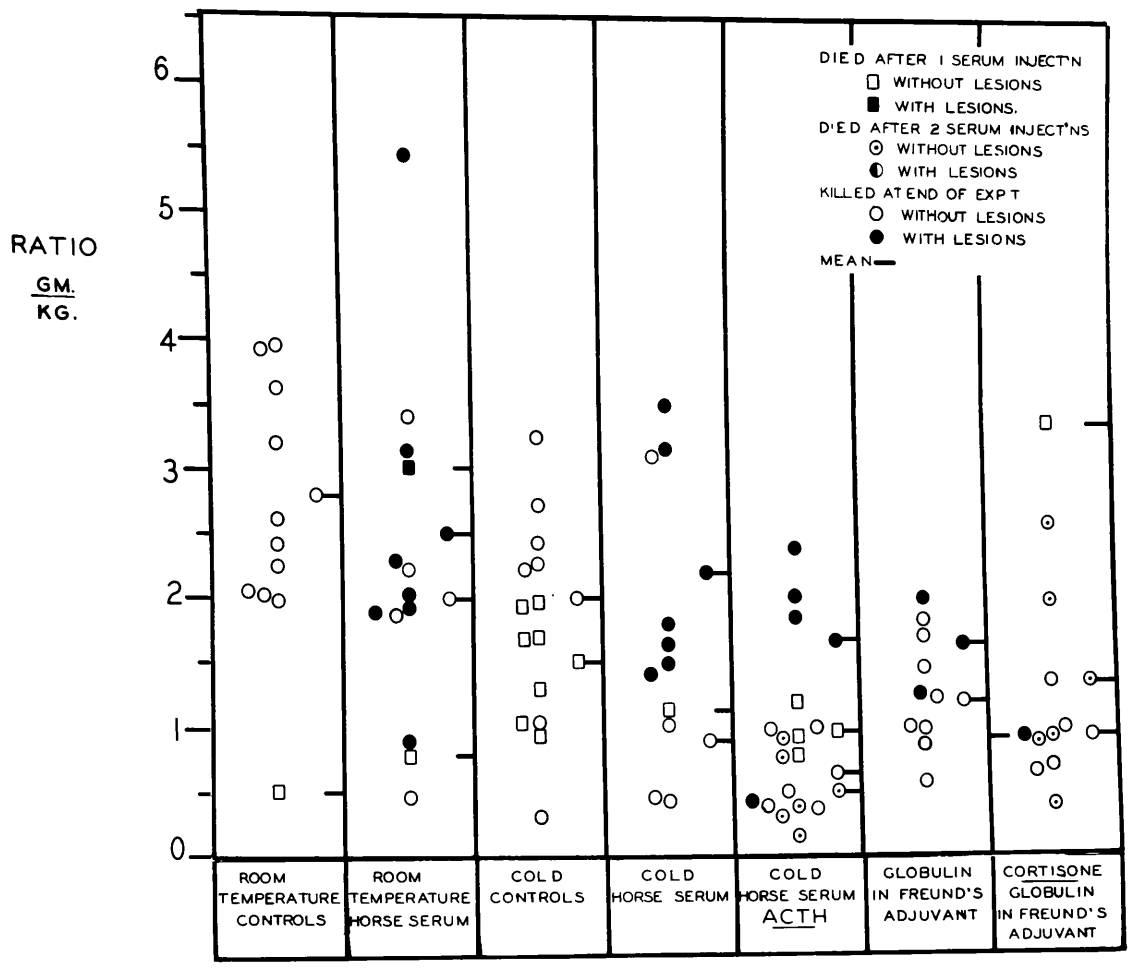
ADRENAL WEIGHT TO CARCASS WEIGHT RATIOS
CORRELATED WITH KIDNEY LESIONS.



Text-Fig.XII.

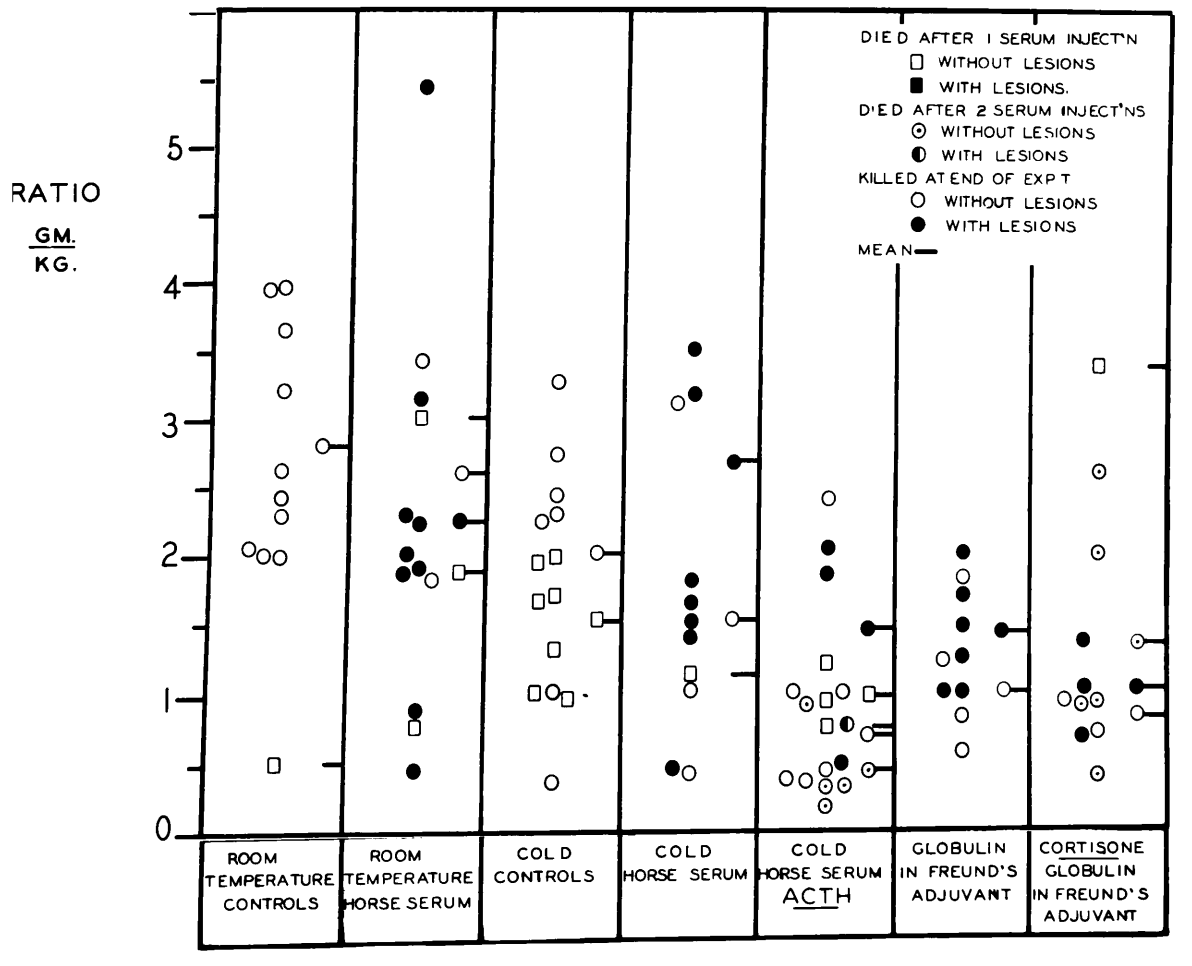
Appendix A.

THYMUS WEIGHT TO CARCASS WEIGHT RATIOS
CORRELATED WITH HEART LESIONS.



Text-Fig.XIII.

THYMUS WEIGHT TO CARCASS WEIGHT RATIOS
CORRELATED WITH KIDNEY LESIONS.



Text-Fig.XIV.

Appendix "B"

Tables I - XXVIII.

TABLE I.

