GLOMERULONEPHRITIS PRODUCED IN RABBITS BY MASSIVE INJECTIONS OF SERUM PROTEINS.

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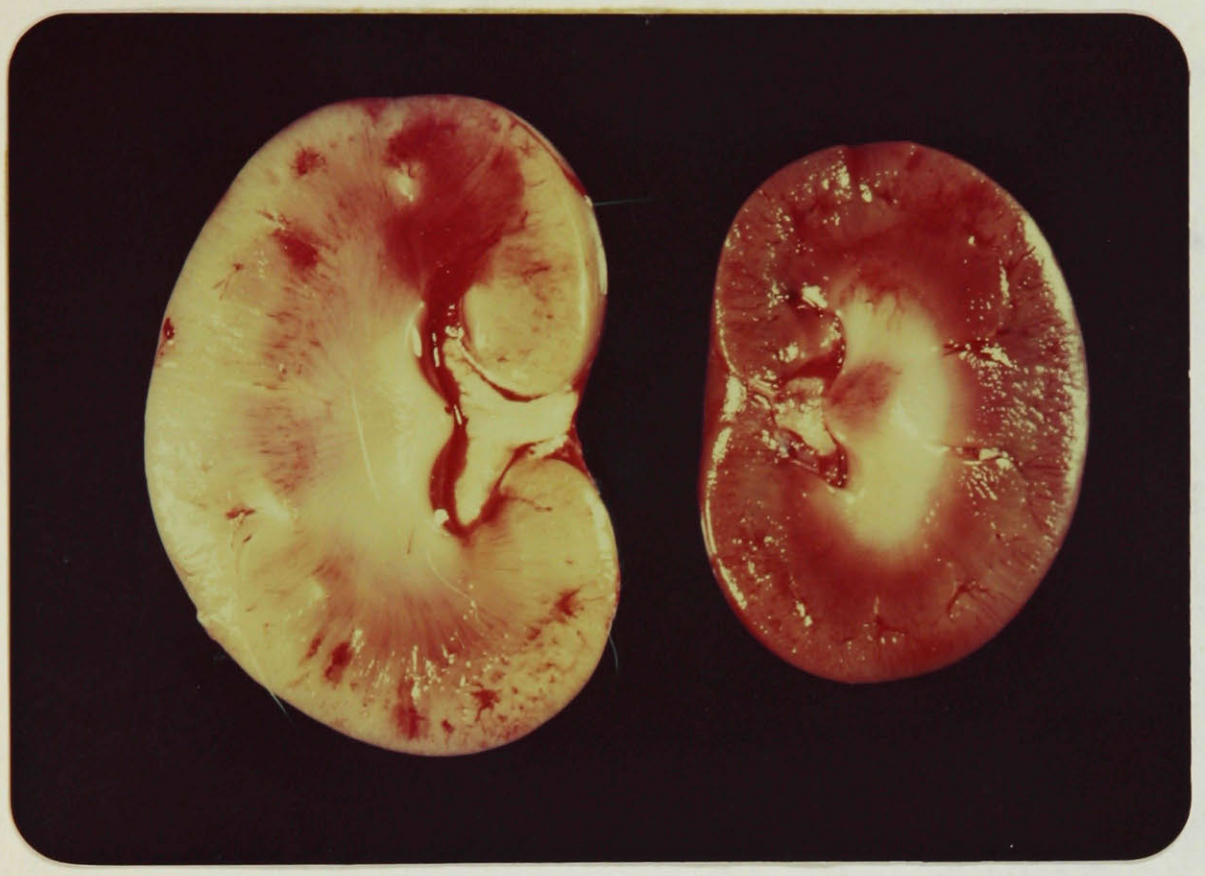

EXPERIMENTAL ACUTE GLOBULIN NEPHRITIS: The kidney on the left is from a rabbit given two massive injections of bovine serum gamma globulin, ten days apart, and killed six days following the second injection. The animal was kept in a cold environment throughout the experiment. The kidney on the right is from a comparable untreated control animal of the same body weight. The kidney of the treated animal shows all the essential gross features found in human acute or subacute diffuse glomerulonephritis.

BRIGHT'S DISEASE

Doctor Richard Bright of Guy's
Had several patients large in size.
Their legs were swollen as could be;
Their eye so puffed they could not see.
To this oedema Bright objected,
And so he had them venesected.
He took a teaspoon by the handle,
Held it above a tallow candle,
And boiled some urine o'er the flame
(As you or I might do the same).
To his surprise, we find it stated,
The urine was coagulated.
Alas, his dropsied patients died.
Our thoughtful doctor looked inside:
He found their kidneys large and white,
The capsules were adherent quite.
So that is why the name of Bright is
Associated with nephritis.

oooOooo

From St. Bartholomew's Hospital Journal.

PREFACE.

The experimental work reported here was carried out at the Pathological Institute, McGill University, during the years 1947 - 50, in partial fulfillment of the requirements for the degree of Ph.D. Throughout the period the writer has been the recipient of Medical Fellowships of the National Research Council of Canada. The pursuit of the project has been greatly facilitated by the generous assistance of the Institute's Director, Professor G. Lyman Duff, who has taken extreme pains to ensure the adequacy of physical facilities for the work. Professor Duff's wide experience and critical insight have been freely at my disposal. I have shamelessly imposed upon him on many occasions, and pursued several ideas of which he was the author.

The project has been under the direct supervision of Professor Robert H. More, who has been in close and expert association with it from start to finish. In addition to giving invaluable assistance in the physical labours, his constant interest and guidance have been tremendously stimulating and helpful. Although Professor More has had much to do with the conception and planning of the experimental work, he should certainly not be held responsible for any shortcomings in its pursuit.

The entire staff of the Pathological Institute have given assistance of one sort or another, greatly facilitating many an otherwise difficult task. Dr. S. D. Kobernick deserves special mention for the assistance he has given on numerous occasions, and for permitting the free use of material from his experiments.

Drs. G. C. McMillan and C. R. McLean have given valuable criticism and help during the experimental work, and have kindly read and criticised portions of this text. Numerous special histological preparations have been made with the expert assistance of Dr. S. Bencosme.

Mr. Harold Coletta has taken all photographs appearing in the text, and has been patient and painstaking with a number of last-minute impositions. Difficult biochemical techniques have been carried out with precision and care by Miss Jacqueline Chaussé, who was assisted from time to time by Misses Irene Williams and Esther Hecht. Under supervision, Miss Chaussé carried out almost single-handed, the work of Experiment V. Microscopic sections were prepared by Mrs. E. Gallagher, Mrs. S. Bencosme, Misses A. Renaud, Y. Latondresse and V. Kostalowa.

Miss Lilian Brown has done the stenographic work with a rare combination of speed and accuracy, and has maintained a cheerful outlook despite the considerable pressure under which this part of the work has been done.

The staff of the Department of Bacteriology and Immunology have been extremely helpful in assisting us to set up immunological tests, and the department's technical facilities have been generously at our disposal. Especially valuable advice was given by Professor E. G. D. Murray and the late Professor Frederick Smith, as well as by Drs. J. W. Stevenson, and J. de Vries.

Through the kind co-operation of Drs. Kenneth Savard and Lena Lewis of the Cleveland Clinic, it has been possible to obtain electrophoretic analyses of serum samples, by which to check the methods used in the experiments.

Seitz filtration of the bovine gamma globulin solutions was carried out by Mr. Turrho Salo and Miss Deirdre Lang, who also checked the sterility of all lots of globulin prepared.

The writer's flights of fancy and uneven temper have been tolerated with extraordinary patience by his wife, who, in addition, has typed the bibliography and helped with many translations from foreign literature.

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I N T R O D U C T I O N

The experiments to be reported in this treatise were carried out with the object of contributing to the general knowledge of the human disease, acute diffuse glomerulonephritis. Albino rabbits were used in all experiments. These animals exhibit many important anatomical and physiological differences from humans. It is therefore obvious that any findings reported will depend for their ultimate validity upon the demonstration that, in certain respects at least, rabbits are capable of responses similar to those of humans, and vice versa. Limitations of time and material resources have not permitted the work to reach this decisive point. Thus, any equivalence existing between the experimental disease in rabbits and the natural disease in humans will have to be reached via deduction, which must, itself, be based upon knowledge of the human disease and its presumed experimental counterpart. The various aspects of human glomerulonephritis will, therefore, be considered in some detail before discussion of artificially induced nephropathies in rabbits is entered.

I. HISTORICAL REVIEW.

The affliction of "dropsy" has been known by one name or another in all ages and cultures whose literature has survived. Early in the 19th century it began to be suspected that disorders of the kidneys might be responsible for certain cases of dropsy. Wells(1), in 1812, observed blood-tinged urine put out by patients with post-scarlatinal dropsy, and also showed that such urine would coagulate when heated. These observations led him to suspect that his patients' urine contained blood serum, and to surmize that the kidneys were the primary focus of disease.

Fifteen years later Richard Bright(2) published the first of a series of reports in which he established all the important clinical features of acute and chronic nephritis, and related these to the gross morbid anatomy of the kidneys at autopsy. "Bright's Disease" (as the condition soon became known) actually included several renal diseases whose clinical behaviour included the features of oedema, proteinuria, blood urea accumulation and hypertension, and which could be shown at autopsy to be associated with either large, pale kidneys in the early phases, or with granular contracted kidneys in chronic cases.

The century since Bright has seen almost continuous division and re-arrangement of the group of conditions originally referred to. Thus, at the present time a large number of clinically and morphologically distinct morbid processes have been identified, and numerous classifications of Bright's "Disease" have been proposed(3,4,5,6 & 7).

In 1840 Rayer(8) suggested an inflammatory basis of Bright's disease. Traube, in 1856(9), differentiated Rayer's "nephrite albumineuse" from such conditions as amyloid kidney and from chronic passive congestion with albuminuria. Gull and Sutton, in 1872(10), described "Hyalin fibroid" arteriolar lesions in certain contracted kidneys, thus initiating the separation of nephrosclerosis from the general group. As knowledge of pathologic anatomy progressed, it became customary to divide chronic nephritis into a parenchymatous and an interstitial form(11). The former was supposed to be characterized clinically by oedema, oliguria and albuminuria, while the latter was dominated by cardiovascular phenomena. Both Weigert(12) and Cohnheim(13) resisted this trend and identified both forms as part of a fundamental process of parenchymal damage, as they are generally regarded today. The earlier concept persisted however, and was perpetuated in the early editions of Osler's text-book(14).

By the turn of the century, although considerable progress had been made in the investigation of Bright's disease, the overall picture was still one of confusion. The observed clinical phenomena often could not be correlated with morphological findings at necropsy, and the need for a comprehensive and integrated classification of the disease began to become apparent. This was done in 1914 by Volhard and Fahr(3) in a monograph on renal disease. Based upon a synthesis of clinical and morphological observations, this work contained the first logical classification of renal diseases. The basis of classification was a division into three main categories: degenerative (nephrotic), inflammatory, and arteriosclerotic diseases. Under inflammatory diseases, diffuse glomerulonephritis was separated from focal and embolic nephritis. This study marked the beginning of a new era in the study of renal diseases, and in the years

that have followed, a prodigious amount of clinical and experimental investigation has been carried out. It is important to realize that until a more or less precise classification had been initiated by Volhard and Fahr, no advancement in the basic knowledge of pathogenesis of the conditions could be expected to occur. Though some of the early confusion of classification still persists, it is now possible to investigate one or other renal disease with reasonable precision and hopes of success.

II. GLOMERULONEPHRITIS IN MAN.

Definition.

Human glomerulonephritis is an acute diffuse inflammation of the kidneys in which the principal damage is to the glomeruli. It is usually associated with the clinical phenomena of oedema, albuminuria, cylindruria and hematuria. The condition commonly subsides with complete recovery in six to eight weeks, but may progress via subacute or latent phases into a chronic and progressive renal damage with manifestations of functional impairment and vascular hypertension(16,17,18 & 19).

It has been suggested that such a definition may be too broad, and that glomerulonephritis should be subdivided into clinical and morphological types having different prognostic outlooks(6,7, & 15). However, since most of the investigation of nephritis has lain within the scope of the above definition, it seems more profitable to review this body of literature as a whole. The etiology, clinical course, and morbid anatomy of human glomerulonephritis will be reviewed in that order. When this has been done, a foundation will have been laid for the subsequent discussion of various artifical nephropathies in experimental animals.

Etiology.

Incidence: Glomerulonephritis cannot be considered one of the common diseases. Seegal et al.(22) calculated the general hospital admission rate for acute glomerulonephritis as lying between 0.47% and 0.62%. Bell(15) estimates nephritis to be the cause of 1% of all deaths beyond one year of age. The relative importance of nephritis is increased by virtue of its tendency to attack the young. Prevention or cure thus offers greater prospect in life-years saved than might the satisfactory attack upon many a commoner ailment in the later age groups.

Age: Acute glomerulonephritis is primarily a disease of children and young adults(15). The greatest numbers of cases occur either in the first(22) or second(21 & 22) decades, with progressively smaller numbers of cases in older persons. Post-scarlatinal nephritis tends to have a maximum incidence in children rather than adolescents(23 & 24). Cases in infancy are rare, though not unknown(18). One case of congenital nephritis has been reported(25) in an infant dying 45 minutes after birth.

Sex: Males of any age are rather more liable to nephritis than females(20,21 & 22). Male dominance in pre-pubertal years was found by Murphy and Rastetter(20). It is of interest that Bell(15), in a rather small series, found no difference in sex ratio in fatal acute nephritis.

Race: Race does not appear to be a predisposing factor in nephritis. However, in the "epidemic" occurrence of war nephritis in World War I, native Indian troops serving in the same divisions as British troops, amongst whom acute nephritis was common, showed an apparently absolute immunity(26).

Geography and Climate: Acute glomerulonephritis appears to occur with about equal frequency at different parallels of latitude. Seegal et al.(22 & 27) found no significant difference in hospital admission rates for nephritis in groups of cases collected from various hospitals in the United States and Canada through a range in latitude from N. 29° to N. 50°. These same authors found a maximum seasonal incidence in the months of February, March and April in more northerly regions, whereas in southern zones the seasonal distribution curve was flatter, with peak incidence in January and February, and a slight secondary rise in August.