AUTOPSY DATA AND ORGAN TO CARCASS WEIGHT RATIOS

GROUP II. ROOM TEMPERATURE CONTROLS

	Ini-tial wt.	Wt. at end	Gain in wt. gm.	Car-cass wt. gm.	Heart wt. gm.	Heart ratio gm./kg.	Kidney wt. gm.	Kidney ratio gm./100	Liver wt. gm.	Liver gm./10	Spleen wt. gm.	Spleen gm./kg.	Adrenal wt. gm.	Adrenal gm./kg/10	Thymus wt. gm.	Thymus gm./kg.	Pituitary wt. gm.	Pituitary gm./kg/100	Lesions Valve	Lesions Artery	Lesions Kidney	Day of death
3	3215	4070	855	3390	9.7	2.861	24.9	.735	187.3	.5525	1.7	.5025	.1976	.583	13.4	3.96	.0314	.926	0	0	0	25
K-40	2940	3570	630	2980	8.3	2.788	24.3	.844	196.5	.685	1.3	.436	.1526	.513	11.6	3.94	.0425	1.427	0	0	0	26
L-3	2070	2925	855	2465	7.7	3.12	20.0	.811	124.3	.499	2.0	.811	.1646	.666	9.0	3.641	.0300	1.213	0	0	0	22
L-17	1975	2460	485	2180	6.1	2.935	17.8	.816	102.	.468	1.5	.6875	.1950	.895	4.3	1.973	.0200	.916	0	0	0	22
L-19	2170	2660	490	2188	9.5	4.33	16.5	.754	108.6	.496	1.2	.547	.1941	.887	7.0	3.2	.0304	1.39	0	0	0	22
L-43	1730	2355	625	1880	5.2	2.763	15.3	.812	118.0	.627	1.8	.951	.160	.851	3.85	2.025	.0263	1.416	0	0	0	26
L-48	1760	2375	615	2070	6.72	3.25	17.3	.836	107.5	.519	1.6	.773	.1554	.751	4.72	2.281	.0234	1.132	0	0	0	26
L-55	1795	2445	650	2076	6.54	3.76	16.0	.771	143.5	.695	1.35	.649	.1564	.754	5.42	2.618	.0221	1.065	0	0	0	26
N-85	1900	2205	305	1695	5.7	3.362	13.2	.778	89.5	.528	.80	.471	.2215	1.306	4.1	2.418	.0355	2.094	0	0	0	25
N-91	1733	1665	-68	1435	clot		13.5	.940	73.5	.512	.85	.592	.180	1.254	.72	.501	.023	1.602	0	0	0	19x
O-74	1355	2290	735	1760	5.6	3.181	15.4	.875	116.2	.660	.80	.454	.1686	.951	3.6	2.045	.0226	1.284	0	0	0	25

x = died spontaneously

TABLE II.

AUTOPSY DATA AND ORGAN TO CARCASS WEIGHT RATIOS.

GROUP II. ROOM TEMPERATURE - HORSE SERUM.

	Initial wt.	Wt. at end	Gain in wt. gm.	Carcass wt. gm.	Heart wt. gm.	Heart ratio gm./kg.	Kidney wt. gm.	Kidney ratio gm./100	Liver wt. gm.	Liver ratio gm./10	Spleen wt. gm.	Spleen ratio gm./kg.	Adrenal wt. gm.	Adrenal ratio gm./10 kg.	Thymus wt. gm.	Thymus ratio gm./kg.	Pituitary wt. gm.	Pituitary ratio gm./100 kg.	Lesions Valve	Lesions Artery	Lesions Kidney	Day of
	2500	2850	350	2500	7.35	2.94	19.3	.772	125.	..5	3.35	1.34	.168	.6725	7.6	3.021	.0318	1.273	++	++	0	24
	2330	2370	40	2025	5.3	2.62	14.8	.7225	82.9	.401	2.3	1.122	.237	1.157	4.1	2.01	.031	1.513	+	++++	+	29
	2035	2600	565	2150	6.4	2.978	19.2	.8949	123.5	.574	1.2	.58	.2756	1.28	11.7	5.44	.029	1.351	+++	++++	+	25
	2425	2675	250	2285	5.7	2.995	17.6	.769	92.7	.406	1.8	.787	.2374	1.037	2.0	.875	.322	1.409	+	+	+	25
L-12	1770	2425	655	2039	6.8	3.328	17.3	.852	82.8	.408	5.8	2.86	.2559	1.261	4.5	2.219	.028	1.38	0	0	+	25
L-46	1860	2770	910	2400	7.6	3.16	19.4	.808	134.7	.512	3.0	1.25	.1138	.474	8.2	3.418	.0284	1.182	0	0	0	25
L-47	1965	2460	495	2195	6.8	3.1	23.2	1.058	106.4	.486	2.5	1.18	.1412	.644	4.2	1.915	-	-	+++	++++	++	25
L-61	1950	2630	680	2187	7.0	3.2	22.5	1.29	157.0	.719	1.9	.869	.145	.663	6.1	2.29	.0331	1.514	+	++	++	25
N-83	2145	3105	960	2414	8.1	3.355	22.9	.948	190.6	.789	2.4	.994	.95	.807	7.6	2.148	.0416	1.723	+++	++	++	24
N-89	1660	1355	-305	1058	5.28	4.99	15.4	1.455	88.7	.838	.95	.897	.2016	1.905	.8	.756	.023	2.173	0	0	0	13x
N-95	2063	2730	667	2200	7.2	3.272	19.2	.872	64.5	.293	1.3	.590	.2494	1.133	4.1	1.863	.0364	1.654	++	++++	+	24
O-55	1512	2205	693	1356	4.0	2.949	16.9	1.246	51.5	.379	1.1	.811	.1595	1.176	.6	.442	.0258	1.902	0	0	0	25
O-58	2360	1655	1356	1865	5.0	2.68	19.3	1.034	98.6	.528	1.6	.857	.2022	1.084	3.35	1.796	.026	1.394	0	0	+	25

x = died spontaneously

TABLE III.

AUTOPSY DATA AND ORGAN TO CARCASS WEIGHT RATIOS

GROUP III. COLD ROOM CONTROLS.

	Initial wt.	Wt. at end	Gain in wt. gm.	Carcass wt. gm.	Heart wt. gm.	Heart ratio gm./kg.	Kidney wt. gm.	Kidney ratio gm./100	Liver wt. gm.	Liver ratio gm./10	Spleen wt.	Spleen ratio gm./kg.	Adrenal wt. gm.	Adrenal ratio gm./kg/10	Thymus wt.	Thymus ratio gm./kg.	Pituitary wt.	Pituitary ratio gm./Kg/100	Lesions Valve	Lesions Artery	Lesions Kidney	Day of death
	2700	3277	577	2750	9.5	3.458	26.3	.956	123.8	.45	2.2	.8	.343	1.248	8.7	3.263	.0396	1.44	0	0	0	29
	2425	2705	280	2080	4.9	2.358	21.2	1.018	137.3	.665	1.7	.817	.3354	1.607	3.5	1.685	.0414	1.99	0	0	0	28x
	1605	1320	-285	990	4.0	4.04	12.7	1.282	49.5	.5	0.5	.55	.1541	1.558	-	-	.019	1.919	0	0	0	9x
	1530	2305	775	1790	7.4	4.15	22.6	1.263	97.0	.552	1.5	.838	.16	.893	4.1	2.286	.0323	1.805	0	0	0	24
L-16	2170	2610	440	1935	7.1	3.667	21.5	1.112	100.0	.517	.85	.439	.4148	2.142	2.5	1.293	.0388	2.02	0	0	0	21x
L-52	2135	1480	-745	1265	5.5	3.24	17.5	1.383	72.5	.574	.5	.395	.255	2.019	-	-	.027	2.118	0	0	0	15x
L-54	2055	1870	-185	1310	7.0	5.34	19.4	1.471	92.8	.708	.7	.534	.145	1.07	.45	.3417	.023	1.756	0	0	0	25
L-63	1795	1780	- 15	1330	7.6	5.22	18.9	1.422	65.5	.51	.85	.639	.1503	1.131	1.4	1.052	.0281	2.113	0	0	0	24
N-86	1696	1752	- 56	1332	6.0	4.5	11.82	.887	-	-	1.3	.974	.1658	1.244	1.4	1.051	.0332	1.957	0	0	0	16x
N-90	2228	2205	- 23	2005	8.73	4.35	22.65	1.13	126.4	5.630	1.05	.523	.1638	.816	3.3	1.645	.0326	1.625	0	0	0	16x
N-93	2305	2200	-105	1825	-	-	23.94	1.311	135.9	.744	1.45	.794	.184	1.008	3.6	1.972	.034	1.863	0	0	0	16x
O-42	2428	3850	1425	2498	9.27	3.11	21.74	.870	129.3	.517	1.8	.720	.228	.912	6.07	2.429	.0336	1.345	0	0	0	27
O-48	2045	2440	395	2035	7.1	3.49	17.8	.874	97.85	.480	1.9	.933	.2370	1.164	5.55	2.727	.0321	1.577	0	0	0	28
O-51	1935	2182	1672	1656	8.2	4.95	15.2	.917	84.0	.507	1.0	.603	.175	1.056	3.2	1.932	.024	1.449	0	0	0	24x
O-63	2230	2027	203	1517	7.31	4.818	17.35	1.143	93.5	.616	1.23	.810	.294	1.938	1.42	.936	.0374	2.465	0	0	0	22x
O-83	2445	2880	435	2460	9.2	3.739	22.9	.930	133.1	.541	.85	.345	.1734	.704	5.5	2.235	.0427	1.735	0	0	0	25

x = died spontaneously

TABLE IV

AUTOPSY DATA AND ORGAN TO CARCASS WEIGHT RATIOS.

GROUP IV. COLD ROOM - HORSE SERUM

Animal No.	Initial wt.	Wt. at end	Gain in wt. gm.	Carcass wt. gm.	Heart wt. gm.	Heart ratio gm./kg.	Kidney wt. gm.	Kidney ratio gm./100	Liver wt. gm.	Liver ratio gm./10	Spleen wt. gm.	Spleen ratio gm./kg.	Adrenal wt. gm.	Adrenal ratio gm./10 kg.	Thymus wt. gm.	Thymus ratio gm./kg.	Pituitary wt. gm.	Pituitary ratio gm./100 kg.	Lesions Valve	Lesions Artery	Lesions Kidney	Day of death
K-26	2340	2692	352	2335	8.4	3.77	21.8	.935	113.9	.488	2.49	1.057	.2240	.959	7.4	3.17	.0304	1.204	0	0	0	29
K-32	2685	3120	435	2548	8.3	3.26	20.4	.80	158.7	.6225	2.4	.942	.296	1.62	7.9	3.11	.0264	1.037	+	+	0	29
L-5	2380	2970	590	2350	10.5	4.47	27.0	1.49	152.0	.647	3.35	1.428	.23	.978	8.0	3.51	.0375	1.597	++	+	+	24
L-6	2465	2590	125	1985	7.5	3.78	25.4	1.28	140.0	.705	1.6	.806	.424	2.135	2.25	1.135	.0371	1.869	0	0	0	9x
L-13	2100	2335	235	1885	7.7	4.08	40.9	2.185	112.0	.594	2.8	1.486	.189	1.002	2.6	1.406	-	-	+++	+++	+++	24
L-51	1935	2090	165	1665	8.9	5.35	21.8	1.31	95.8	.576	1.75	1.052	.170	1.021	3.0	1.802	.031	1.862	+	+	+	24
L-57	1750	2090	340	1650	8.9	5.38	18.2	1.102	110.0	.627	1.7	1.03	.1864	1.13	2.7	1.635	.0355	2.15	+++	+++	++	24
L-64	1885	2000	115	1600	6.68	4.17	17.7	1.105	89.4	.558	1.45	1.104	.115	.719	1.55	1.032	.029	1.812	0	0	0	24
L-79	1668	1559	-118	1200	5.9	4.916	13.2	1.1	81.32	.677	1.3	1.083	.1536	1.280	.5	.416	.027	2.25	0	0	++	25
N-82	1559	1510	- 47	1285	7.4	5.76	14.72	1.145	72.0	.560	1.22	.949	-	-	.6	.466	-	-	0	0	+	25
N-92	2015	2330	315	1900	8.33	4.331	21.97	1.156	100.0	.526	1.5	.789	.1804	.949	2.84	1.494	.0364	1.915	+	0	+++	25

x = died spontaneously

TABLE V.

AUTOPSY DATA AND ORGAN TO CARCASS WEIGHT RATIOS

GROUP V. COLD ROOM - HORSE SERUM - ACTH.

ini-	Wt.	Gain	Car-	Heart		Kidney		Liver		Spleen		Adrenal		Thymus		Pituitary		Lesions			
;ial	at	in	cass	wt.	ratio	wt.	ratio	wt.	ratio	wt.	ratio	wt.	ratio	wt.	ratio	wt.	ratio	Valve	Artery	Kidney	
wt.	end	wt.	wt.	gm.	gm.	gm.	gm.		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.				
		gm.	gm.		kg.		gm/100		gm/10		kg.		kg/10		kg.		kg/100				
2315	2775	460	2300	8.6	3.74	26.1	1.135	133.9	.574	2.56	1.113	.2899	1.258	5.5	2.395	.038	1.652	+	+	0	29
2565	2330	-235	1810	5.85	3.232	21.4	1.182	96.2	.532	1.1	.675	.65	3.59	1.7	.938	-	-	0	0	0	15
2055	2565	510	2020	7.9	3.91	26.8	1.327	132.0	.654	2.8	1.387	.264	1.307	4.2	2.04	.0361	1.789	+	+++	++	24
2210	2595	385	2005	9.1	4.548	27.2	1.358	119.0	.594	2.1	1.09	.439	2.197	3.7	1.849	.053	2.648	+	+	0	24
L-20 1975	2190	215	1640	6.8	4.15	21.6	1.318	93.7	.572	2.7	1.646	.3123	1.904	.7	.426	.0354	2.283	0	0	0	24
L-44 1765	1630	-135	1250	-	-	16.6	1.329	77.2	.617	.65	.521	.285	2.28	1.4	1.2	.0274	2.192	0	0	0	14x
L-49 1650	1460	-190	1095	4.68	4.27	12.4	1.133	67.1	.614	.7	.639	.307	2.805	.85	.776	.0170	1.553	0	0	0	13x
L-67 1775	1715	- 60	1247	7.2	5.27	17.4	1.396	54.5	.437	.9	.722	.186	1.492	1.2	.914	.0418	3.35	0	0	0	24x
N-80 1741	1360	-351	1255	6.3	5.02	17.1	1.362	68.3	.544	1.0	.796	.245	1.952	.4	.318	.017	1.354	0	0	0	19x
N-96 2003	1855	-148	1395	7.8	5.59	19.5	1.397	91.5	.655	1.1	.788	.2016	1.446	.5	.358	.038	2.724	0	0	0	25
N-97 2000	2185	185	1585	7.7	4.86	20.0	1.261	111.0	.700	2.32	1.463	.4666	2.943	.6	.378	.03	1.892	0	0	0	24
O-43 2366	1960	-376	1517	5.14	3.38	14.67	.967	76.15	.501	1.23	.810	.3645	2.402	.74	.487	.025	1.647	0	0	+	25
O-53 2207	2280	73	1855	6.4	3.450	21.95	1.183	92.6	.499	.65	.350	.2328	1.254	1.85	.997	.030	1.617	0	0	0	25
O-54 2255	2167	- 88	1803	6.0	3.327	24.47	1.357	95.55	.529	1.62	.898	.203	1.125	1.84	1.020	-	-	0	0	0	25
O-66 2260	1967	-293	1610	8.15	5.062	19.1	1.186	102.0	.633	4.03	2.503	.298	1.850	1.0	.743	.025	1.552	0	0	++	20x
O-76 2105	1805	-300	1395	6.6	4.907	19.75	1.469	70.2	.521	.9	.666	.3064	2.278	.5	.371	.265	1.970	0	0	0	25
O-80 2000	1543	-457	1310	5.6	4.274	13.4	1.022	54.8	.418	.5	.381	.2785	2.125	.2	.152	.0212	1.616	0	0	0	25x

x = died spontaneously

TABLE VI

AUTOPSY DATA AND ORGAN TO CARCASS WEIGHT RATIOS.