Familial and Hereditary Nephritis: Few investigators appear to have been impressed with any familial or hereditary predisposition to nephritis. There is one rather unconvincing report(28) of three sons and one daughter with nephritis in the same family, and the authors refer to other similar occurrences. A family of 27 members in three generations, of whom 16 were said to have nephritis, has also been reported(29). These and other isolated reports(30) all seem open to the objection that the investigation of the patients was often cursory, and that it is not clearly established that the condition was truly glomerulonephritis.

Epidemic Nephritis: In the true sense of the word epidemic nephritis cannot be said to occur. However, in the American Civil War, and in World Wars I and II, acute nephritis did occur among soldiers with far greater frequency than could have been reasonably expected in equivalent civilian populations(26,31,32 & 33). Widely known as "trench nephritis", the term war nephritis appears preferable, since numbers of cases occurred among troops never exposed to trench warfare(31). The peak incidence was

ten cases in a single battalion (about 1,000 men) over a period of four months(26).

"Epidemic" acute nephritis has also been reported in civilian populations(34 & 35). The most recent outbreak occurred in Amsterdam in 1945(35). In this instance 38 acute nephritics were admitted to hospital during the year, compared to a normal average admission rate of six cases per year.

Exposure to Cold: Recent exposure to cold, or to cold and dampness, has been a frequently noted item in the histories of patients with acute nephritis(2,18,36 & 37). Temporary albuminuria has been observed after prolonged bathing in cold water(38), but partial immersion for short periods has not had this effect(39). It has been assumed(18) that the role of cold in the pathogenesis of nephritis is that of a predisposing factor, acting by the production of reflex ischemia of the upper respiratory mucosa(40) with resulting increased liability to infection. At the present time it can only be stated that cold appears to play some part in the pathogenesis of acute nephritis, but further evidence is needed before it can be known how important it may be, or by what mechanism it may act.

Diet and Social Factors: These have been but little investigated in relation to glomerulonephritis. In war nephritis during World War I it was noted that the disease was consistently less common among officers than in enlisted men(31). The Amsterdam outbreak of 1945(35) appears to support this conclusion that diet may have an important bearing upon the pathogenesis of acute nephritis. Nearly all the cases in this outbreak were admitted to hospital between May 1945 and September 1945. This coincides with the period

TABLE I.

PRECEEDING CONDITIONS IN NEPHRITIS

(after Fishberg)

Primary condition.	Southby and Stanton[6] (children).	Clausen[7] (children).	Hill[8] (children).	Volhard.[9]	Lichtwitz.[10]	Longcope.[11]	Total
Angina	29	45	14	17	28	24	157
Scarlet fever	0	26	4	19	3	4	56
Pneumonia	11	10	2	6	4		33+
Bronchitis	0	0	0	0	0	4	0+
Otitis and mastoiditis	0	5	4		0		9+
Influenza	2	1	0	8	5	0	8+
Rhinitis	1	*	0		4	0	5+
Cold (exposure)	0	0	1		2	0	3+
Sinusitis	0	4	0	0	0	13	17
Diphtheria	0	4	0	0	0	0	4
Rheumatic fever	0	0	0	2	0	0	2
Purpura	0	0	2	2	0	0	4
Impetigo and pyodermia	17	0	4	0	19	0	40
Infection of wounds and lymph nodes	0	0	0	7	1	3	11
Pregnancy	0	0	0	4	2	0	6
Erysipelas	0	1	0	1	0	0	2
Tonsillectomy	0	0	2	0	0	0	2
Osteomyelitis	0	1	0	0	0	0	1
Measles (during epidemic)	15	0	0	0	0	0	15
Congenital defects causing obstruction?	0	2	0	0	0	0	2
Tuberculosis	0	0	0	3	1	0	4
"Intestinal catarrh"	0	0	0	0	1	0	1
Unknown	27	3	15	2	26	?	73+
Total	102	102	48	71	96	48	455+

during which all of Western Holland, and Amsterdam in particular, was recovering from some seven months of semi-starvation(41). It might be suggested that the increase in proportion of the population existing in an actively anabolic state might in some way have rendered them more liable to acute nephritis. This might also explain the fact that the average age of patients in this outbreak was about fifteen years above that normally encountered in the same area. The above evidence is no more than suggestive, but indicates that investigation of individual dietary status prior to attacks of acute glomerulonephritis might be carried out with profit.

Infection: Most cases of acute glomerulonephritis follow an infection. It is usually an infection of some part of the respiratory tract, and often streptococcal(18,21-24,42-48)(see Table I). It was early pointed out that acute nephritis was common during convalescence from scarlet fever(23,24). It has since been shown that acute nephritis may follow otitis and mastoiditis, pneumonia, bronchitis, rhinitis, sinusitis, erysipelas, osteomyelitis and a variety of other infections(42-48). Beta Streptococci have been isolated from the respiratory tract of the majority of a group of 40 patients with acute or subacute nephritis(48). Alpha Streptococci were found in a small number of these same patients. A high percentage of positive skin reactions to streptococcal filtrates has been found(49) in patients with acute nephritis, while such reactions were rare in normal patients. Toxic substances of presumed streptococcal origin have also been demonstrated in the urine of patients with scarlet fever(50). Pneumococcal infections have also, though less frequently, been followed or accompanied by the development of acute nephritis(15,52 & 53).

It should be noted, however, that most of the above reports list cases in which no organism or history of recent infection could be elicited, even after careful search. In the Amsterdam outbreak referred to earlier(35), the majority of patients had had no previous infection, and none could be demonstrated on careful examination. Furthermore, the incidence of acute nephritis following upper respiratory infection is low(54), and appears to bear no relation to the severity of the infection(18).

A striking feature of nephritis following infection is that the renal complication almost invariably appears when the infectious process is on the wane(18,56). This is particularly true of post-scarlatinal nephritis, where the commonest time of onset is between 18 and 21 days after the initial sore throat(57).

Association with Other Diseases: The association of nephritis with respiratory tract infection has already been noted. Acute nephritis may also occur in association with subacute bacterial endocarditis(58,59), and has been said(58) to be commoner in association with this condition than with any other disease, with the possible exception of scarlet fever. It has been further observed that nephritis is more than fifteen times as common in the bacteria-free stage of subacute bacterial endocarditis as in the bacterial stage(59).

True glomerulonephritis appears to be rare in association with other diseases. In view of the widespread suspicion that rheumatic fever may be another disease associated with streptococcal infection, it is interesting to note that acute glomerulonephritis is extremely rare in this condition(59,60).

Summary

Acute diffuse glomerulonephritis is a not uncommon disease, affecting mainly children and young adults. Males of all ages contract the disease more frequently than females. No race appears to be immune. The maximal seasonal incidence is in midwinter, and geographical location does not appear to predispose to the condition. Reports of familial and hereditary nephritis seem based upon questionable diagnostic criteria, and it is doubtful whether these factors predispose to the condition. Epidemic nephritis is not uncommon in war-time among troops, but is relatively uncommon in civilian populations. Exposure to cold appears to play an etiologic role in acute nephritis, but the importance of this factor has not been fully determined. Diet and social factors may influence the appearance of acute nephritis, but few studies have attempted to evaluate these items. The most important single etiologic factor in nephritis seems to be a recent acute infection which is usually respiratory, and often streptococcal. Staphylococcal and pneumococcal infections may also be followed by the development of nephritis. Scarlet fever is fairly commonly complicated by acute nephritis during convalescence. Nephritis may occur as a complication of subacute bacterial endocarditis, but is rarely associated with other diseases.

The Clinical Features of Acute Diffuse Glomerulonephritis in Man.

The clinical picture of acute glomerulonephritis is one of endless variations on a central theme. The theme consists of the sum of clinical and biochemical phenomena which may result from varying degrees of diffuse glomerular damage. Any of these manifestations may dominate the clinical picture, or all may be obscured by unrelated, non-renal phenomena. The disease may run its course in a few days or weeks, it may last for months or become chronic, and fulminant cases may have a fatal outcome in a few days or weeks.

Onset: This may be acute or insidious. Fishberg(18) lists seven modes of onset as being of principal importance:

1. Latent phase, asymptomatic, except for positive urinary findings.

2. Those with oedema as the primary symptom. Swelling of eyelids usually noticed first, with oedema of feet or genitalia less commonly encountered.

3. Bloody or scanty urine as first symptom. May be associated with frequency, urgency or lumbar pain.

4. Initial symptoms those of acute infection. There is fever, sore throat, chills, headache and bodily pains.

5. Cerebral symptoms may announce the disease, especially in children. Headache, convulsions, transitory palsies and vomiting are the chief of these.

6. Dyspnoea is the common initial symptom in war nephritis(26), but is rare in civilian cases.

7. Insidious onset with weakness, pallor, loss of appetite, thirst, and perhaps slight oedema, as the only symptoms.

Ellis and collaborators(6) have subdivided nephritis into two main groups, having different clinical and morphological manifestations. The clinical basis of this division into types 1 and 2 nephritis is summarized in the accompanying table.

TABLE II.

	Type 1 Nephritis	Type 2 Nephritis
Age Incidence .	A disease of children and young sdults.	Much wider age distribution.
Ætiological Background	History of acute infection, usually of upper respiratory tract, almost always present.	History of acute infection almost entirely lacking.
Onset	Abrupt Accompanied by constitutional symptoms ; malaise, vomiting and headache.	Insidious Constitutional symptoms are inconspicuous.
Gross Hæmaturia at Onset	Always	Never
Œdema . .	Present at onset, affecting face and feet. Not progressive. Of short duration.	Invariably the presenting sign. Progressive. Persistent.
Prognosis . .	82 per cent. Make a Complete Recovery.	95 per cent. Die from the Disease.

The principal feature of the division is the fact that cases with abrupt onset (type 1) in general have a far more favourable prognosis than those cases in which the onset is insidious (type 2). The morphologic aspects of this subdivision will be discussed later.

Symptoms and Physical Findings: Initially, the chief complaints of the patient with acute nephritis will vary with the mode of onset as outlined above. Haematuria, puffiness of the face, headache and oliguria may be the initial complaints. In addition, there may be more generalized oedema, visual disturbances, dyspnoea, delirium, convulsions, coma and death in more severe cases(17,18).

The blood pressure in acute nephritis is normal more often than not(17). However, in 25% of cases there is a moderate transient elevation of systolic and diastolic pressure, and in 10% of patients the systolic pressure may reach 180 - 200 mm. Hg., or even higher. When present, blood pressure elevation begins early in the disease.

Cardiac insufficiency may occur in severe cases with elevation of blood pressure, and, in acute nephritis, has been found a common cause of death(17). Anaemia is rare in acute nephritis, and the white blood cell count is normal in the absence of infection.

Urinary Findings: In acute nephritis evidence of renal damage is always found on urinalysis. The urine volume may be normal, but more commonly is reduced, and in some 5% of cases admitted to hospital, there

is anuria. The specific gravity of the urine in the acute disease is usually normal. Albuminuria is of constant occurrence, the daily urinary protein output being usually in the range of 2 - 4 gm. though up to 10 gm. may be excreted in some cases(61). Blackman(62) found that patients excreting globulin in significant amounts had usually a worse prognosis.

Some degree of haematuria is another constant finding. Though this may be only detectable on microscopic examination, some 40% of acute nephritics have gross haematuria(17). Microscopic examination of urinary sediment discloses tubular casts of all kinds, hyaline, waxy, granular, and casts made up predominantly of red blood cells.

Chemical Findings in the Blood: Non-protein nitrogen, urea, creatinine, etc., frequently do not depart from their normal concentration in the blood. The blood urea nitrogen rises above 100 mg.% in less than 5% of cases, and only 2 - 3% of patients with acute nephritis die in uraemia(17).

Plasma protein changes (as determined by electrophoresis) have recently been summarized by Leutscher(63). Even when first examined, there is usually a considerable fall in serum albumin, presumably related to excessive loss of this protein in the urine. There may be a rise in globulin, which, in the absence of infection, is due to a rise in the gamma globulin fraction. Alpha and beta globulin fractions are usually normal. Later, in the so-called nephrotic stage of the disease, both

albumin and gamma globulin fractions are reduced, and there is an elevation of alpha and beta globulins. Leutscher(63) considers such a pattern diagnostic of this phase of the disease.

Diminution of complementary activity of the serum in acute nephritis has also been reported(64,65), and it has been suggested(65) that this finding may help in differentiating acute nephritis from an acute exacerbation of chronic nephritis.

Differential Diagnosis: Acute diffuse glomerulonephritis can often be differentiated only with difficulty from several other renal diseases. Where this has not been carefully done, completely erroneous statistical studies may find their way into publication. The most important diseases which may simulate acute nephritis are the following:-

Essential hypertension,

Orthostatic albuminuria,

Sulfonamide poisoning,

Embolic nephritis,

Pyelonephritis,

Tuberculous nephritis,

Renal stone or tumour.

Prognosis: Recovery is the commonest outcome of acute glomerulonephritis(16,17,18,19,67,78,80,83) and death during the acute stage is exceptional(17). Complete recovery appears to be the rule in post-scarlatinal nephritis(23). In acute glomerulonephritis following other upper respiratory infections (Ellis' type I nephritis), the outlook is still good, with about

80% of patients making eventual complete recovery(7). Ellis'(6) type II nephritis, where the onset is insidious tends to run a much more malignant course, with fatal outcome in about 95% of cases(7).

Where recovery does not occur, the disease may go into a latent phase, from which recovery may still be possible(15), or which may progress into an active chronic state. Another possibility is continuous progression through a subacute phase into active chronic nephritis. The frequency with which one or other of these outcomes occurs has been computed by a number of authors(20,66-83), whose findings have been summarized by Bell(15)(see Table III).

TABLE III.

OUTCOME OF ACUTE GLOMERULONEPHRITIS

Author	Total number	Died in acute stage, per cent	Number followed	Latent chronic, per cent	Active chronic, per cent	Died in chronic stage, per cent	Recovered, per cent	Comment
Van Slyke, *et al.*, 1930	23	0	23	47.8	8.7	21.7	21.7	
Rosemoller, 1928	26	4	25	28	12	4	56	
Lyttle and Rosenberg, 1929	51	11.8	45	0	15.5	0	84.5	
Blackfan, 1926	24	12.5	21	0	0	0	100	
Clausen, 1925	102	19.6	82	0	10.9	0	89	
Guild, 1931	180	15.5	34	0	26.5	0	73.5	
Erenberg, 1911	40	0	40	0	0	0	100	Under 15 years old
Erenberg, 1911	16	0	16	25	0	0	75	15 to 30 years old
Hill, 1919	49	4.1	47	12.7	0	0	87.3	
Longcope, 1938	116	6	116	32	6.9	6	50	
James, 1921	67	0	67	13.4	0	0	87.6	
Osman,	56	0	56	35.7	0	0	64.3	
Hume, 1928	281*	not reported	281	44.7	7.1	2.5	45.5	War nephritis
Tallerman, 1932	27	7.4	25	48	0	0	52	
Gros, 1929	211*	not reported	211	21.8	28.4	5.2	44.5	War nephritis
Murphy and Rastetter, 1938	150	8	127	0	41.7	0	58.3	
McPhee, 1932	90	5.5	48	0	14.6	0	85.4	
Richter, 1936	100	5	77	0	15.5	4	80.5	
Folkers, 1935	68	8.8	41	0	19.6	24.4	56	
Hayman and Martin, 1940	77	9.1	52	17.3	9.6	5.8	67.3	
Murphy and Rastetter, 1938	150	15.3	150	0	35.3	0	49.3	

* Deaths during the acute stage not included.