GROUP VIII. BOVINE GAMMA GLOBULIN IN FREUND'S ADJUVANT.

Animal No.	Initial wt.	Wt. at end	Gain in wt. gm.	Carcass wt. gm.	Heart wt. gm.	Heart ratio gm./kg.	Kidney wt. gm.	Kidney ratio gm./100	Liver wt. gm.	Liver ratio gm./10	Spleen wt. gm.	Spleen ratio gm./kg.	Adrenal wt. gm.	Adrenal ratio gm./kg/10	Thymus wt. gm.	Thymus ratio gm./kg.	Pituitary wt. gm.	Pituitary ratio gm./kg/100	Lesions Valve	Lesions Artery	Lesions Kidney	Day of death
0-46	2420	2650	230	2325	7.4	3.182	18.0	.774	92.1	.396	1.9	.817	.129	.554	2.3	.989	.032	1.376	0	0	++	22
0-50	2225	2670	445	2227	6.0	2.694	26.4	1.162	119.0	.534	2.55	1.145	.194	.871	2.8	1.257	.027	1.212	0	+	++	22
0-52	1992	2342	350	1850	4.65	2.513	17.6	.451	90.0	.486	.95	.513	.1718	.928	3.15	1.703	.0294	1.589	0	0	+	23
0-64	2078	2380	302	2000	5.55	2.775	23.7	1.185	111.15	.555	3.0	1.5	.223	1.165	4.1	2.05	.0298	1.490	0	0	+++	23
0-67	2225	2472	247	2135	6.2	2.903	19.0	.0889	99.0	.465	1.7	.796	.1708	.80	3.9	1.826	.0262	1.227	0	0	++	22
0-69	2380	2860	480	2325	6.1	2.623	15.7	.675	102.0	.438	3.1	1.333	.195	.838	3.4	1.462	-	-	0	0	++	23
0-71	2490	2680	190	2250	6.8	3.022	19.9	.884	133.6	.593	1.95	.866	.1566	.696	1.3	.577	.0322	1.431	0	0	0	22
0-79	2240	2400	160	2125	6.4	3.011	16.6	.781	87.5	.411	1.2	.564	.2035	.957	2.6	1.223	.0285	1.311	0	0	0	22
0-81	1865	2078	213	1805	5.75	3.185	14.7	.814	70.4	.390	1.2	.664	.1501	.832	1.95	1.080	.0366	2.027	0	0	+	23
0-82	2195	2880	685	2260	5.85	2.588	23.4	1.035	99.8	.441	1.6	.707	.227	1.004	1.85	.818	.032	1.415	0	0	0	22

AUTOPSY DATA AND ORGAN TO CARCASS WEIGHT RATIOS.

GROUP IX. BOVINE GAMMA GLOBULIN IN FREUND'S ADJUVANT AND CORTISONE.

Animal No.	Initial wt.	Wt. at end	Gain in wt. gm.	Carcass wt. gm.	Heart wt. gm.	Heart ratio gm./kg.	Kidney wt. gm.	Kidney ratio gm./100 gm.	Liver wt. gm.	Liver ratio gm./10 gm.	Spleen wt. gm.	Spleen ratio gm./kg.	Adrenal wt. gm.	Adrenal ratio gm./10 kg.	Thymus wt. gm.	Thymus ratio gm./kg.	Pituitary wt. gm.	Pituitary ratio gm./100 kg.	Lesions Valve	Lesions Artery	Lesions Kidney	Day of death
0-44	2410	1365	-1045	1185	5.1	4.303	15.1	1.274	66.1	.553	.7	.590	.074	.624	1.1	.928	.0159	1.341	0	0	0	20x
0-47	2028	1993	- 35	1467	-	-	20.2	1.376	198.0	1.349	.15	.102	.0918	.625	3.8	2.590	.0379	2.583	0	0	0	23
0-49	2360	1893	- 467	1442	8.88	6.102	24.7	1.712	158.35	1.097	.86	.596	.145	1.005	2.86	1.983	.0325	2.253	0	0	0	20x
0-56	2120	1102	-1018	895	4.45	4.972	13.26	1.481	45.0	.05	.4	.447	.08	.893	.35	.391	-	-	0	0	0	21x
0-59	1975	1885	- 90	1562	6.7	4.289	23.5	1.504	107.5	.688	1.6	1.024	.0965	.617	5.3	3.393	.0265	1.696	0	0	0	13x
0-60	1977	1597	- 380	1295	3.96	3.057	14.27	1.101	90.17	.695	.46	.355	.102	.787	1.17	.903	.0125	.965	0	0	0	21x
0-61	1675	1190	- 485	993	6.0	6.042	15.4	1.550	80.5	.081	.35	.352	.13	1.309	small		.0238	2.392	0	0	0	19x
0-65	2055	2115	60	1602	7.2	4.494	19.1	1.192	261.0	1.629	1.0	.624	.0905	.564	1.65	1.029	.0208	1.298	0	0	+	26
0-68	2535	2250	- 285	1575	8.7	5.523	6.2	1.028	212.75	1.35	.7	.444	.095	.603	2.15	1.365	.0299	1.898	0	0	+	26
0-70	2070	1925	- 145	1483	5.35	3.607	17.8	1.200	198.2	1.336	.75	.505	.0714	.481	1.4	.944	.019	1.281	+	0	0	27
0-73	2490	2710	220	1830	6.7	3.661	22.0	1.202	233.25	1.274	1.1	.601	.124	.677	1.3	.710	.0305	1.666	0	0	0	27
0-78	2145	2330	185	1636	6.65	4.064	22.45	1.372	206.45	1.261	.65	.397	.0845	.516	1.25	.675	.028	1.711	0	0	+	27

x = died spontaneously

TABLE VIII.

AVERAGE DAILY FOOD INTAKE

Room Temperature Horse Serum Group

Animal No.	Control Period	After I/V H.S. I	After I/V H.S. II	Whole Experiment
K-27	115.0 (10)	131.5 (16)	135.0 (1)	125.5 (27)
K-29	119.5 (10)	107.6 (16)	78.1 (8)	104.1 (34)
L-7	129.2 (7)	145.0 (11)	149.2 (7)	141.7 (25)
L-8	152.8 (7)	139.5 (11)	49.3 (8)	115.3 (26)
L-12	143.7 (7)	145.0 (11)	146.2 (8)	145.0 (26)
L-46	160.4 (10)	144.0 (11)	148.8 (8)	150.9 (29)
L-47	138.0 (10)	135.9 (11)	108.8 (9)	128.5 (30)
L-61	129.8 (10)	151.5 (11)	177.2 (9)	152.0 (30)
N-83	123.0 (9)	191.3 (11)	195.0 (8)	170.0 (28)
N-95	155.0 (9)	160.9 (11)	125.5 (8)	148.9 (28)
O-55	92.7 (9)	73.7 (11)	1.0 (8)	159.0 (28)
O-58	15.7 (9)	127.0 (11)	129.7 (8)	91.9 (28)
Mean	120.9 (116)	133.5 (149)	119.4 (90)	97.7 (355)

Note: Figures in brackets = No. of days.

TABLE IX.

AVERAGE DAILY FOOD INTAKE.