About 5% of patients with acute nephritis die in this stage of the disease(17). Loeb(17) states that cardiac insufficiency is the commonest cause of death in acute glomerulonephritis, but in the same communication lists the causes of death (presumably in order of importance) as:-

1. Hypertensive encephalopathy with convulsions.
2. Cardiac decompensation, often terminating in pulmonary oedema.
3. Uraemia.
4. Bronchopneumonia or sepsis.

However, most authors(18,46,68) list uraemia (perhaps including hypertensive encephalopathy) as the chief cause of death, with pneumonia and cardiac decompensation as less frequent modes of exit.

Relation of Nephritis to Lipoid Nephrosis: The concept of nephrosis was introduced by Müller(84), and it seems possible that if he could have lived to see the confusion that the term has caused he might wish he had never thought of it. Originally intended to designate renal diseases characterized by damage to the tubular epithelium, it has come to be known as "lipoid nephrosis" or the "nephrotic syndrome". Clinical features of the condition were chronic massive albuminuria, with depletion of serum albumin and marked renal oedema. Examination of kidneys from such cases shows accumulation of lipids in the tubular epithelium and the glomeruli are usually considered normal. Haematuria, hypertension and azotemia were not supposed to develop in nephrosis. Numbers of cases satisfying these criteria have appeared in the literature, but it has

been found that if such cases are followed they may develop hypertension and azotemia, and show at autopsy the pathological features of chronic nephritis of Ellis' type II (7).

Moschcowitz(85) shares with Bell(15) the belief that lipoid nephrosis nearly always represents one of the biological progressions of of glomerulonephritis, although he concedes that instances may occur where the nephrotic picture may result from extra-renal factors causing hypo-proteinaemia.

The Morphology and Pathogenesis of Acute Glomerulonephritis.

"The pathogenesis of diffuse glomerulonephritis is a problem of fundamental importance. It is intimately related to that crux of renal doctrine, high blood pressure, and thereby impinges on the great questions of the general pathology of circulation. It is closely related also to the more remote associations of renal disease, namely oedema, uraemia and retinitis. Finally, by way of the morphological changes in the kidney itself, hitherto usually accepted as inflammatory, it is closely linked with the process, so momentous in general pathology, inflammation."

(Volhard, 1931)(86).

Although anatomic changes may be found elsewhere in the body in patients dying of acute glomerulonephritis(e.g. cerebral oedema, cardiac dilatation, pericarditis, pulmonary congestion or pneumonia), these will not be dealt with here. The principal alterations are to be found in the kidney, and these will be dealt with in some detail.

Gross Changes: Where death has occurred during the first few days of the disease, the kidneys may show no gross abnormality. Usually however, there is some degree of diffuse renal enlargement. The consistency is softer than normal, and the renal substance has a pallor. The capsule is not adherent, and beneath it numerous tiny haemorrhages may be found.

When cut, the cortico-medullary distinction is sharp, and the pallor is restricted to the cortex, whereas the medulla may appear congested. The tissue may appear moist, and the markings indistinct. In the cortex, the glomeruli may show themselves as greyish translucent points, or, where haemorrhage or congestion are prominent, may appear as small red pin-points. In other instances the glomeruli may be invisible in the swollen parenchyma. Tubules containing blood may appear as sharp red streaks.

Both kidneys are always more or less equally involved in the process.

Microscopic Changes: Although alterations in the renal tubules, interstitial tissue and blood vessels may be found in acute glomerulo-nephritis, these changes are of secondary importance to the changes taking place in the glomeruli.

The first accurate portrayal of the fundamental glomerular lesion in acute hephritis was made by Langhans in 1879(87). Most text-book descriptions are based upon this and other early publications(88,89,3). The principal glomerular alteration noted by these investigators was increased cellularity and enlargement of the tufts. Swelling was sometimes seen to result in herniation of the tuft into the mouth of the proximal tubule(26). The capillary walls were distended and their lumina largely filled by cells generally taken to be endothelial, leaving little space for blood, which was scanty or absent. Polymorphonuclear leucocytes were

also seen in varying numbers, but were less numerous than endothelial cells. An occasional capillary was occluded by thrombus. In somewhat more advanced stages thickening of the capillary walls was noted(89). Proliferation, degeneration and desquamation of the glomerular epithelium was also usual, and in the later stages an accumulation of cells partly encircled the glomerulus: the familiar 'crescent'.

An important contribution to the knowledge of the finer morbid anatomy of acute nephritis was made in 1929(90,91) by Leone McGregor. Using an aniline blue staining technique, she was able to demonstrate the frequent development of a fine meshwork of intracapillary fibrillae, passing between the enlarged and proliferated endothelial cells, and connected with the basement membrane of the capillary. This observation was confirmed by Gray(5), and has become an important criterion in the histological diagnosis of the disease.

Within the capillaries themselves, a number of alterations may be found. Most conspicuous, and most frequently described(5,15,18,26, 102 & 103) is a reduction in the amount of blood in the lumina. This is largely the result of swelling and proliferation of endothelial cells(92). Occasional glomerular tufts may contain thrombi(93), or be plugged with masses of polymorphonuclear leucocytes(100). With suitable staining, fat droplets may be seen in the endothelial cells or in the overlying epithelial cells(26). Hyaline droplets within the cytoplasm of the glomerular epithelial cells are also sometimes seen(94).

In the renal tubules the commonest abnormality is haemorrhage(92), which is generally accepted as coming from the glomeruli. Hyaline droplets may be found in the proximal convoluted tubular epithelium, but are often absent. Fat droplets may also be found in proximal and distal tubules in patients dying after a less acute course.

The principal facts outlined above are not in great dispute. It is only where attempts have been made to determine the earliest alterations, and to suggest a sequence of events, where disagreement has appeared. This discord is to be expected in view of the obvious fact that at the time even 'early' lesions are observed, the initial manifestations may already have passed. It is also apparent that the morphological changes seen in patients dying of acute nephritis may be of such intensity to render insecure any judgement of the process in patients who recover. Furthermore, should an investigator accurately describe the earliest morphological change to be seen, he is always open to the criticism that the changes to which he refers are too slight and insignificant to warrant a diagnosis of acute nephritis.

Most efforts to unravel the pathogenesis of the glomerular damage in nephritis begin with the microscopic picture of distended capillary loops, containing little or no blood, and partly or completely occluded by swollen and proliferated endothelial cells(15,86,90,91,92,93,95). Volhard(86) ascribed this appearance to an allergically conditioned spasm of the afferent

arteriole, with ischemic irritation as the cause of the glomerular anaemia and proliferative changes. Dunn(92) disagrees with this interpretation, pointing out that the remainder of the renal cortex is but little altered in acute nephritis, and that the intertubular capillaries may even be congested(26,96). Dunn reasons that the tubules depend for their blood supply upon the flow through the efferent glomerular arteriole and that ischemic glomerular damage therefore implies much more marked tubular degeneration than is actually found.

Several cases of acute nephritis, dying within a few days of the onset of symptoms have been described in which the most striking glomerular change has been capillary dilatation and congestion(92,95,97, 98,99). Dunn(92) refers to these, and presents his own observations of cases in which capillary dilatation and congestion were virtually the only alteration. From this he concludes that "The fundamental change in acute....glomerulonephritis is.... a uniform dilatation of the..... capillaries. The.....change is the fundamental element which links glomerulitis to the manifestations of acute inflammation in other tissues."

Bell(15,93,100) believes the fundamental glomerular lesion to be obstructive and ischemic. Unlike Volhard(86), he places the initial alteration within the glomerulus, with circulatory obstruction the result of basement membrane fibrillation and endothelial increase, though compression by crescent formation, thrombosis, or plugging of the capillaries with leucocytes may also contribute. Though agreeing with Dunn(92) and Gray(5)

that such cases may occur, he is unwilling to accept congestion alone(15) as the basis for a diagnosis of acute nephritis.

MacCallum(101) disagrees with most observers that the cellular increase in the glomeruli is mainly intracapillary (endothelial), and believes that the proliferated cells are actually intercapillary connective tissue cells.

The recent study of Bright's disease by Ellis and his colleagues at the London Hospital may represent the most important contribution to the knowledge of nephritis since the publication of Volhard and Fahr in 1914. Some six hundred cases were observed during a period of twenty years, with autopsy observations in two hundred of these. On the basis of this study, cases of nephritis were classed in two main groups as Type I and Type II nephritis. Cases of Type I occurred mainly in children and young adults, almost always gave a history of acute upper respiratory infection, with abrupt onset of symptoms of nephritis with haematuria. Oedema, if present in these cases, was of short duration and not progressive, and 82% of this group made a complete recovery. Morphological features were generally those of Fahr's(96), extracapillary glomerulonephritis. The principal characteristics were diffuse and uniform involvement of the entire cortex, with considerable proliferation of the glomerular capillary endothelium and exudation of leucocytes. In cases having a duration of a few weeks or more, there is free proliferation of the epithelium of Bowman's capsule with conspicuous crescent formation. This lesion was never found

in the absence of haematuria, and the London group believe it is the result of encapsulation of effused blood.

Type II nephritis was found to occur over a much wider age distribution, and history of acute infection was almost entirely lacking. The onset was insidious, and gross haematuria never occurred. Oedema was invariably the presenting sign and was persistent and progressive, and ultimately 95% of patients died of the disease. The morphologic changes in Type II nephritis were considered to be characteristic. Every glomerulus showed a slowly progressive intracapillary hyalinization due to hyaline thickening of the capillary basement membranes. The process extended from the vascular pole to the periphery, and large amounts of hyaline material were found in the central parts of the tuft. Glomerular enlargement was much more marked than in Type I nephritis, and the normal lobulation was accentuated. Proliferation of vascular endothelium was mild, and crescent formation rare. Ultimately there is hyalinization of most of the glomeruli, with a fine interstitial fibrosis throughout the kidney. In general, the morphologic features of Type II nephritis correspond with those of Fahr's intracapillary type(96).

The validity of the London Hospital classification has recently been carefully put to test, and confirmed in all its essentials(19). As yet it is not known whether the two characteristic clinical and morphological pictures represent two entirely different etiologic and pathogenetic entities, or whether they are merely variations of the same fundamental process.

SUMMARY.

Minimum Clinical and Morphological Criteria required for Diagnosis of Acute Glomerulonephritis.

The review of the literature thus far presented allows us to lay down certain criteria upon which a diagnosis of acute glomerulonephritis in man or experimental animals may be accepted or rejected.

Among the clinical features, the urinary findings are of primary importance, and of these albuminuria is a fundamental finding which must be present. Gross or microscopic haematuria should also occur in most cases. All other clinical features of acute nephritis are variable, and their absence need not exclude the diagnosis. These include oedema, oliguria, elevation of blood pressure, cardiac insufficiency, retention of nitrogen, and hypoalbuminaemia.

In the judgement of groups of cases, it seems essential to expect that a varying, but definite proportion, of clinical cases of acute nephritis must progress into subacute and chronic phases of the disease, manifest either by the so-called nephrotic syndrome, or by progressive renal failure with azotemia and hypertension. However, the complete and permanent recovery of the majority of the group in no way invalidates a diagnosis of nephritis, since that is to be expected in most series.

On the morphological side, the sine qua non is diffuse involvement of all the glomeruli in both kidneys. The changes found vary somewhat with the duration of symptoms, but the principal alteration should consist of increased cellularity of the glomerular tufts, with reduction in their blood content. The capillary walls should be distended in the early phases, and their lumina obtruded by swelling and proliferation of endothelial cells. Epithelial accumulations may also form, to the extent of partial encirclement of the glomeruli by crescents. Splitting and fibrillation of the capillary basement membrane should also be found. In addition, capillary thrombi, or polymorphonuclear leucocyte accumulation may also be found. Changes in the tubules are inconstant and not essential to the diagnosis.

It seems wise for the present, in both human and experimental nephritis to test the validity of the London Hospital classification, and to designate nephritides as either intra- or extracapillary types, or as Type I and Type II nephritis.

III. GLOMERULONEPHRITIS IN EXPERIMENTAL ANIMALS.

Historical.

In the middle of the last century Semmola(104) promoted the idea that albuminuria in Bright's disease was the result of an alteration in the serum albumin, rendering it more diffusible than usual. He believed that the renal damage in the disease was due to this abnormal albumin excretion. This theory lead to a number of experiments quite similar to those being carried out in this field today. Laboratory animals were injected with chicken egg albumin(105-109) in efforts to reproduce acute nephritis. In a few instances inflammatory changes in the kidneys were noted, but in general the results of the experiments were considered unsatisfactory. Other experimentors of the same period(107,110,111) injected animals with blood, urine and transudates from "kidney patients", with similar unsatisfactory results.

Spontaneous Nephritis in Laboratory Animals.

Evaluation of the morphologic results of any experiment can only be reached with due regard for spontaneous lesions occurring in the particular species. The topic of spontaneous renal lesions in laboratory animals has been reviewed by Horn(112). True glomerulonephritis in animals is exceedingly rare(113-115). It has been noted more frequently in the rat than in other animals(15,115). Mallory and Parker(116) interpreted as spontaneous a diffuse glomerular endothelial proliferation with tuft enlargement found in rabbits injected subcutaneously with powdered metallic zinc. Leiter(117) found a

spontaneous interstitial nephritis "very easy to distinguish from..... glomerulonephritis" in more than 40% of normal rabbit kidneys.

Transfer of Nephritis from Man to Animals.