Cold Room Control Animals
(those surviving whole experimental period only)

Animal No.	Control Period	Cold Room Period	Whole Experiment	Lesions Valve	Artery	Kidney
K-31	124.2 (7)	191.4 (27)	177.6 (30)	0	0	0
L-4	62.3 (4)	148.4 (22)	135.1 (26)	0	0	0
L-54	132.8 (7)	125.2 (22)	127.2 (27)	0	0	0
L-63	133.5 (7)	123.5 (22)	125.9 (29)	0	0	0
O-42	39.8 (6)	206.5 (23)	172.0 (29)	0	0	0
O-48	93.6 (6)	158.5 (22)	144.6 (28)	0	0	0
O-83	146.6 (6)	188.2 (21)	178.9 (27)	0	0	0
Mean	108.6 (43)	161.5 (160)	153.5 (200)			

Note: Figures in brackets = No. of days.

TABLE X.

AVERAGE DAILY FOOD INTAKE

Cold Room Horse Serum Group
(survivors of whole experiment only)

Animal No.	Control Period	Cold Room Control Period	After I/V H.S. I.	After I/V H.S. II	Whole Experiment	Lesions. Valve	Artery	Kidney
K-26	120.7 (7)	125.0 (3)	155.1 (16)	151.1 (8)	144.4 (34)	0	0	+
K-32	187.1 (7)	140.6 (3)	196.4 (16)	141.25(8)	176.6 (34)	+	+	0
L-5	158.7 (4)	155.0 (3)	213.7 (16)	202.6 (2)	197.0 (25)	++	+	+
L-13	158.7 (4)	163.3 (3)	142.7 (11)	95.71(7)	134.58(25)	+++	+++	++++
L-51	147.1 (7)	126.3 (3)	130.9 (11)	155.0 (8)	140.9 (29)	+	+	+
L-57	143.6 (7)	103.3 (3)	112.3 (11)	140.0 (8)	126.5 (29)	+++	++++	++
L-64	155.7 (7)	141.6 (3)	99.0 (11)	158.1 (8)	133.4 (29)	0	0	0
N-79	123.3 (6)	113.3 (3)	129.5 (11)	108.1 (8)	120.3 (28)	0	0	++
N-82	114.1 (6)	140.0 (3)	161.8 (11)	120.0 (8)	137.3 (28)	0	0	+
N-92	156.6 (6)	135.0 (3)	162.3 (11)	140.0 (8)	145.3 (28)	+	0	+
Mean	143.36(81)	126.6(30)	150.15(125)	136.76(73)	145.7(289)	+ 153.5 (6)	+ 143.3(8)	
						0 133.8 (4)	0 155.0(2)	

Note: Figures in brackets = No. of days.

TABLE XI.

AVERAGE DAILY FOOD INTAKE

Cold Room Horse Serum ACTH Group.
(survivors of whole experiment only)

Animal No.	Control Period	ACTH	ACTH & Cold	After I/V H.S. I	After I/V H.S. II	Whole Experiment	Lesions. Valve	Artery	Kidney
K-30	181.2 (4)	103.3 (3)	155.0 (3)	166.3 (16)	150.6 (8)	157.8 (34)	+	+	0
L-1	160.0 (1)	105.0 (3)	131.6 (3)	124.5 (11)	188.7 (8)	144.2 (26)	+	+++	++
L-18	75.0 (9)	95.0 (3)	120.0 (3)	153.1 (11)	199.6 (8)	151.1 (26)	+	+++	+++
L-20	100.0 (1)	100.0 (3)	116.6 (3)	103.6 (11)	150.0 (8)	118.8 (26)	+	+	0
L-67	168.7 (4)	121.6 (3)	85.0 (3)	157.7 (11)	113.5 (7)	136.6 (28)	0	0	0
N-96	123.3 (3)	66.6 (3)	120.0 (3)	149.7 (11)	137.8 (8)	131.4 (28)	0	0	0
N-97	93.3 (3)	128.3 (3)	155.0 (3)	160.4 (11)	105.6 (8)	117.1 (28)	0	0	0
0-43	85.4 (3)	81.6 (3)	72.3 (3)	69.8 (11)	107.1 (8)	83.6 (28)	0	0	+
0-53	80.0 (1)	65.0 (1)	62.6 (3)	131.6 (11)	139.0 (8)	120.5 (24)	0	0	0
0-54	134.3 (3)	68.0 (3)	23.3 (3)	97.2 (10)	130.0 (8)	99.6 (27)	0	0	0
0-76	133.0 (3)	66.6 (3)	61.0 (3)	102.3 (11)	119.7 (8)	102.3 (28)	0	0	0
0-80	134.3 (3)	99.3 (3)	34.3 (3)	109.3 (11)	47.2 (8)	85.12(28)	0	0	0
Mean	132.0(48)	93.3(34)	93.9(36)	123.4(136)	131.9(95)	121.1(331)	+ 143.0(4)	+ 126.3(3)	
							0 109.4(8)	0 118.8(9)	

Note: Figures in brackets = No. of days.

TABLE XII.

AVERAGE DAILY FOOD INTAKE.

Adjuvant, Globulin Group.

Animal No.	Control Period	After Adj.Glob.I.	After Adj.Glob.II.	Whole Experiment	Lesions. Valve	Artery	Kidney
0-46	160.3 (6)	122.0 (9)	130.7 (10)	123.9 (25)	0	0	++
0-50	138.9	124.2 (10)	129.5	129.8 (26)	0	+	++
0-52	135.3	107.0	95.6 (11)	108.6 (27)	0	0	+
0-64	147.6	116.9	110.4	121.1	0	0	+++
0-67	85.0	111.7	138.6	116.7	0	0	0
0-69	143.5	120.5	132.7	130.6	0	0	++
0-71	136.0	99.5	102.0 (10)	108.9 (26)	0	0	0
0-79	157.5	60.3	124.4	107.4	0	0	0
0-81	88.0	126.4	73.8 (9)	98.28(25)	0	0	+
0-82	137.6	186.1 (9)	87.5 (10)	135.0	0	0	0
Mean	124.1 (60)	113.3 (98)	110.6 (103)	118.0 (261)	+ 129.8(1)	+ 118.7(6)	
					0 116.8(9)	0 117.0(4)	

Note: Figures in brackets = No. of days.

TABLE XIII

AVERAGE DAILY FOOD INTAKE (grams)

Adjuvant Globulin Cortisone Group

Animal No.	Control Period	Cortisone only	After Adj.Glob. I.	After Adj.Glob.II.	Whole Experiment	Lesions. Valve	Artery	Kidney
0-47	131.0 (3)	150.6 (3)	150.6 (10)	158.8 (10)	151.5 (26)	0	0	0
0-65	138.0	144.0	156.4	125.5 (9)	141.6 (25)	0	0	+
0-68	61.6	45.6	156.3	101.8 (10)	111.6 (26)	0	0	+
0-70	136.3	189.6	153.5	108.2 (11)	137.1 (27)	(+)	0	0
0-73	86.0	39.3	196.0	145.7	145.9	0	0	0
0-78	126.0	201.6	166.5	147.6	158.2	0	0	+
Mean	113.1 (18)	108.4 (18)	160.6 (60)	125.0 (62)	141.1 (158)			

TABLE XIV

Appendix B

SKIN TESTS

AVERAGE DIAMETER IN MILLIMETERS OF 24 HOUR READINGS
CORRELATED WITH LESIONS OF HEART AND KIDNEYS.