Apart from the attempts to produce experimental nephritis by means of various bacteria and their products (see below), relatively little has been done in attempting direct transfer of glomerulonephritis from man to animals. Injection of laboratory animals with urine, blood and transudates from patients with Bright's disease have already been referred to(107,110,111). Schober(117) claimed to have produced acute nephritis in rats by the injection of a protein-free serum ultra-filtrate from human acute nephritics, but Reader(118), attempting to confirm this observation, met with failure.

Experimental Nephritis Produced by Bacteria or their Products.

The common association between upper respiratory infection and acute glomerulonephritis in humans makes it logical that a considerable amount of investigation should have been carried out in efforts to reproduce the disease in animals by treating them in various ways with bacteria or their products. The results of these experiments have been rather less satisfactory than might have been expected.

Partly for this reason, and partly due to the fact that an experimental nephritis closely resembling the human disease has been satisfactorily produced by other methods (see below), the bacterial approach to experimental nephritis has been largely abandoned in the past ten to twelve years. The early literature on this aspect of the subject, and indeed, most of the

information available, has been capably reviewed by Horn(112) and Ahlström(141).

Numerous efforts to reproduce the morphological picture of nephritis by sensitizing various animals (usually rabbits) to a number of bacterial antigens have been completely unsuccessful(120-125). The only renal lesion encountered by Nye and Parker(119) in rabbits injected with various dead bacteria was accumulations of "giant cells" in the glomerular tufts. It seems likely that these represented response to small bacterial emboli. Bell and Hartzell(126) gave repeated injections of a culture of Strep. viridans to a male monkey over a period of more than a year. The animal developed albuminuria, but failed to show hypertension or uraemia. However, when killed after 18 months, the kidneys showed lesions equivalent in all essentials to human chronic glomerulonephritis. This work does not appear to have been repeated.

Masugi and Isibasi(127) found that repeated injection of either living or dead E. coli organisms produced diffuse glomerular alterations in rabbits. These changes consisted of capillary endothelial proliferation, intracapillary hyaline masses, and leucocyte accumulation. Though difficult to judge from the photographs in the publication, the lesions may well be similar to those of human intracapillary glomerulonephritis. Glomerular damage was only found in animals who had received at least 14 injections of E. coli, and similar, though less marked lesions appeared in one animal injected 20 times with Staphylococcus toxin. Morphologically these lesions seem closer to those of the human disease than those reported by others using bacterial antigens, and it is unfortunate that the work has not been repeated.

Intracutaneous inoculation with a culture of haemolytic Staphylococcus aureus was reported by Domagk and Neuhaus(128) to have caused glomerular damage. The changes appear more consistent with a focal glomerulitis however, than with diffuse glomerulonephritis.

Kô(129) reported glomerular hypaemia and cellular proliferation, associated with tubular casts and interstitial infiltration in dogs injected with haemolytic streptococci by intravenous route and tonsillar inoculation. In both groups there was albuminuria after four days, but the renal damage was more severe in the animals receiving tonsillar inoculation. The lesions in all cases, however, were focal in character. Focal acute glomerulitis has been reported in experimental brucellosis in dogs(130).

Other investigators have attempted to reproduce the picture of glomerulonephritis by injecting various organisms into the renal artery of normal and sensitized animals(131,132). The injection of a suspension of living Streptococci into rabbits caused an acute focal glomerulitis(131), which was, however, more widespread and more severe in a previously sensitized group of animals. Similar lesions resulted from the injection of various strains of Strep. viridans directly into the renal artery and again the damage was more widespread and of commoner occurrence in sensitized animals(132).

On the assumption that the products of bacterial growth might be important in the pathogenesis of acute nephritis, a number of experimentors have attempted to produce the disease by injecting animals with bacterial toxins or autolysates. Duval and Hibbard(133) injected rabbits by intravenous, subcutaneous and intraperitoneal routes with a lysate of Strep. scarlatinae,

and reported the appearance of glomerular necrosis, hypaemia, capillary thrombosis, and in addition, crescent formation and casts. Only a small number of animals was used in these experiments, and the lesions appear more consistent with focal glomerulitis than diffuse glomerulonephritis. Other workers(134) were unable to reproduce these results, and reported finding no renal lesions in the treated animals which were not also found in controls. In a later communication, Duval(135) reported the production of acute and chronic nephritis in dogs injected with a toxic product of Strep. scarlatinae. The changes were said to consist of albuminous and fatty degeneration of the glomerular endothelium, frequently accompanied by thrombosis and capillary rupture. Although Duval stated that the lesions were similar to those of human scarlatinal nephritis, lack of illustrations does not allow the reader to judge this. The importance of the hypersensitive state is, however, suggested by the assertion that the renal lesion was always aggravated by a second injection.

Diffuse renal lesions were reported in rabbits by Hückel following the injection of Dick toxin into the renal vein(136). However, in a later paper he stated that these lesions consisted mainly of interstitial inflammation, and were not equivalent to human glomerulonephritis(137).

Blackman and others(138) reported an acute glomerulitis of focal character in rabbits injected with pneumococcal autolysate. In further experiments(139) Blackman reported finding necrosis, calcification and regenerative changes in the tubular epithelium of rabbits injected with a single dose of pneumococcal autolysate. Repeated doses of the same material resulted in

glomerular haemorrhages, fibrin exudation and capillary thrombi, in addition to the tubular changes seen after a single dose. Blackman concludes from these findings that the nephrosis produced by a single injection, and the glomerulitis resulting from repeated injections are essentially the same disease, and that the determining factors appear to be the amount of toxin administered and the degree of immunity of the animal.

Rich et al.(140) injected rabbits intravenously with a filtrate of a Strep viridans culture from a patient with subacute bacterial endocarditis, and killed the animals 24 hours after injection. The kidneys showed haemorrhages into the glomerular capsular spaces and into the tubules. Although similar changes did not occur in animals injected with other bacteria, acute experiments of this type lacking more extensive observations, cannot have great bearing upon the pathogenesis of human nephritis.

Ahlström(141) describes diffuse glomerular lesions in rabbits resulting from the injection of staphylolysin into the renal artery of rabbits previously sensitized to horse serum. The lesions consisted of glomerular intracapillary plasmatic and fibrin exudate, together with an increase in polymorphonuclear leucocytes. Similar changes did not appear in animals treated with horse serum alone or with staphylolysin alone. Ahlström concludes that his findings demonstrate the importance of allergy in nephritis, and suggests that in addition, a purely toxic initiating factor may be necessary. Although the lesions illustrated by him have many of the characteristics of human nephritis, they appear too acute to support any but tentative conclusions in this respect.

In summary, therefore, it may be stated that the use of bacterial antigens, or bacterial products, have usually led only to the appearance of focal glomerulitis. Possible exceptions to this statement may be the experiments of Bell and Hartzell(126), and those of Masugi and Isibasi(127). Experiments involving direct injection into the renal artery or vein seem open to criticism on the ground that the insult to the kidney is probably far more intense than any conceivable in normal physiology. Such experiments appear to have done no more than demonstrate that the renal lesions so induced, may be rather more severe in previously sensitized animals, and their importance in relation to human glomerulonephritis is as yet undetermined.

Nephritis Produced by Foreign Protein Injections.

Injection of animals with foreign protein, and, in particular, with foreign serum or its fractions, has resulted in diffuse glomerulonephritis in a number of reported instances. This approach to the problem has been much more profitable than have those investigations in which bacteria have been used.

This portion of the review will be concerned mainly with a critical analysis of the morphological end-point of these experiments: the renal lesions. In most instances an attempt will be made to evaluate the type of nephritis produced as either intra- or extra-capillary glomerulonephritis, along the lines suggested by the London Hospital classification. At a later point a number of related topics will be introduced. These will include such immunologic phenomena as antibody formation, antigen persistence, allergy and anaphylaxis, and some consideration of what is becoming known as tissue reactions of hypersensitivity. This discussion will impinge upon such broad fields as immunology and immunochemistry, protein chemistry and dietetics. Obviously these cannot be given comprehensive treatment in a work of this nature. They will, therefore, only be introduced after a purely morphological evaluation has eliminated much that is unrelated to the problem.

In 1913, Longcope(142) reported an experimental nephritis in dogs, cats, rabbits and guinea pigs which had been injected with repeated small doses of horse serum and egg white. In rabbits the changes consisted of degeneration and necrosis of the loops of Henle, collecting tubules, and less

frequently, convoluted tubules. Accumulations of round cells were found in the intertubular connective tissue. It now seems likely that many of the changes observed by Longcope were of spontaneous nature. This work is nonetheless of historic importance, in that it has inspired much of the investigation done in subsequent years. Longcope's findings could not be confirmed by a number of investigators(143,144,145), however, similar results were reported by Boughton(146).

Hepler and Simmonds(147) inoculated the kidneys of normal and sensitized rabbits with horse serum, and observed a rapidly developing haemorrhagic and necrotic inflammatory response in the sensitized animals; a reaction which did not occur in non-sensitized controls. These findings do no more than suggest a capacity in the kidney for local reactions of hypersensitivity (Arthus phenomena).

Masugi and Sato(148) injected unilaterally nephrectomized rabbits with progressively increasing intravenous doses of horse serum. Beginning with an initial injection of 2 c.c. subsequent injections were given at 5 day intervals, with each dose consisting of 2 c.c. more than its predecessor. The total number of injections varied from 4 to 9. During treatment all animals developed albuminuria, haematuria and cylindruria. At autopsy diffuse glomerular changes were found in all animals. These consisted of increased glomerular volume, tuft swelling, anaemia, oedema and protein exudate within the tufts. Increased numbers of glomerular nuclei were seen in some animals, and epithelial crescent formation was occasionally met with. Capillary thrombi

were sometimes found in glomeruli. These changes are well illustrated in the published report, and the most striking changes appear to be swelling and broadening of the glomerular tufts. From the photographs, the changes appear to be mainly intra-capillary in character, and therefore to correspond more or less with Ellis(6) type II nephritis.

Rich and Gregory(149) injected rabbits with 10 c.c. of horse serum per kilo of body weight. The animals were given 1 c.c. of serum 17 days later, and a second massive injection, equal to the initial dose, on the 19th day. When killed a week after the second injection seven of the nine animals in the group were said to have lesions of acute diffuse glomerulonephritis. The glomerular lesion was characterized by condensation of the tufts, epithelial proliferation and haemorrhage into Bowman's capsule and the tubules. The single photograph of a glomerulus appearing in the report shows marked protein exudate into the capsular space. Further reports concerning nephritis in animals treated in this way have not come from Rich's laboratory, although a number of experiments have been reported in which rabbits were injected with horse serum.

Letterer(150) sensitized frogs to foreign protein and then made direct observations of the glomeruli during administration of intravenous "shock" doses of the same protein. He noted a transient glomerular capillary stasis, followed by ischemia and some glomerular enlargement. Letterer interpreted his observations as being consistent with spasm of the vasa afferentia, glomerular capillaries and vasa efferentia. He felt this evidence

lent support to Volhard's contention that the renal lesion of Bright's disease was based upon allergically conditioned vasospasm.

Recently Ehrich, Siefter and Forman(151) have reported diffuse glomerular damage in rabbits injected with large amounts of foreign sera. The animals had not been subjected to preliminary nephrectomy. Although it is not stated whether the injected serum was normal or immune, the assertion that the horse serum used had a globulin content of between 3.4 and 4.0 per cent is in keeping with its having been non-immune normal serum(193). One series was injected with 10 ml. of horse serum per kg. of body weight, and the animals were killed in groups, 11, 14 and 19 days after injection. Nine of ten animals killed after 11 days were said to have shown mild diffuse intra-capillary glomerulitis (graded as + by the authors). Ten of fourteen killed after 14 days showed rather more marked lesions of similar character (graded + to ++). All eight of those killed after 19 days showed + to ++ lesions of the same type.

In a second experiment rabbits were injected with 20 ml. of horse serum per kg. of body weight, and were killed at intervals of 3, 5, 7 and 14 days. One of four animals killed after three days showed changes classed as severe intra-capillary glomerulitis(++++) 2 out of 4 killed at five days had similar but mild lesions (0 - +). All animals killed at 7 and 14 days had ++ intra-capillary glomerulitis.

A third series was given two injections of horse serum, each injection amounting to 10 ml. per kg., with doses nineteen days apart. Animals were

killed at 26 and 34 days after the initial injection. Only two of the total series of ten animals showed evidence of renal damage, interpreted as mild extra-capillary glomerulitis.

A final series was injected with (presumably) normal duck serum. One group received single doses varying from 1.8 to 4.2 ml. per kg. A second group was given 8.5 to 9.0 ml. per kg. in divided doses over a three-day period, and a third group was given 10 ml. per kg. in a single dose. When the animals were killed 15 or 17 days after the commencement of treatment, questionable intracapillary glomerulitis was found in one of six animals receiving small amounts of serum, and all animals given the larger dose had somewhat more marked lesions of similar character.

From the illustrations in the publication it is apparent that in all instances where glomerulitis appeared, it was rather mild. The authors' attempt to classify the lesions as intra- and extra-capillary nephritis is commendable, but no great difference between the two types of lesion is apparent in the illustrations. Furthermore, since the extracapillary type of lesion only occurred in two animals, it does not seem that the authors are justified in drawing the conclusion that this type of lesion is characteristic of an acute Arthus-type reaction, whereas the intracapillary lesion represents a subacute Arthus phenomenon. In general, the glomerular lesions in these experiments appear considerably less severe than those shown by Masugi and Sato(148).

McLean et al.(154) injected unilaterally nephrectomized rabbits with 10 ml./kg. of horse serum in two doses. Apart from the nephrectomy, these experiments differed from those of Rich and Gregory(149) in that the interval between injections was shortened to eleven days. When killed one week after the second injection, all of the eight animals injected showed a diffuse glomerulitis of quite severe degree. The lesions were characterized by marked glomerular swelling, basement membrane damage, and considerable epithelial proliferation of both glomerulus and capsule.

Further interesting data on horse serum nephritis come from the same laboratory(154). One series of rabbits was injected with small daily intravenous doses (0.5 c.c.) over prolonged periods. One group of 7 intact (i.e. non-nephrectomized) animals was treated in this manner for a period of from 10 to 13 months. Four animals died during the course of treatment, and at autopsy showed severe subacute and chronic diffuse glomerulitis. Two other animals developed terminal uremia, and showed similar lesions. Another group of 8 rabbits were given the same treatment for six months and then subjected to unilateral nephrectomy. The kidneys removed at this time showed no histological evidence of diffuse glomerulitis, but all of the six animals surviving the procedure had lesions of nephritis after another two months of treatment. Eight other animals were treated similarly for three months, nephrectomized and killed after a further month of treatment. Two animals died at the time of nephrectomy, and both showed lesions of diffuse glomerulitis. Four of the six remaining animals had similar lesions when killed one month after nephrectomy. A final group was unilaterally nephrect-

omized and then subjected to the same form of treatment for three months. Three of these animals had diffuse glomerulitis at autopsy, and in one of these the lesions were considered severe.

The writer has been kindly afforded opportunity to examine some of the sections from these various groups. In general, the glomerular lesions are similar throughout the series. The most striking change consists of a diffuse, irregular nodular thickening of the capillary basement membranes, with some splitting and fibrillation. Associated with this is a striking increase in the quantity of cytoplasm of the epithelial cells, without marked hyperplasia. In animals of the first group described (non-nephrectomized, treated for 10 to 13 months), who survived the longest, there is also some proliferation of capsular epithelium, with occasional crescent formation. Throughout, however, the most prominent change is in the glomeruli themselves. The tufts are increased in volume, and sometimes adherent. There appears to be an increase in leucocyte content of the glomeruli. In general, the changes appear to the writer to correspond most closely to human intracapillary glomerulonephritis, although it cannot be denied that an extracapillary component is often distinctly in evidence.

Wissler, Smull and Lesh(152) attempted to determine whether the type and severity of lesions induced by horse serum injection was dependent upon one or other of the protein fractions in the serum. Groups of rabbits were injected intravenously with 0.65 gm. per kg. of each of several serum fractions. The injected material consisted of a 6.5% solution of the particular protein used. The animals were given a second similar injection fifteen days

after the first, and were killed one week later. In addition to whole serum, fractions of the following composition were injected:

(a) A fraction consisting mainly (89%) of serum gamma globulin.

(b) A fraction consisting mainly of alpha, beta and gamma globulins, but low in albumin.

(c) A fraction consisting of whole serum, from which most of the alpha globulin had been removed.

(d) A fraction consisting mainly of serum albumin.

At autopsy, three of eight rabbits injected with the serum gamma globulin fraction ('a' above) showed slight diffuse glomerular swelling, with "variable" thickening of basement membranes and questionable endothelial proliferation. In addition, a focal, necrotizing glomerulitis was seen in some of these animals. This lesion was also encountered in animals given whole serum, and, in higher incidence, in animals injected with the albumin fraction. The diffuse glomerulitis occurring in the gamma globulin-treated animals is illustrated in the report, and appears very mild indeed, perhaps doubtful. In the absence of further experiments, this work cannot be said to demonstrate that the gamma globulin fraction of horse serum is mainly responsible for the development of glomerulitis. Experiments in which larger quantities of this fraction are injected would, however, appear advisable.

In investigations carried out in this Institute by More and Kobernick(153) intact rabbits were injected with horse serum in two massive doses, 16 days apart. These animals were exposed to a cold, dry environment (about 20°F.) throughout the experimental period. When killed 22 and 25 days

after the initial serum injection, eleven of twelve animals in the group showed lesions of diffuse extracapillary glomerulonephritis, and the lesions could be classed as severe in four of these. The writer has examined this material, and is convinced that the lesions are clearly of the extracapillary variety.

In an exactly similar experiment, carried out at normal room temperature (65° to 70°F.) the same group(153) injected intact rabbits with double the amount of horse serum used previously (i.e. 20 c.c./kg.). The interval between injections was the same, and the time of killing was as before. At autopsy three of four animals treated in this way showed diffuse extracapillary glomerulitis, of the same character as seen in the previous experiment.

Silfversiöld(195) has reported that the symptoms of nephritis following horse serum injection by Masugi's technique, may be prevented or inhibited if the animals are treated with heparin prior to the serum injection. This work was done on the theory that development of the renal lesions might in part be due to the formation of glomerular capillary thrombi. Small numbers of animals were used in these experiments, and histological descriptions and photographs do not allow one to judge the effect of heparin therapy. The work does not appear to have been repeated.

The experiments outlined above include the principal reported instances of diffuse glomerulonephritis induced in animals by horse serum injection. It is therefore of interest that a number of studies have been

published in which no renal lesions have been found after horse serum injection.

Vaubel(145) found no significant renal alterations in animals injected repeatedly with horse serum by subcutaneous route. Another group of rabbits was injected intravenously with 2 c.c. of horse serum at eight day intervals for a total of eight injections. A third group received from 7 to 10 injections increasing from 2 c.c. to 10 c.c. per dose, at five day intervals. Two other animals were given 10 and 13 injections ranging from 2 c.c. to 20 and 30 c.c. per dose. In none of these animals did Vaubel find evidence of nephritis. It should be pointed out that only a few of these animals were ultimately given the same amount of serum as that injected by Masugi and Sato(148). The lack of renal lesions in Vaubel's animals might be explained on several grounds. Nevertheless, the most striking difference between his animals and those of Masugi and Sato(148) seems to be that those of the latter group had been subjected to unilateral nephrectomy before treatment.

In another series of experiments carried out at this Institute(155), 77 rabbits were injected with two to seven massive doses of normal horse serum at eighteen day intervals. When the animals were killed, four to seven days after the last injection, none were found to have glomerulitis.

Bovine serum has not been nearly so widely employed in experiments of this nature. However, the recent application of mass production methods to the cold-alcohol fractionation technique of Cohn(194) has made available large quantities of highly purified serum protein fractions. These materials

as antigens had the advantage of greater purity and a high degree of immunological specificity, and they are now being used quite extensively.

The most important study involving the use of bovine serum protein fractions is that of Hawn and Janeway(156). Rabbits were injected intravenously with 10 c.c. per kg. of a 10% saline solution of purified bovine gamma globulin. Other groups were injected with similar doses of purified crystalline bovine albumin, and whole bovine serum. Diffuse glomerulitis appeared only in the group treated with purified gamma globulin. The lesions occurred in all animals killed one week after injection, and were featured by increased cellularity of the glomerular tufts. Swelling of the capillary endothelium was also noted, with partial occlusion of the capillary lumina. Intracapillary coagula were sometimes seen. The lesions were most marked in animals killed one week after injection, and the authors state that "healing" lesions were found in animals killed after this time. The lesions illustrated appear to show the changes referred to, but do not seem very severe.

The work of Hawn and Janeway has been extended by the observations of More and Waugh(157). This work will be described in detail in the experimental section of this thesis, and will only be touched upon here. It was found that a severe diffuse extracapillary glomerulitis could be produced in high incidence in unilaterally nephrectomized rabbits injected intravenously with two massive doses of purified bovine serum gamma globulin. The injections were given eleven days apart, and animals were killed one week after the last injection.

In a similar experiment, McLean(154), found no nephritis in intact rabbits injected with two massive doses of purified bovine gamma globulin, 16 days apart.

Summary: From the foregoing review it is apparent that both intra- and extracapillary glomerulonephritis can be produced in rabbits by injecting them intravenously with foreign sera. Several factors seem important in determining whether or not nephritis will appear in animals so treated. Although it is not possible to decide the relative importance of each of these, the following seem to be the principal factors involved in this question:

(a) The amount of antigen injected. Small amounts given over a relatively short period have not caused nephritis(145), but daily small doses have resulted in nephritis(154). Large doses have given rise to nephritis in some cases(153), but not in others(155).

(b) The interval between injections appears of considerable importance particularly where large amounts are given. Thus, More and McLean(155) found no nephritis in rabbits injected at 18 day intervals, but McLean did get nephritis, using an 11 day interval(154).

(c) Unilateral nephrectomy appears to enhance the liability of the remaining kidney to nephritis. Thus, Masugi and Sato(148) got nephritis in unilaterally nephrectomized animals, whereas Vaubel(145), using a similar dose schedule, got no nephritis in non-nephrectomized animals. More and Waugh(157) got nephritis in unilaterally nephrectomized animals, whereas McLean(154) got no such lesions in intact animals treated similarly.

From the evidence at hand, it can be further suggested that continued small doses of foreign serum(154,148) tend to give rise to the intracapillary type of glomerulitis, whereas a few massive injections(149,151,152,157) appear more liable to result in the extracapillary variety.

Nephritis Produced by Injection of "Anti-kidney" Antibodies.

A number of investigations have been concerned with the nephritis which follows injection of anti-kidney sera. In essence these consist of repeated injection of animals of one species with a kidney extract from a different species. After a period of time the sera of the injected animals acquire the capacity of *in vitro* reaction with the original kidney extract. Injection of such a serum into an animal of the species which contributed the kidney extract, gives rise to symptoms and lesions of acute and chronic glomerulonephritis.

This approach was introduced by Lindemann(158), and was further explored by Pearce(159). About thirty years later Masugi(160,161,162) reported a severe experimental nephritis, resembling human nephritis, produced by the injection of anti-kidney serum. Masugi attributed his successful results to the fact that in preparing his kidney extract he took the precaution of first perfusing the kidney free of blood. This had not been done by Lindemann or Pearce, who had found that fatal toxic reactions usually killed animals injected with the extract before anti-kidney antibodies of high titre could be produced. These reactions were almost completely eliminated by preliminary perfusion.

In Masugi's original work, anti-rabbit-kidney serum was produced in ducks, and anti-rat-kidney serum was produced in rabbits. Injection of either of these sera into the appropriate animal (rabbit or rat), resulted in a diffuse and quite intense glomerulonephritis. Clinically, the condition was characterized by albuminuria, haematuria and cylindruria in the acute stages, and, after a more prolonged course, left sided cardiac hypertrophy (presumably hypertensive), oedema and azotemia.

In the kidneys the earliest evidence of damage was said to be a distension of the glomerular capillaries with homogeneous protein material. In addition, glomerular leucocytosis, anaemia, and endothelial swelling were usually noted. In more seriously damaged kidneys fibrinous and hyaline deposits were found in the glomerular tufts. In the most severe cases, Masugi noted congestion of the glomerular capillaries and afferent arterioles, and necrosis of parenchyma and vascular walls. Chronic progressive forms were also seen, with proliferation of capsular epithelium and crescent formation.

The lesions of this type of nephritis are well illustrated in publications by Masugi and his collaborators, and in the works of other investigators. In rabbits, the glomerular lesions appear to correspond most closely to those of human Type I nephritis, or to Fahr's extracapillary glomerulonephritis. As in the human disease, the features of endothelial swelling and proliferation, together with basement membrane fibrillation are found, and in addition there is more or less marked extracapillary epithelial proliferation involving both

glomerulus and capsule. The clinical course of the disease also appears to resemble that found by Ellis in Type I nephritis. There is a rather acute onset seven to ten days after the injection of the serum, with haematuria as a prominent symptom. It may also be significant that this type of nephritis bears a strong resemblance to that produced by massive injections of foreign serum(154,157).

Masugi's original observations have been confirmed in all important details by a number of other investigators(163-167). The entire subject of "nephrotoxic" nephritis has been reviewed by Smadel(168), who points out the similarities of the disease in rabbits and rats. Haematuria and glomerular capillary thrombosis have been considered characteristic of the disease in rabbits(160,163). These changes may occur in rats, but are attributed by Smadel(169) to factors other than nephrotoxin. Proliferation of cellular elements of the glomeruli is prominent in rabbits(163,170) but not in rats(169). Clinical evidence of renal damage appears within a few hours of injection in rats, while in rabbits these manifestations do not occur for 7 - 10 days(168,170). Mild hypertension may occur(162,166) during the acute stage in rabbits, but is inconstant(171), whereas it does not occur in rats.

Chronic nephritis may follow acute nephrotoxic nephritis in both rabbits(161,166,167) and rats(165,172). Smadel and Swift(173) have found that rats of different strains may vary in susceptibility to nephrotoxic nephritis. Similar experiments have not been done in rabbits, but this possibility should be considered in planning of experiments.

Diet has been found to influence the course of nephrotoxic nephritis in rats. Those maintained on a low protein diet tended to recover promptly from the acute nephritis, whereas animals on intermediate or high protein diets developed correspondingly more severe nephritis, which usually became chronic(174).

A number of attempts have been made to alter the course of nephrotoxic nephritis. Smadel and Swift(175) noted no effect on the disease by administration of sulfonamides. The observation that administration of testosterone gave rise to renal hypertrophy(176), led LeCompte(177) to study the effect of this hormone upon nephrotoxic nephritis in rats. Hypertrophy and hyperplasia of tubular epithelium were found in the kidneys of animals so treated, but there was no evidence of any effect of the hormone on the nephritis.

On the other hand, administration of desoxycorticosterone acetate (DCA), together with sodium chloride, seemed to intensify nephrotoxic nephritis in rats(178). This effect was not found when DCA alone was given.

The experiments of Sarre and Wirtz(179) suggest that the nephrotoxic serum acts upon the kidney in an extraordinarily short time. They found that clamping of one renal artery for 10 to 15 minutes during and after the injection of nephrotoxic duck serum, rabbits failed to develop lesions in that kidney, although severe lesions would appear in the opposite organ. The remarkable nature of this finding is further attested when one remembers that the earliest evidence of renal damage does not appear for at least a week after the injection.

Kay(171) found that exposure to heavy doses of X-radiation inhibited the development of nephrotoxic nephritis in rabbits. This effect was reversed if the animals were injected with small amounts of rabbit anti-duck-serum immediately after the injection of nephrotoxin. Since X-radiation inhibits antibody production, these results suggest that the development of nephrotoxic nephritis is dependent upon a more or less intact antibody producing mechanism. Should this be the case, then the entire assumption that anti-kidney antibodies are responsible for the nephritis is brought into question. If the injected antibodies are in fact responsible for the nephritis, then one would expect the phenomenon to be one of simple passive transfer, in which an antibody producing mechanism need not be involved. The demonstration by Ehrich et al.(151) that nephritis can be induced in rabbits by large injections of normal duck serum appears to support this idea.

In an effort to inhibit a supposed immunologic mechanism in nephrotoxic nephritis, Reubi(180) administered some of the "anti-histamine" drugs, notably a Ciba preparation known as "Antistin". He reports that rabbits treated with Antistin either before or after the injection of anti-kidney serum, showed significantly less albuminuria than control animals injected with nephrotoxin alone. Reubi goes on to say that the histological changes were diminished or absent in Antistin-treated animals. Only small numbers of animals were used in these experiments, whose results have not thus far been confirmed.