GROUP I

Animal No.	Sal				HS 1/1				1/10				1/100				1/1000				ACTH				Cort			
	R	O	H	N	R	O	H	N	R	O	H	N	R	O	H	N	R	O	H	N	R	O	H	N	R	O	H	N
K-38+	0								25	25	0	0	10	10	0	0		0										
K-40+	0								40	40	0	0	20	20	0	0	8	8	0	0								
L-3	0				0				0				0				0				0				0			
L-17	0				0	15	0	0	0				0				0				0				0			
L-19	0				0				0				0				0				0				0			
L-43	0				0				0				0				0				0				3	3	0	0
L-48	0				10	10	0	0	0				0				0				0				0			
L-55	0				0				0				0				0				0				0			
N-85	0				0				0				0				0				0				0			
O-74	0				0				0				0				0				0				0			
Mean	0				1.2	3.1			32.5	32.5	0	0	15	15	0	0	8	8	0	0	0				0			

\+ - Repeated skin tests
R - Redness
O - Oedema
H - Haemorrhage
N - Necrosis
Cort. - Cortisone
Sal - Normal saline

TABLE XV

SKIN TESTS

AVERAGE DIAMETER IN MILLIMETERS OF 24 HOUR
READING CORRELATED WITH LESIONS OF HEART AND KIDNEYS

GROUP II

Animal No.	Saline R	Saline O	Saline H	Saline N	HS 1/1 R	HS 1/1 O	HS 1/1 H	HS 1/1 N	1/10 R	1/10 O	1/10 H	1/10 N	1/100 R	1/100 O	1/100 H	1/100 N	1/1000 R	1/1000 O	1/1000 H	1/1000 N	ACTH R	ACTH O	ACTH H	ACTH N	Cort. R	Cort. O	Cort. H	Cort. N	Lesions Val	Lesions Art	Lesions Kid.
L-29	0								20	20	0	0	10	10	0	0	0	0											+	++++	+
L-7	0	14	14	0	0	5	0	0	0				0				0				0				0				+	++++	+
L-8	0					8	0	0	0				0				0				0				0				+	+	+
L-12	0	22	22	0	0			0					0				0				0				0				0	0	+
L-46	0	35	48	24	2	14	20	0	0				0				0				0				0				0	0	0
L-47	0	42.5	47.5	10.5	5.5	14	20	0	0	0			8	0	0		0				0				0				++	++++	++
L-61	0	26	30	9.5	0	10.5	13	0	0				0				0				0				0				+	++	+++
N-83	0	0	13.5	7.5	0	0	7	3.5	0	5.5	11		0	0	7		12.5	0	0		0				0				++	++	+
N-95	0	0	19	0	0	0	11	0	0				0			0	9	0	0		0				0				++	++++	+
O-55	0	0	27.5	11	0	18	18	0	0	11.5	11.5	0	0	6.5	0		0	0	3	3	0	0			0				0	0	+
O-58	0	28.5	28.5	0	0	10	18.5	0	0				0				0				0				0				0	0	0
Mean	0	18.66	27.8	6.9	0.9	9	11.6	0.3	0	2.4	3.7	0	0	1.2	1.9	0	0	0.3	0.3						0						

R - Redness O - Oedema H - Haemorrhage N - Necrosis Cort. - Cortisone Val - Valve Art - Artery Kid. - Kidney

TABLE XVI

Appendix B

SKIN TESTS

AVERAGE DIAMETER IN MILLIMETERS OF 24 HOUR READINGS
CORRELATED WITH LESIONS OF HEART AND KIDNEYS.

GROUP III

Animal No.	Sal R O H N	HS 1/1 R O H N	1/10 R O H N	1/100 R O H N	1/1000 R O H N	ACTH R O H N	Cort R O H N
K-31+	0		8 8 0 0	0 1 0 0	0		
L-4	0	0	0	0	0	0	0
L-54	0	0 9 0 0	0	0	0	0	0
L-63	0	10 5 25 0	0	0	0	0	0
0-42	0	0	0	0	0	0	0
0-48	0	0	0	0	0	0	0
0-83	0	0	0	0	0	0	0

+ - Repeated skin tests
R - Redness
O - Oedema
H - Haemorrhage
N - Necrosis
Cort. - Cortisone
Sal - Saline

TABLE XVII

SKIN TESTS

AVERAGE DIAMETER IN MILLIMETERS OF 24 HOUR
READING CORRELATED WITH LESIONS OF HEART AND KIDNEYS

GROUP IV

Animal No.	Saline R	Saline O	Saline H	Saline N	HS 1/1 R	HS 1/1 O	HS 1/1 H	HS 1/1 N	1/10 R	1/10 O	1/10 H	1/10 N	1/100 R	1/100 O	1/100 H	1/100 N	1/1000 R	1/1000 O	1/1000 H	1/1000 N	ACTH R	ACTH O	ACTH H	ACTH N	Cort. R	Cort. O	Cort. H	Cort. N	Lesions Val	Lesions Art.	Lesions Kid.
K-26	0								15	15	0	0	0				0												0	++	+
K-32	0								5	20	0	0	0				0												+	+	0
L-5	0	22.5	22.5	7.5	0	0	11	0	0								0				0				0				++	+	+
L-13	0	35	35	9	0	13.5	13.5	2	0				0				0				0				0				+++	++++	++++
L-51	0	20	20	0	0	7.5	7.5	0	0				0				0				0				0				+	+	+
L-57	0	27.5	27.5	0	0.5	11	0	0	0				0				0				0				0				+++	++++	++
L-64	0	12.5	31.5	0	0	0	9	0	0				0				0				0				0				0	0	0
M-79	0	22	22	0	0	7	18.5	0	0	0	13.5	0	0	0	10	0	0				0				0				++		
M-82	0	10	0	0	0	8.5	8.5	0	0	6.5	6.5	0	0	3	7	0	0				0				0				+	0	+++
L-92	0	0	42.2	0	9				0	7.5	4	0	4.5	7.5	0	0					0				0						
Mean	0	18.7	25.9	2.1	1.2	8.2	11.4	0.2	0	2.2	2.2	1.3	0	2.5	8.2	0	0				0				0						

R - Redness O - Oedema H - Haemorrhage N - Necrosis Cort. - Cortisone Val - Valve Art. - Artery
Kid. - Kidney

TABLE XVIII

SKIN TESTS

AVERAGE DIAMETER IN MILLIMETERS OF 24 HOUR READING CORRELATED WITH LESIONS OF HEART AND KIDNEYS

GROUP V

Animal No.	Saline					HS 1/1					1/10					1/100					1/1000					ACTH					Cort.					Lesions		
	R	O	H	N		R	O		H	N	R	O		H	N	R	O		H	N	R	O		H	N	R	O		H	N	R	O		H	N	Val.	Art.	Kid.
K-30	0										20	20		0	0		0					0														+	+++	0
L-1	0		35			35		9		0	17.5	17.5					0				0					15		0	0						+	+++	++
L-18	0		30			10		0		0	11	11		0	0		0					0					0				0				+	++++	+
I-20	0		10			35		0		0	12	12		0	0		0					0					0				0				+	+	0
I-67	0		6			18.5		0		0	0	7		0	0		0					0					0				0				0	0	0
N-96	0		6.5			27.5		0		0	0	13.5		0	0		0					0					0				0				0	0	0
N-97	0		12.5			19.5		6.5		0	5	9.5		0	0	0	9.0		0	0	3	6.5		0	0		0				0	0			0	0	0
O-43	0		0			43.5		6.5		0						8	8		0	0		0					0								0	0	+
O-53	0		20			20		0		0	0	10		0	0		0					0				6	6		0	0					0	0	0
O-54	0		10			23.5		0		0	12.5	12.5		0	0	0	8		0	0		0				5	5		0	0					0	0	0
O-76	0		0			21		8.5		0	10.5	10.5		0	0		0					0				0	4.5		0	0					0	0	0
O-80	0		6.5			19.5		0		0	6	6		0	0		0					0				5.5	5.5		0	0					0	0	0
Mean	0		12.4			24.8		2.95		0	8.6	11.8		0	0	2.7	8.3		0	0	3	6.5		0	0	4.1	5.2		0	0	0				0	0	0

R - Redness O - Oedema H - Haemorrhage N - Necrosis Cort. - Cortisone Val. - Valve
Art. - Artery Kid. - Kidney

TABLE XIX

SKIN TESTS

AVERAGE DIAMETER IN MILLIMETERS OF 24 HOUR
READING CORRELATED WITH LESIONS OF HEART AND KIDNEYS

GROUP VIII

Animal No.	Saline	Adj.Glob.1/1			1/10			1/100	1/1000	Lesions		
	R O H N	R	O	H N	R	O	H N	R O H N	R O H N	Valve	Artery	Kidney
0-46	0	28.5	28.5	0 0	8.5	13.5	0 0	0	0	0	0	++
0-52	0	27.5	27.5	0 0	18.5	18.5	0 0	0	0	0	0	+
0-64	0	26	26	0 5	18.5	18.5	0 3	0	0	+	0	+++
0-67	0	28	28	0 0	10.5	0	0 0	0	0	0	0	0
0-69	0	35	55	0 0	25.5	25.5	0 0	0	0	0	0	++
0-71	0	42	42	0 0	13	24.5	0 0	0	0	0	0	0
0-79	0	11	50	0 6.5	11.5	22.5	0 0	8 8 0 0	0	0	0	0
0-81	0	23	23	0 0	11	11	0 0	0	0	0	0	+
0-82	0	20	20	0 9	12	12	0 6.5	9 0 0 0	0	0	0	0
Mean	0	30.8	33.3	0 2.3	14.3	16.2	0 1.5	85 4 0 0	0	0	0	0

R - Redness
O - Oedema
H - Haemorrhage
N - Necrosis
Adj.Glob. - Adjuvant Globulin

TABLE XX

SKIN TESTS

AVERAGE DIAMETER IN MILLIMETERS OF 24 HOUR READING CORRELATED WITH LESIONS OF HEART AND KIDNEYS

GROUP IX

Animal No.	Saline R O H N	Adj.Glob. 1/1 R	O	H	N	1/10 R	O	H	N	1/100 R O H N	1/1000 R O H N	Lesions Valve	Artery	Kidney
0-47	0	6	25	0	0					0	0	0	0	0
0-65	0	37	37	0	0	16.5	16.5	0	0	0	0	0	0	+
0-68	0	0	11.5	17	0		0			0	0	0	0	+
0-70	0	22.5	22.5	0	0	12.5	12.5	0	0	0	0	0	0	+
0-73	0	21.5	21.5	0	0	8.5	8.5	0	0	0	0	0	0	0
0-78	0	26	26	0	0	13.5	13.5	0	0	7 0 0 0	0	0	0	+
Mean	0	18.8	23.9			13.7	13.7	0	0	7 0 0 0	0			

R - Redness
O - Oedema
H - Haemorrhage
N - Necrosis
Adj.Glob.-Adjuvant Globulin

TABLE XXI

SKIN TESTS

AVERAGE DIAMETERS OF ALL GROUPS CORRELATED WITH LESIONS OF HEART AND KIDNEYS

	HS 1/1				1/10				1/100				1/1000				ACTH				Cort.			
	R	O	H	N	R	O	H	N	R	O	H	N	R	O	H	N	R	O	H	N	R	O	H	N
Gp. I.																								
Animals without lesions*					8	8	0	0	0	1	0													
Gp. II																								
All animals	18.7	27.8	6.9	0.9	9.1	11.6	0.3	0	2.4	3.7	0	0	1.2	1.9	0	0	0.3	0.3	0	0	0			
Animals with lesions	14.9	24.8	5.5	0.3	6.9	11.2	0.4	0	6.7	10.1	0	0	4.5	7.2	0	0								
Animals without lesions	31.7	38.7	12	1.0	12	19.5	0	0	0	0	0	0	0	0	0	0								
Gp. III.																								
All animals without les.*					33.5	32.5	0		15	15	0		8	8	0									
Gp. IV.																								
All animals	18.7	25.9	2.1	1.2	8.2	11.4	0.2	0	2.2	9.2	1.3	0	2.5	8.2	0	0		0				0		
Animals with lesions	19.2	24.6	2.7	1.6	8.6	10.8	0.3	0	3.2	7	2	0	3	7	0	0								
Animals without lesions	17.2	26.7	0	0	3.5	14	0	0	0	13.5	0	0	0	10	0	0								
Group V.																								
All animals	12.4	24.8	3.8	0	8.6	11.8	0	0	2.7	8.3	0	0	3	6.9	0	0	4.1	5.2	0	0		0		
Animals with lesions	18.7	30.9	7.7	0	15.1	15.1	0	0	8	8	0	0	0	0	0	0	0	0	0	0				
Animals without lesions	8.8	21.4	2.5	0	4.9	9.9	0	0	0	8.5	0	0	3	6.5			4.1	5.2	0	0				
Gp. VIII +																								
All animals	26.8	33.3	0	2.3	14.3	16.2	0	1.05	8.5	4	0	0						0				0		
Animals with lesions	28	32	0	1	16.4	17.4	0	0.6	0	0	0	0												
Animals without lesions	25.2	35	0	7.1	11.7	14.7	0	1.6	8.5	4	0	0												
Gp. IX +																								
All animals	18.9	23.9	0	0	13.7	13.7	0	0	7	0	0	0						0				0		
Animals with lesions	21.4	24.2	0	0	14.2	14.2	0	0	7	0	0	0												
Animals without lesions	13.7	23.2	0	0	8.5	8.5	0	0	0	0	0	0												

* Repeated skin tests of 2 animals in each group.

\+ Tested to graded dilutions of bovine gamma globul.

R - Redness. O - Oedema. H - Haemorrhage. N - Necrosis. Cort. - Cortisone.

TABLE XXII

RING TESTS

GROUP 1 - MALE NON-CONTROLS

Animal No.	Dil'n Antigen	Day of Experiment 0	12	13	14	15	20	23	25	26
K-38	1/1	0							0	
	1/40	0							0	
K-40	1/1	0							0	
	1/40	0							0	
L-3	1/1	0						0		
	1/40	0						0		
L-17	1/1	0						+		
	1/40	0						0		
L-19	1/1	0						+		
	1/40	0						0		
L-43	1/1	0	0	0	0	0			0	
	1/40	0	0	0	0	0			?	
L-48	1/1	0	0	0	0	0			0	
	1/40	0	0	0	0	0			0	
L-55	1/1	0	0	0	0	0			0	
	1/40	0	0	0	0	0			0	
N-85	1/1	0	0	0	0	0			0	
	1/40	0	0	0	0	0			0	
N-91	1/1	0		0	0	0				
	1/40	0		0	0	-				

TABLE XXIII

RING TESTS

GROUP II. WARM ROOM - HORSE SERUM

Animal No.	Antigen Dil'n	Day of Experiment 0	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Lesions: Valves	Arteries	Kidneys
		1/v HS I											1/v Desens								1/v HS II						
	1/1	0				0							+							+	x				++	++	0
K-29	1/40	0				0							+							+							
	1/1	0				0							+							+		+				
K-27	1/40	0				0							+							+		+			++	++++	+
	1/1	0	0				0			0			+							+							
L-7	1/40	0	0				0			0			+							+					+++	++++	+
	1/1	0	0				0			0			+							+							
L-8	1/40	0	0				0			+			+							+					+	+	+
	1/1	0	0				0			0			+									+			0	0	+
L-12	1/40	0	0				0			+			+									+					
	1/1							0	0	0	0	+		0								+			0	0	0
L-46	1/40							0	0	0	1	+		0								+					
	1/1							0	+	+	+	+		0									+				
L-47	1/40							0	+	+	+	+		0									+		+	++	++++	++
	1/1							0	+	+	+	+											+				
L-61	1/40							0	+	+	+	+											+		+	+	++	++
	1/1									+	+	+		+									+				
N-83	1/40							0		+	+	+		+									+		+	+++	++	++
	1/1							0																			
N-89	1/40							0																	0	0	0
	1/1							0		0	0	+		0									+				
N-95	1/40							0		0	+	+		0									+		+	++	++++	+
	1/1			0	0	+	+	+												+		+					
O-55	1/40			0	0	0	+	+												+		+			0	0	0
	1/1			0	0	+	+	+												+		+					
O-58	1/40			0	0	0	+	+												+		+			0	0	+

x - died spontaneously.