Strehler(181) claimed to have induced a "nephrotic" tendency in rabbits rendered nephritic with anti-kidney serum. After the serum injection, the animals were given daily feedings of cholesterol in oil, together with 10 mg./kg. of tetra-methyl thiourea. Animals treated in this way did not develop hypertension, but had severe albuminuria and hypoalbuminemia, together with elevation of blood cholesterol. Although these findings are those of the human nephrotic syndrome, the method by which the changes were induced suggests a combined physiological insult rather than a single disease process.

The work of Pressman and Keighley(182) indicates that there is in fact, a considerable degree of specificity to nephrotoxic serum. In their experiments, radio-iodine (iodine 131) was combined with rabbit anti-rat-kidney serum without interfering with its capacity to precipitate kidney extract. Rats injected with this material and killed 48 hours later showed the main concentration of radioactivity (and presumably of antibody) in the kidney, with lesser amounts in liver, lung, spleen and other organs. A comparable experiment with anti-ovalbumin serum showed the highest concentration of radio-activity in the liver, with a large residue remaining in the blood. Although it cannot be concluded from these experiments that the distribution of radio-activity did in fact coincide with the distribution of antibody, more or less specific removal of the anti-kidney serum by the renal tissue is implied.

By estimation of the phenol red:inulin clearance ratio in dogs with nephrotoxic nephritis, Fouts et al. (183) found evidence of increased renal blood flow and decreased glomerular filtration during the development of the

disease. Similar observations were made by Gukelberger(184) in rabbits. The latter author found correspondence between the clinical estimate of glomerular blood flow, and the appearance of the glomeruli in frozen sections stained with a benzidine technique. He concluded that nephrotoxic nephritis begins with passive hyperaemia in the glomeruli, with ischemia ensuing only after some days, as a result of endothelial proliferation. Primary arteriolar spasm could not be detected in these experiments. Although the methods of measurement used by both investigators may be questioned, it is perhaps significant that the same result was attained by both.

Anti-sera to other organs than kidney have usually failed to give rise to nephritis. Masugi(161) did not observe renal damage in rats treated with rabbit anti-rat-liver serum, and Smadel(169) found the same to be true of anti-sera to rat heart, liver, serum and erythrocytes.

Seegal and Loeb(185) however, in an experimental investigation directed at toxaemia of pregnancy, found that rats injected with an anti-placenta serum prepared in rabbits, developed chronic glomerulonephritis indistinguishable from that induced by anti-kidney serum. Eighteen of thirty-two animals so treated developed a chronic diffuse sclerosing glomerulitis. In further investigations the same workers found that the incidence and severity of this nephritis could be enhanced by administration of DCA. Pregnancy was also found to potentiate the disease, which, once developed, was not influenced by abortion.

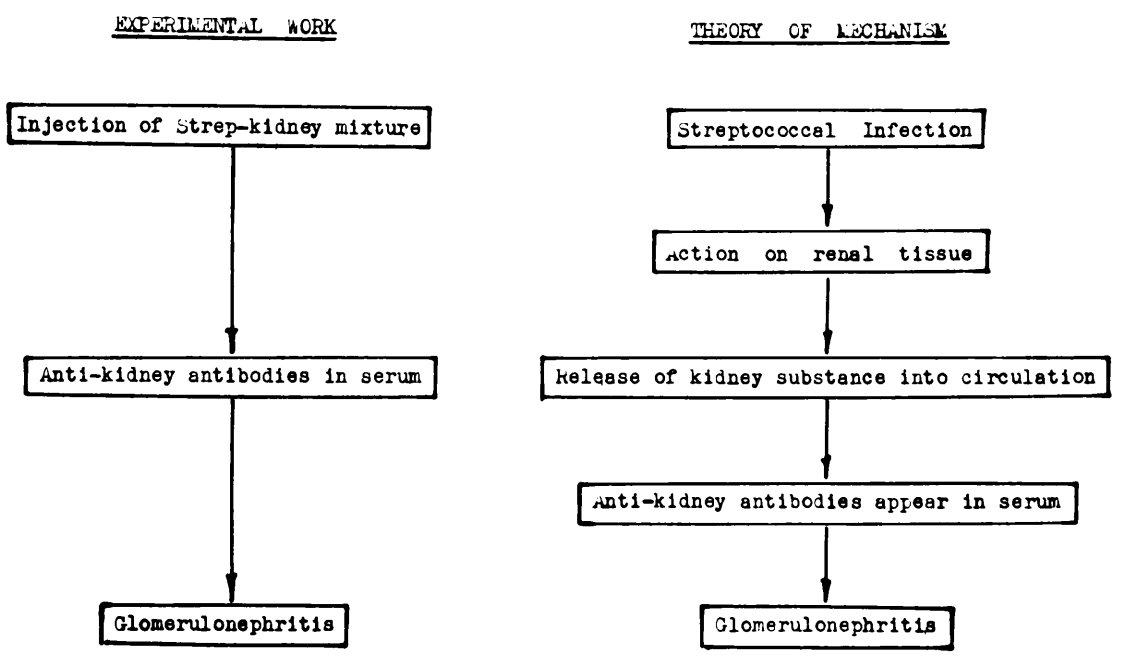
The implication of most of the above work has been that antibodies to renal substance were responsible for this type of experimental nephritis. It

is therefore understandable that investigations should have been conducted to determine whether such a mechanism might operate in human nephritis. The purpose of these investigations appears to have been to demonstrate a link between streptococcal infection and anti-kidney antibodies. The initial work in this direction was that of Schwentker and Comploier(196), who injected rabbits with a mixture of ground homologous kidney and killed streptococci. Using the highly sensitive collodion particle agglutination technique to detect serum precipitins, they were able to demonstrate more or less specific anti-kidney antibodies in the serum of these animals.

From these results it was suggested that streptococcal infection might in some way render a person's kidney antigenic, and that antibodies to this streptococcus-kidney combination might then give rise to acute nephritis. Experimental support for this concept was added by the experiments of Cavelti and Cavelti(187-190). They found that injection of a streptococcus-kidney mixture would give rise to significant amounts of anti-kidney antibodies (demonstrable by the collodion particle technique), in both rabbits and rats. In rats these antibodies appeared in 50% of treated animals, and diffuse glomerulitis was reported in about the same proportion of animals. Unfortunately the reports do not state the degree of correspondence between the appearance of antibodies and the occurrence of lesions. Lesions in rabbits in whom anti-kidney antibodies were found, are not described. Nevertheless, a rather neat hypothesis of the pathogenesis of acute nephritis has been constructed on the basis of these experiments.

Table IV.

SUMMARY OF CAVELTI EXPERIMENTS



The left half of the table indicates the experimental findings of the Caveltis, while the theory as to the chain of events is on the right. At the present time, this theory has not progressed beyond this attractive blue-print stage. Indeed, the whole concept is brought into question by repetitions of the Cavelti experiments in which the results were completely negative(191,192). The above conclusions may thus, for the moment, be pigeon-holed as 'premature'.

Summary.

The injection of appropriate animals with rather large amounts of anti-kidney sera prepared in heterologous species has resulted in acute and chronic nephritis in a number of instances. In most, if not all cases, the renal disease has been a diffuse extra-capillary glomerulonephritis, in which epithelial proliferation is prominent. Development of the disease is accompanied by the clinical phenomena of acute haemorrhagic nephritis in man: haematuria, proteinuria and cylindruria. In some instances the course of the disease has been influenced by the strain of animal used. The disease also tends to be less severe in animals maintained on a low protein diet.

Attempts to inhibit the disease other than by dietary manipulation have met with indifferent success. Efforts to demonstrate a chain of events in which streptococci and anti-kidney antibodies are implicated, have been inconclusive.

Radio-isotope studies have suggested a certain amount of organ specificity in anti-kidney sera. Other experiments have indicated that the primary action of anti-kidney serum occurs within a few minutes of its injection, even though symptoms and lesions are not manifest for several days after this. The question of whether the injected anti-kidney antibodies are actually responsible for the nephritis, or whether the disease is simply another instance of foreign serum nephritis, must be left open for the moment.

Functional studies have suggested that the initial change in this type of experimental nephritis is one of glomerular hyperaemia, and decreased filtration.

IV. THEORETIC CONSIDERATION OF ACUTE DIFFUSE GLOMERULONEPHRITIS.

Introduction.

Most of the information thus far presented has been of a factual nature, with theoretic considerations introduced only to clarify the selection of the experimental approaches. We should now be in a position to attempt to consider synthetically some of the information from human and experimental animal sources, with a view to defining some of the gaps in knowledge and theory, and perhaps to explain some apparent discrepancies. In the following section, a number of theories concerning etiology and pathogenesis of acute nephritis will be considered in the light of the evidence at hand.

Nephritis as a Bacterial Disease.

Reference has been made to the frequent association between acute nephritis and infection in man. The infection almost always involves the upper respiratory tract, and is frequently a streptococcal tonsillitis. It has also been pointed out that both the urine and kidneys are usually bacteria-free in patients with nephritis. Thus, from almost the beginning, acute nephritis has not usually been considered a bacterial disease in the ordinary sense. It has, from time to time, been suggested that the condition might be brought about by a filterable virus(35), but little evidence has been forthcoming on this score.

Kylin(56), in an effort to explain the fact that symptoms of renal damage usually appear when the upper respiratory infection is on the wane, suggested the disease was brought about by release of split proteins, toxins, antitoxins and tissue degeneration products. He postulated the absorption of this brew from the site of infection, and its extension to the kidney via the blood stream. He assumed a difference in toxicity of these substances, and in host susceptibility to explain the low incidence of nephritis following throat infections, and the lack of correlation between the severity of the infection and subsequent development of nephritis. In explaining the occurrence of nephritis in the absence of preceding infection, he reasons adroitly that since the renal lesions are indistinguishable from those in which infection has been found, that the exciting cause was probably the same: i.e. an undetected respiratory infection.

Such a theory has sufficient elasticity to render difficult its proof or disproof. It does not go so far as to distinguish which of the offending substances may be the important one, or to suggest that they must present an united onslaught. The mode of action upon the kidney is no further indicated than the suggestion of 'toxicity'.

Nephritis as a disease of Hypersensitivity.

Most of the evidence from humans and experimental animals tend to support the thesis that acute diffuse glomerulonephritis is in some way linked in its development with the state of hypersensitivity. In 1912 Escherich and Schick(55) pointed out that the incubation period in post-scarlatinal nephritis

is approximately that required for antibody formation in the human organism. They suggested that nephritis is an immunity manifestation, related to antibody formation, and therefore an allergic disease. The theory postulates that the scarlet fever organism persists in latent form after the disappearance of acute manifestations, and only becomes pathogenic for the kidney when it can react with its specific antibody, i.e. after this antibody has had time to form. They point to the analogy with serum sickness in man, where the manifestations appear coincident with the rise of antibody in the serum, and disappearance of the antigen (horse serum).

Along similar lines were the findings of Friedemann and Deicher(197) in 1928. They demonstrated manifold occurrence of antibacterial antibodies in cases of scarlet fever nephritis, and showed that such antibodies were rare in non-nephritic scarlet fever convalescents. The suggestion was made that post-scarlatinal nephritis is a result of premature formation of anti-bacterial antibodies, without simultaneous production of anti-endotoxin. The endotoxin was thus 'prematurely' liberated by bacteriolytic action of the antibacterial antibodies, and free to produce kidney damage.

Volhard(43,86) explained the anatomical picture of nephritis on the basis of allergically conditioned spasm of the pre-glomerular arterioles, analogous to the spasm of the pulmonary vessels in anaphylactic shock. This concept was tentatively supported by Rueff's observation of an increased vasoconstrictor irritability in the skin vessels of convalescent scarlet fever patients(198).

Though the authors referred to differ in their suggestions of the mechanism by which nephritis develops, most of them accept the idea that glomerulonephritis is in some way or another a manifestation of immune reaction. It should be pointed out, however, that up to the present, the only connection between the state of hypersensitivity and acute nephritis that has been established is one of occasional association. It has not yet been demonstrated that because hypersensitivity and nephritis may occur together, one is necessarily the cause (or effect) of the other.

In experimental nephritis, the evidence is rather more strongly in favour of a hypersensitive mechanism. All of the experimental nephritides discussed in this review have been produced by the administration of an antigenic 'foreign' protein. Perhaps the most striking feature of these artificial nephropathies is the fact that a number of quite different antigens have produced an almost identical morphological picture. The list now includes normal horse serum, normal duck serum, anti-kidney sera, anti-placenta serum, and horse and bovine gamma globulins. A few of these substances can be shown to contain antibodies capable of reacting with kidney tissue of the animal into which they are injected, but the majority contain no such antibodies. Furthermore, the course and development of the disease induced by sera containing anti-kidney antibodies, is the same in all essentials as that produced by non-immune sera. Finally, the inhibition of antibody production by X-ray therapy has been shown to inhibit simultaneously the nephritis resulting from anti-kidney serum injection. These facts, and others already cited, argue against the assumption that nephritis is the result of

some action of organ-specific antibodies.

It seems profitable, at this point, to consider briefly some of the features of tissue reactions in animals sensitized to foreign materials. Hypersensitivity may be defined as an altered bodily response to parenteral contact with an alien antigen, the altered response being the result of previous exposure to the same material(199). Hypersensitive tissue reactions have been divided broadly into the so-called tuberculin-type and anaphylactic type. In the former, local inoculation of the antigen results in slowly progressive inflammatory reaction characterized by damage and death of cells. Since this reaction occurs even in avascular tissues, such as cornea or tissue culture preparations, it has been interpreted as an intrinsic cellular sensitivity(199). Anaphylactic hypersensitive reactions, on the other hand, are characterized by a prompt local inflammatory response, and appear to depend for their development upon intact vascular supply. Various other aspects of these reactions are dealt with by Rich(199). It is sufficient to point out here that animals sensitized to foreign serum generally exhibit the anaphylactic type of hypersensitivity.

In anaphylactic hypersensitivity, there appears to be a direct relationship between the degree of sensitivity and the amount of antibody in the circulating blood(200). In the experimental nephritides induced by foreign protein injection, it has been stated that rather large amounts of material must be injected before the renal lesions can be expected to appear. It would therefore be of interest to know whether the quantitative antibody response is

related to the amount of antigen injected. Search of the literature fails, however, to divulge much information upon this point, at least with reference to inoculation of rabbits with foreign serum. Topley and Wilson(193) found that rabbits injected with progressively increasing amounts of Salmonella paratyphi B. showed correspondingly greater degrees of antibody response within the limits of dosage allowed by the toxicity of the antigen. Masugi and Sato(148) found a rough correspondence between the amount of horse serum injected intravenously into rabbits in divided doses, and the precipitin titre. This titre also appeared to bear a rough, though by no means absolute, relation to the severity of the nephritis induced.

The findings of Hawn and Janeway(156) may also be related in a somewhat different way to quantitative antibody production. In rabbits injected with bovine gamma globulin antibodies became demonstrable in the serum one week after injection, whereas animals given equivalent amounts of bovine albumin failed to develop antibodies before the second or third week. This suggests that the rate, and perhaps also the quantity of antibody production, varies with the antigen employed. Again, it was the animals with the most rapid antibody response, i.e. those injected with bovine gamma globulin, in which lesions of diffuse glomerulitis appeared. This line of reasoning leads us to the tentative assumption that the development of nephritis is in some way linked with the quantitative aspect of antibody development: the greater, or the more rapid, the antibody production, the greater the liability to nephritis.

If we examine the evidence from experiments in which the appearance of nephritis has been inhibited or enhanced, they are in general agreement with this proposition. Thus, Kay(171) inhibited antibody production in rabbits by X-radiation, and simultaneously inhibited nephritis. Artificial replacement of antibody in these animals led to development of the lesions.

The inhibition of experimental nephritis by restriction of dietary protein requires rather more detailed explanation. It is now generally agreed that antibodies are themselves serum proteins, and in most instances, occur in the gamma globulin fraction(201,202). Immune serum gamma globulins have been found to show few, if any, important chemical differences from non-immune globulin(203). Schoenheimer et al. (204) have shown in studies using radio-isotope amino acids that the globulin fractions of the serum participate in the general metabolic exchanges to the same extent as do other serum and tissue proteins. They also demonstrated that antibody protein in immunized rats did not differ in this respect from other proteins. Cannon et al.(205) found that in young rabbits the capacity for antibody formation was significantly diminished when the animals were maintained on a diet deficient in protein. Similar results have also been obtained in rats(206). Thus, the inhibition of nephritis by lowering the intake of dietary protein would appear to have coincided with, if not indeed been dependent upon, impaired antibody formation.

The increase in severity of experimental nephritis observed by Knowlton et al.(178) brought about by DCA therapy, might be similarly explained, although here the evidence is conflicting. Dougherty et al.(207) noted an

increased rate of antibody release as a result of treatment with adrenal cortical extract. Other investigators, however, have not been able to confirm these findings(208,209,210).

Allusion has already been made to the Amsterdam outbreak of acute nephritis, occurring during a period of dietary rehabilitation following prolonged semi-starvation. It may again be suggested that antibody response was involved. If it is assumed that the prolonged dietary restriction resulted in decreased antibody formation in the victims, it may be further postulated that infections were probably more frequent and persistent. During the period of rehabilitation, immune responses may have been extreme, and, in some instances, appear to have been associated with development of acute nephritis.

The foregoing is obviously but the thinnest web of circumstantial evidence, and is not intended as more than a working hypothesis, subject to rejection or modification in the light of further evidence.

The Relation of Nephritis to the "Collagen Diseases" and "Tissue Reactions of Hypersensitivity".

Within recent years a concept has been put forward to the effect that the collagenous tissues of the body represent an organic "system", various diseases of which exhibit features in common, whatever part of the "system" is involved(211,212,213). The diseases which thus far have been said to fit into this concept have included periarteritis nodosa, disseminated

lupus erythematosus, diffuse scleroderma, dermatomyositis, rheumatic fever, rheumatoid arthritis, Buerger's disease and serum sickness. Exception has been taken to the "system" concept by Duff(215) who prefers, on perfectly rational grounds, to designate them merely as tissue diseases. Debates arising out of this concept need not be considered here, beyond the point of implying that, as with other new ideas, the entire matter is in a rather fluid state at the present time.

The main interest in these diseases appears to arise from the attitude that, since they have common features in their morphology, they may arise on the basis of similar etiologic and pathogenetic factors. Even before the collagen disease concept appeared, Klinge(214), Vaubel(145) and others had suggested that experimental lesions associated with the hypersensitive state usually exhibited common morphological aspects. In most of the experimental work already referred to, in which foreign protein has been injected into animals(148,149,151,152,153,154,155,156,157,216), extrarenal granulomatous lesions have been found, principally in the heart and blood vessels. In the heart the lesions have consisted of swelling and disruption of collagen, accumulation of lymphocytes and large mononuclear cells, with occasional giant cell formations. The lesions were found between the bundles of myocardial fibres, in the endocardium, valves and valve rings. In the blood vessels the changes have consisted of segmental fibrinoid necrosis of innner adventitia with extension to the muscle of the media, and, associated with these changes, infiltration with lymphocytes, plasma cells, large mononuclear cells, and occasional polynuclear leucocytes. The changes have

generally been considered an experimental counterpart of human periarteritis nodosa. A number of investigators have felt that the cardiac lesions represented experimental Aschoff nodules, comparable to those of human rheumatic fever. It has been pointed out(216) that the resemblance between the experimental lesions and those of rheumatic fever is no more than a similarity, and that typical Aschoff bodies have not as a rule been produced experimentally.

Nevertheless, on the basis of experimental lesions, associated with the state of hypersensitivity, it is now widely considered that many of the so-called collagen diseases may be classified broadly as tissue reactions of hypersensitivity. Any such deduction is at the moment no more than speculative, and Duff(215) emphasizes that the grouping together of the diffuse collagen diseases must for the present be regarded as a purely morphological correlation.

Thus, the most that can be said of glomerulonephritis in relation to the collagen diseases is that, in experimental animals, the lesions of nephritis tend to be associated with the state of hypersensitivity and with cardiac and vascular damage which are considered by some equivalent to the collagen diseases in man. The fact that, except in the case of periarteritis nodosa, true glomerulonephritis in man is rarely associated with any of the collagen diseases, may be used to argue either that the experimental extra-renal lesions are not true equivalents of human collagen disease, or that these experimental nephritides are not true counterparts of human nephritis. On purely morphological grounds, the former argument appears more reasonable.

The Relation of Nephritis to the "General-Adaptation-Syndrome" of Selye.

On the basis of a considerable body of experimental work, Selye (217-224) has put forward the concept that a number of apparently unrelated, non-specific systemic phenomena, represent in fact, an integrated response to a variety of noxious stimuli (injury, exposure to cold, exercise, etc., etc.,). Various phases of this response are included under the term 'General-Adaptation-Syndrome'. The reaction includes morphologic alterations in the adrenal cortex, thymus and lymphatic tissue, and is accompanied by effects upon carbohydrate, nitrogen and mineral metabolism. Selye(224) recognizes three main phases of the response: (1) the Alarm reaction, (2) the Stage of Resistance, and (3) the Stage of Exhaustion. In the Alarm reaction there is lipid depletion and necrobiotic changes in the adrenal cortex, associated with reduction in the volume of the gland. Thymic and lymphatic tissue undergo rapid and extensive atrophy, due mainly to a reduced content of lymphoid cells. In addition, acute ulcers in the gastro-intestinal tract may also occur. There is increased blood coagulability, leucocytosis and haemoconcentration. After an initial rise, blood sugar values fall below normal. There is evidence of pronounced breakdown of body protein, reflected in increased blood N.P.N. values. Blood chlorides are markedly depressed, with more or less parallel changes in blood sodium, whilst potassium values tend to rise. During the stage of resistance, most of these changes tend to return to normal. If the noxious stimulus continues to operate, the capacity for resistance diminishes, and the changes of the alarm phase reappear and usually lead to death (Stage of Exhaustion).

From the results of his experiments, Selye has worked out a tentative mechanism whereby adaptation changes are brought about. The suggested chain of events is that stress (burns, exposure to cold, trauma, etc.,) act via unknown pathways upon the anterior lobe of the pituitary, causing increased production of adrenal corticotrophic hormone. The resulting discharge of adrenal cortical hormone gives rise initially to the Alarm Reaction phenomena of thymolymphatic atrophy (discharge and breakdown of lymphocytes), and is manifest morphologically in the adrenal cortex in lipid (hormone) depletion and necrobiotic cellular changes. As the noxious stimulus continues, hyperplasia of the adrenal cortex occurs, and the lipid content of the cells returns to normal with restoration of normal physiologic and biochemical balance (Stage of Resistance). Involutional changes in the adrenal cortex are found again in the stage of exhaustion. The main point of Selye's thesis appears to be that the non-specific reactions of adaptation are mediated by the pituitary adrenal axis.

The thesis has been extended(223) now to suggest that a number of human diseases may be in fact the morphological manifestations of prolonged or perverted reactions of adaptation. Experimentally, administration of a mineralocorticoid (DCA) to unilaterally nephrectomized rats lead to changes interpreted as periarteritis nodosa, nephrosclerosis and rheumatic carditis, lesions which were particularly prominent when the sodium/chloride ratio was allowed to rise through simultaneous administration of NaCl. Selye refers to these conditions as "Diseases of Adaptation"(223), and has since extended the list to include acute nephritis and other diseases(224). The entire

concept is set forth with great lucidity and considerable persuasiveness in Selye's Textbook of Endocrinology(224). The validity of the hypothesis as applied to human disease, however, has yet to be shown conclusively.

Within the framework of the adaptation theory it might be expected that various forms of stress would have some influence upon the course of an experimental nephritis. In view of the frequent association between exposure to cold and the appearance of acute glomerulonephritis, it seems profitable to consider some of the effects of exposure to cold upon animals and man, with special reference to renal function and disease.

Lassar(225) observed that chilling caused diarrhoea and albuminuria with hyaline casts in animals, and commented on an interstitial inflammation in the kidneys found at autopsy. Myer et al.(226) observed albuminuria and haematuria in dogs immersed in cold water, and at autopsy found hyaline and haemorrhagic areas of necrosis in the kidneys. In experiments in which direct observations of renal and aortic blood temperatures were recorded, Nedzel(227) found that chilling dogs with ice resulted in rapid and marked fall in kidney temperature within 10 minutes. This fall was more marked than that occurring in the aortic blood, but tended to approximate the blood temperature if the kidney were "denervated" before the experiment by stripping of the perivascular nervous plexuses. From this, Nedzel concluded that a marked renal vasoconstriction could occur physiologically during exposure to cold, but that this was dependent upon nervous stimulation.

Foord(228) found that repeated chilling effected a moderate increase in agglutinin production in rabbits. Similar results had been obtained earlier by Graziani(229) in experiments in which animals were maintained continuously in a cold dry atmosphere throughout the period of immunization.

Thus most of the work cited in this section would suggest that exposure to cold might have the effect of potentiating an experimental glomerulonephritis. This will be further considered in the Discussion following the report of Experiments.

V. EXPERIMENTAL WORK.

Introduction.

The experiments reported here have had as their principal objective the study of experimental acute diffuse glomerulonephritis. The initial task was, therefore, the selection of a method by which the disease could be induced in a high proportion of animals, and preferably in considerable severity.

It was necessary to select an experimental animal. In the review just presented, nephritis has been said to have been produced artificially in a number of species, but particularly in the rabbit and the rat. In view of the fact that nephritis may appear spontaneously in the rat(15,112,114,115) and that different strains have been shown to vary in their susceptibility to the disease, it was felt that the rabbit would probably be more satisfactory. Spontaneous glomerulonephritis has not been reported in this animal, but quite acceptable artificial nephritides have been induced. It is a large enough animal to allow individual and quite extensive clinical studies (blood chemistry, urinalysis), which are more difficult in the rat by virtue of the small quantities of material obtainable. It is, on the other hand, small enough to be economical in upkeep, so that considerable numbers can be used for statistical studies. Finally, there was the fact that facilities on hand for animal experiments with rabbits were completely adequate, and a minimum of 'working-in' of new techniques would be required.

In the choice of means, several alternatives presented themselves. Injecting animals with horse serum had given varying results in the hands of different investigators. Some had reported nephritis, and others had not. The use of nephrotoxins (anti-kidney sera) seemed more promising, but involved a complexity in immunological study which was considered beyond our facilities. The use of the Cavelti technique of injecting streptococcus-kidney mixtures involved bacteriological facilities which were, at the time, inaccessible. The work of Hawn and Janeway(156), in which nephritis had been reported in rabbits following a single massive injection of purified bovine serum gamma globulin, seemed to offer a promising new technique. In that report the suggestion was implied (though not actually made) that the use of purified serum protein fractions tended to give predominantly one type of lesion or another, and in the case of bovine gamma globulin, principal emphasis was placed upon glomerulonephritis. A further advantage seemed to lie in the use of a single purified antigen, allowing perhaps greater degree of immunologic control.

A possible disadvantage in the use of bovine gamma globulin was the rather mild and evanescent nature of the lesions in Hawn and Janeway's animals. For this reason, means by which the renal disease might be intensified were considered. In the portion of the review dealing with experimental nephritis induced by foreign protein injection, it has been pointed out that lesions of nephritis seem to be more frequent when animals were injected with large doses of foreign protein. Furthermore, it will be recalled that in nearly all the work, except that of Hawn and Janeway, two or more injections of the

foreign protein have been given. It was, therefore, decided to give our experimental animals two massive injections of purified bovine serum gamma globulin instead of the one used by Hawn and Janeway. In the spacing of the injections, there was little information to serve as a guide. However, Hawn and Janeway had found that antibodies to gamma globulin appeared much earlier than to other serum fractions used, and on this basis, the conventional 17 - 19 day interval was pared down in our case to 10 or 11 days.

It had also been noted that Masugi's unilaterally nephrectomized rabbits developed nephritis following horse serum injection, whereas Vaubel's non-nephrectomized animals on similar dose schedules had not. It was therefore decided to carry out unilateral nephrectomy in our animals before treatment was initiated, in the hope that any renal lesions might thus be intensified.

These, then, were the main features of the first experiment: unilaterally nephrectomized rabbits were to be injected with two massive intravenous doses of purified bovine serum gamma globulin, ten or eleven days apart. It was decided to kill the animals six or seven days after the last injection, since with other techniques, lesions had been found to be well developed by this time.

The first experiment did indeed produce an acute diffuse glomerulonephritis of considerable severity in a high proportion of animals. It may seem facetious to tell now of its planning in the manner just done, but the line of reasoning which was followed was actually that which has been given.