TABLE XXIV

RING TESTS

GROUP III - COLD ROOM CONTROL

Animal No.	Dil'n Antigen	Day of Experiment 0	8	10	12	13	14	15	16	19	21	22	23	25
		Cold Room from day 4												
K-28	1/1	0												
	1/40	0										x		
K-31	1/1	0												0
	1/40	0												0
I-2	1/1	0	0											
	1/40	0	0	x										
L-4	1/1	0												
	1/40	0												
L-16	1/1	+									0			
	1/40	0									0	x		
L-52	1/1	0	0	0	0	0								
	1/40	0	0	0	0	0x								
L-54	1/1	0	0	0	0	0	0							0
	1/40	0	0	0	+	0	0							0
L-63	1/1	0	+	0	0	0	0							0
	1/40	0	0	0	0	0	0							0
N-86	1/1	0		0	0	0								
	1/40	0		0	0	0x								
N-90	1/1	0		0	0	0								
	1/40	0		0	0	0x								
N-93	1/1	0		0	0	0								
	1/40	0		0	0	0x								
O-42	1/1	0		0	0	0	0							0
	1/40	0		0	0	0	0							0
O-48	1/1	±		0	±	0	0							0
	1/40	0		0	0	0	0							0
O-51	1/1	0		0	±	0	0							
	1/40	0		0	0	0	0							
O-63	1/1	0		0	0	0								
	1/40	0		0	0	0								
O-83	1/1	0		0	0	0	0				0			0
	1/40	0		0	0	0	0				0			0

x - died spontaneously.

TABLE XXV

RING TESTS

GROUP IV. COLD ROOM + HORSE SERUM

Animal No.	Antigen Dil'n	Day of Experiment 0	5	7	8	12	13	14	15	16	17	18	19	22	23	24	25	Lesions Valves	Arteries	Kidneys.
		1/v HS I								1/v Desens					1/v HS II					
		Cold Room from day 4																		
	1/1	0			0									+			+			
K-26	1/40	0			0									+			+	0	++	+
	1/1	0			0				+					+			+			
K-32	1/40	0			0				+					+			+	+	+	0
	1/1	0	0		0	+					+				+					
L-5	1/40	0	0		0	+					+				+			++	+	+
	1/1	0	0		0	0		x												
L-6	1/40	0	0		0	0												0	0	0
	1/1	0	0		0	0					+				+					
L-13	1/40	0	0		0	0					+				+			+++	++++	++++
	1/1					0	0	+	+	+		+			+					
L-51	1/40					0	0	+	+	+		0			+			+	+	+
	1/1					0	+	+	+	+		+			+					
L-57	1/40					0	+	+	+	+		+			+			+++	++++	++
	1/1					0	0	+	0	+		0			+					
L-64	1/40					0	0	0	?	+		0			-			0	0	0
	1/1					0		+	+	+		+				+				
N-79	1/40					0		+	+	+		+				+		0	0	++
	1/1					0		+	+	+		0				+				
N-82	1/40					0		+	+	+		0				+		0	0	+
	1/1					0		+	+	+		+				+				
N-82	1/40					0		+	+	+		0				+		+	0	+++

x - died spontaneously

TABLE XXVI GROUP V **COLD, HORSE SERUM, ACTH.**

Animal No.	Antigen Dil'n	Day of Experiment 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Lesions: Valves	Arteries	Kidneys
									1/v HS I										1/v Desens								1/v HS II				
									Cold Room from day 4																						
		ACTH																													
	1/1	0							0								+							+			+			
K-30	1/40	0							0								+							+			+	+	+++	0
	1/1	0							0								+														
Y-36	1/40	0							0								+	x											0	0	0
	1/1	0							0				+						+					+							
L-1	1/40	0							0				0						+					+					+	+++	++
	1/1	0							0				0						+					+							
I-18	1/40	0							0				0						+					+					+	+++	+
	1/1	0					0		0				+						+					+							
L-20	1/40	0					0		0				0						+					?					+	+	0
	1/1												0	+	0																
L-44	1/40												0	0	0	x													0	0	0
	1/1												0																		
I-48	1/40												0	x															0	0	0
	1/1												0	0	+	?	0		0												
L-67	1/40												0	0	0	0	0		0	x									0	0	0
	1/1												0		+	+	+		0												
N-80	1/40												+		+	+	+		0	x									0	0	0
	1/1												0		+	+	+		0							+					
N-96	1/40												0		+	+	+		+							+			0	0	0
	1/1												0		+	+	+		+							x					
N-97	1/40												0		+	+	+		+										0	0	0
	1/1			0	0	0	0	0	±									+		+											
O-43	1/40			0	±	0	0	0	+									+		+									0	0	+
	1/1			0	0	0	0	0	+									+		+											
O-53	1/40			0	0	+	0	0	0									+		+									0	0	0
	1/1			0	+	+	0	0	+									+		+											
O-54	1/40			0	+	0	0	0	+									+		+									0	0	0
	1/1			0	0	0	+	+																					0	0	++
O-66	1/40			0	±	0	+	+										x													
	1/1			0	0	0	0	+										+		+											
O-76	1/40			0	0	0	0	+										+		+									0	0	0
	1/1			0	0	0	0	+										+													
O-80	1/40			0	0	0	0	+										+									x		0	0	0

x - died spontaneously

TABLE XXVII

Appendix B

ADJUVANT TESTS

GROUP VIII - GLOBULIN, ADJUVANT

Animal No.	Dil'n Antigen	Day of Experiment 7	8	9	10	12	15	23	Lesions
0-46	1/1	0	0	+	+	+	+	+	
	1/40	0	0	0	+	+	+	+	0 0 ++
0-50	1/1	0	0	+	+	+	+	+	
	1/40	0	0	+	+	+	+	+	0 0 ++
0-52	1/1	0	0	+	+	+	+	+	
		0	0	0	0	+	+	+	0 0 +
0-64	1/1	0	0	+	+	+	+	+	
	1/40	0	0	+	+	+	+	+	+ 0 +++
0-67	1/1	0	0	+	+	+	+	+	
	1/40	0	0	0	0	+	+	+	0 0 0
0-69	1/1	0	0	+	+	+	+	+	
	1/40	0	0	0	0	+	+	+	0 0 ++
0-71	1/1	0	0	+	+	+	+	+	
	1/40	0	0	0	0	-	-	+	0 0 0
0-79	1/1	0	0	0	+	+	+	+	
	1/40	0	0	0	+	+	+	+	0 0 0
0-81	1/1	0	0	+	+	+	+	+	
	1/40	0	0	0	+	+	+	+	0 0 +
0-82	1/1	0	0	+	+	+	+	+	
	1/40	0	0	0	+	+	+	+	0 0 0

Adjuvant Globulin I given on day 4
Adjuvant Globulin II given on day 14

TABLE XXVIII.

RING TESTS

GROUP IX. ADJ. GLOB., CORTISONE.

Animal No.	Antigen Dil'n	Day of Experiment 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	23	Lesions Valves	Arteries	Kidneys
		Cortisone																		
		Adj. glob.I															Adj.glob.II			
	1/1							0	0	0	±	0	0							
0-44	1/40							0	0	0	0	0	0					0	0	0
	1/1							0	0	0	+		+			+	+			
0-47	1/40							0	0	0								0	0	0
	1/1							0	0				+			0				
0-49	1/40							0	0									0	0	0
	1/1							0	0	0	0	±	0			0				
0-56	1/40							0	0	0	0		±					0	0	0
	1/1							0	0											
0-59	1/40							0	0	x								0	0	0
	1/1							±	0	0	±	+	+							
0-60	1/40							0	0	0	0	0				x		0	0	0
	1/1							0	0				+							
0-61	1/40							0	0									0	0	0
	1/1							0	0	+	0	+	+			+	+			
0-65	1/40							0	0	+								0	0	+
	1/1							0	0	0	+		+			+	+			
0-68	1/40							0	0	0								0	0	+
	1/1							0	0	+	+		+			+	±			
0-70	1/40							0	0									0	0	0
	1/1							0	0	+	+		+			+	+			
0-73	1/40							0	0									0	0	0
	1/1							0	0	0	+	+	+			+	+			
0-78	1/40							0	0	0								0	0	+

x - died spontaneously

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