In a second experiment, the work of Hawn and Janeway was repeated, using a small number of unilaterally nephrectomized animals. The purpose of this work was to determine whether the results of these authors could be re-duplicated, and also to estimate the morphologic character of the early lesions of nephritis. It was found, as had been suspected, that the early lesions were extremely mild and difficult to assess. For that reason, in subsequent experiments we reverted to the two injection technique which had been employed in the first experiment.

Before experiments designed to inhibit or prevent the experimental disease were planned, a third experiment was undertaken in an effort to further intensify the nephritis. It was felt that if this could be accomplished, the ideal point would have been reached where it could be predicted that every animal treated would develop the disease, and it would then be feasible to do small-scale, but statistically valid experiments, in which a large number of variables could be introduced one by one, with the principal purpose of inhibiting the lesions. Since the effect of cold in the pathogenesis of nephritis in both man and animals appeared to have been but little studied, it was decided to expose some animals to a cold, but tolerable, environment during the period when they could be expected to be developing the disease. During these experiments various clinical and morphological data were collected which were considered of value in plotting the course and development of the disease.

This experiment again seemed successful, with now 100% of animals treated coming down with nephritis, and with an apparent increase in the severity of the disease. Therefore, a third experiment was carried out in which groups in the cold and at room temperature were treated simultaneously, together with untreated control groups. The purpose of this experiment was simply to expand the body of clinical and morphological information already accumulated, and to increase the size of the group of animals treated in the cold to the point where the increase in incidence and severity of the lesions might be more striking.

Insofar as the result of this experiment had been anticipated, it was not quite so successful. Although all animals treated in the cold still developed nephritis, the lesions were, in general, no more severe than those in most animals treated at room temperature.

A final experiment was therefore planned at this point, in which control was added in one important respect. Previous groups of animals had been allowed to eat and drink ad libitum. It had now become obvious that animals in the cold were unable to drink as much as their counterparts at room temperature, due to the fact that their water supply froze before they could drink more than a portion of it. Since animals on a dry diet seemed to have more or less parallel food and water intakes, in the final experiment both food and water supply were restricted to the point where every animal in each group (one at room temperature, one in the cold room) consumed his entire supply each day throughout the experiment. Although the restriction of dietary

intake was a relatively mild one, the results of the experiment were startling in that not a single animal of either group developed nephritis.

This outline is intended merely to establish the thread of continuity joining the experiments to be reported, since the logic of their arrangement might not otherwise be apparent. The details of each experiment will be first presented, following which a systematized description of all results will be presented. The results will then be discussed within the framework of the review of the literature already given.

EXPERIMENT I.

EXPERIMENTAL PRODUCTION OF ACUTE DIFFUSE GLOMERULONEPHRITIS.

Materials and Methods: Twenty-eight rabbits were used in all. Of these, eighteen were treated with bovine serum gamma globulin, and ten were kept as controls. Animals of different strains and both sexes were used. They had an average weight of about 2.5 kg. at the time treatment was instituted. Before receiving any treatment all animals were unilaterally nephrectomized. Nephrectomies were done from 3 to 6 weeks before globulin treatment was begun. The treated animals were given two massive intravenous injections of purified bovine serum gamma globulin, with a 12 day interval between injections. Possible fatal anaphylactic reactions were avoided by desensitizing the animals with a small intravenous injection of globulin about 18 hours before they were to receive the second massive dose.

The treated animals were injected intravenously with 1 gm. per kilo of bovine globulin, given as a 10% solution in normal saline. Eleven days later they were slowly injected intravenously with the desensitizing dose, which consisted of 1.0 c.c. of the concentrated solution diluted to 5 or 10 c.c. total volume in normal saline. On the following day, a second massive intravenous dose was given, equaling in amount the initial dose.

Blood urea nitrogen estimations were made on eight animals before treatment was begun, and again immediately before the animals were killed. Similar estimations were made on two other animals before killing only.

Morphologic Studies: All animals were killed by intravenous air injection one week after the second large dose of globulin. Autopsies were performed immediately, and tissues were fixed in Bouin's and Helly's fluids. Histological sections were cut at 3 to 4 mu thickness, and stained with haematoxylin and eosin, haemalum-phloxin-saffron, Mallory-Heidenhain, Mallory's phosphotungstic acid haematoxylin, and according to McManus' periodic acid routine(230).

Tissues upon which routine histological examinations were made were kidney, heart, thymus, adrenals, spleen and liver. Additional sections were made in some cases from lung, intestine, mesenteric blood vessels, striated muscle and thyroid.

Immunological Studies: Blood was drawn from ten of the eighteen treated animals on three occasions; before the first globulin injection, before the desensitizing injection on the 11th day of the experiment, and finally just before the animals were killed. The blood was centrifuged and the serum drawn off. A simple qualitative ring test technique, similar to that employed by Hawn and Janeway(156) was used in all tests. In this technique a small quantity of test antigen is carefully layered over the serum in a small test tube (ours were 3 X 40 mm.). The tubes were incubated at 37°C. for one hour and promptly read. The formation of a clear-cut white ring at the interface between serum and antigen was recorded as positive. Reactions were roughly graded from zero to four plus. Tests were made on these ten sera for the following:-

1. Antibodies to bovine serum gamma globulin. 0.05% globulin solution was used as test antigen.

2. Antibodies to rabbit kidney. The serum of each rabbit was tested against saline extract of perfused, ground, rabbit kidney. 20%, 10% and 2% kidney extracts were used. The kidneys removed from the animals prior to globulin therapy had been washed free of blood by perfusion with normal saline at the time of removal. They had then been stored at -20°C. until required for serological testing. The serum of each rabbit was tested then against extract of the rabbit's own kidney, as well as against the pooled extracts of several kidneys.

3. Antibodies to rabbit liver. This served as a control of test 2 above. 20%, 10% and 2% extract of ground, perfused rabbit liver was used as test antigen.

In addition to these tests, each lot of bovine serum gamma globulin was tested against the individual and pooled rabbit kidney extracts, and also against rabbit liver extract and against sera of all the animals before treatment was initiated. All serological tests were controlled by corresponding tests with normal saline.

Intradermal skin sensitivity tests were carried out in all animals just before killing. For this purpose, a shaved area on the back of the animal was injected intradermally with 0.1 c.c. of each of the following substances:-

(a) bovine gamma globulin (10% solution), (b) rabbit kidney extract (20% saline suspension of the rabbit's own kidney), (c) rabbit liver extract (20% saline suspension), and (d) normal saline. In ten of the animals this test was done 2 days before killing. In the other eight globulin-treated animals the remaining kidney was surgically removed before skin testing. It was hoped thus to eliminate the possibility that the remaining kidney might be absorbing any kidney antibody that might have been present, rendering it undetectable by skin tests. Unfortunately these animals failed to survive the second nephrectomy for long enough periods to allow accurate reading of the tests.

Blood Coagulation Studies: Coagulation time observations were made on globulin-treated and control animals. The capillary tube technique was used in all determinations, and times were recorded to the nearest 1/10th of a minute. In this technique blood from a fresh cut in a small ear vein was drawn into a glass tube of capillary bore. The tube was mounted upright in a piece of plasticine, and small bits of it broken off every few seconds. The coagulation time was recorded as the time at which, when the tube was broken, a thin strand of clot pulled away with the broken end. Daily observations were made in most instances, and hourly recordings were made on several occasions. Estimation of the normal coagulation time was based upon thirty-four observations made on the eighteen animals which were subsequently injected with globulin.

Controls: Ten animals comprised the control group. All were unilaterally nephrectomized. Two developed post-nephrectomy infections and were killed and autopsied one month after operation. Three animals of this group

were allowed to survive nephrectomy for 7 months, in order to control the possibility of spontaneous appearance of renal lesions following this treatment alone. The remaining five animals were treated in exactly the same manner as those of the experimental group, with the exception that instead of injections with bovine serum gamma globulin, they received equivalent amounts of normal saline.

All control animals were killed and autopsied by the same technique as was used in the globulin-treated group, and histological preparations from these animals formed the basis for estimation of the degrees and types of damage found in the treated group.

EXPERIMENT II.

THE EARLY MORPHOLOGIC ALTERATIONS IN EXPERIMENTAL GLOBULIN NEPHRITIS.

Materials and Methods: Eight albino rabbits of both sexes weighing between 1.8 and 2.8 kg. were used. Before any treatment was given, all animals were unilaterally nephrectomized, and allowed from four to six weeks to recover. They were then placed in metabolism cages to allow daily collection of urine for analysis. Urinalysis was done daily throughout the experimental period. Normal urinary findings for these rabbits were first determined by observations during a five-day control period. Urine was examined for protein by boiling and acidifying, 24 hour output was measured, and microscopic examination was carried out.

Five of the animals were injected with bovine serum gamma globulin, and three were kept as controls. The treated animals were injected intravenously with 1 gm. per kg. of purified bovine serum gamma globulin, given as a 10% solution in normal saline. Except for one animal which died in the course of treatment (5th day), all were killed by air embolism on the eighth day after the globulin injection. Tissues were fixed in Bouin's solution and in Zenker-formol, and sections prepared as in the previous experiment.

EXPERIMENT III.

THE EFFECT OF EXPOSURE TO COLD UPON EXPERIMENTAL GLOBULIN NEPHRITIS.

Materials and Methods: Eighteen albino rabbits of both sexes were used in all. Of these, ten were treated with bovine serum gamma globulin, and eight served as controls. Three to five weeks before any treatment, all animals were unilaterally nephrectomized. Three days before the first globulin injection, the backs and sides of the animals were shaved clean, and they were placed in a refrigerated room where they remained for the rest of the experimental period. Owing to the lack of an airlock, the temperature in this room fluctuated considerably. During the daytime, when the door was being opened frequently, it would rise to between 35° and 40°F. whereas at night, it would fall to 15° or 20°F. Throughout the experimental period they were fed Purina Fox Chow ad libitum, and were given fresh water twice a day.

Although all animals were handled in about the same way, the treated animals were divided into two groups of five each, treated at different times. Certain observations were made on one group and not on the other, and there were minor differences in experimental technique between the two groups. Procedures carried out in the first group (Group A) will be described first, and deviations from this routine in Group B will then be indicated. The average weight of animals of both groups the day treatment was initiated was 2.2 kg., within a range of 1.5 to 2.3 kg.

Group A. These animals were injected intravenously with 1 gm. of bovine serum gamma globulin per kg. of body weight, given as a sterile 10% solution in normal saline. Ten days later they were injected slowly with 10 c.c. of 1% gamma globulin as a desensitizing measure, and, 18 - 20 hours later were given a second massive injection, equaling the initial dose. All were killed by air embolism 6 days after the second injection (17 days after the first).

The animals were bled from the ear artery on three occasions: before being placed in the cold room, before the desensitizing injection, and three days after the second massive injection. Serum was separated, and ring tests were made to detect antibodies to the injected protein, using the same method as that in Experiment I.

Urine was collected daily from each animal via a special funnel bottom in the cage, covered by a fine screen grid. The 24 hour urine output was recorded. The reaction of the urine was roughly estimated with pH paper. Urinary protein was determined by boiling, then acidifying the urine with 10% acetic acid, and was recorded in the conventional zero to four plus. In addition, microscopic examination was carried out on all samples, and the presence or absence of tubular casts or red blood cells especially noted.

Group B. The treatment of this group differed from that of Group A only in one respect. Instead of receiving their second massive globulin injection 18 to 20 hours after the desensitizing dose, it was given within one or two hours. Since these animals also were killed 6 days after the

second injection, the total duration of the experiment was therefore about one day less. Additional observations were made on these animals as outlined below.

Morphologic Studies: All animals were killed by air embolism. Autopsies were performed immediately and tissues were fixed in Müller-formol. Histological sections were prepared as in Experiment I. Body weight, thymus, heart, kidney and spleen weights were recorded at autopsy for animals of both groups.

Immunologic Studies: The animals were bled before being placed in the cold room, and daily, in small amounts, for the first eight days after the initial globulin injection, and for three days after the second injection. Ring tests were carried out daily after the first and second injections, until the day antibodies to the injected globulin were first detected. All tests were controlled with normal saline, as in Experiment I. These tests became positive the 8th day after the first, and the 3rd day after the second injection.

Urinalysis: The only difference in this procedure from that followed in Group A consisted of the use of a finer quantitative measurement of urinary protein output. The Kingsbury-Clark albuminometer was used, involving a 1:4 dilution of clear centrifuged urine with 3% sulphosalicylic acid. After standing for 10 minutes, samples so treated were compared with turbidity standards, and the quantity of urinary protein recorded in mg. per 100 c.c. of urine.

Blood Studies: Oxalated samples of blood were drawn before the animals were placed in the cold room, before the first injection and one week after the first injection. Wintrobe hematocrit estimations were made on these samples. In addition, blood urea nitrogen estimations were made on some animals in this group. Serum samples from some animals, at different intervals, were frozen, and stored at -20°C. for several weeks, after which estimations of serum proteins were carried out. The technique used in all these estimations is detailed in Appendix.

Two treated animals were bled by cardiac puncture at the time of killing, their sera separated from the clot and pooled. A similar sample from two control animals was also prepared. Through the kindness of Dr. Lena Lewis of the Cleveland Clinic, Cleveland, Ohio, it was possible to obtain electrophoretic analyses of these sera.

Blood coagulation time determinations were made morning and afternoon on all globulin-treated animals throughout the experimental period. The technique employed was the same as described in Experiment I.

Controls: Of the eight animals in the control group, three were kept as controls for Group A, and five for Group B. Control animals were bled in like amount, and at similar intervals to the treated animals, and were maintained in the cold room under identical circumstances for the same period of time. These animals were killed and autopsied at the same time as the treated animals, and histological sections were prepared for comparison with those of the treated groups.

EXPERIMENT IV.

THE EFFECT OF EXPOSURE TO COLD UPON EXPERIMENTAL GLOBULIN NEPHRITIS.

Materials and Methods: Twenty-three albino rabbits of both sexes were used in all. All were unilaterally nephrectomized two to four weeks before any treatment was instituted. They were then divided into four groups, three of six animals each, and one of five. Two groups were placed in the refrigerated room, as in the previous experiment, and one of these received two massive injections of bovine serum gamma globulin, ten days apart. As in the previous experiment, the animals received a desensitizing injection of dilute globulin solution an hour or so before the second massive injection was given. The animals were killed six days after the second injection. The second cold room group was handled in the same way as the treated group, bled at the same intervals, and subjected to parallel observations throughout, and killed at the same time. The remaining two groups of animals were handled at room temperature in exactly the same manner as the two groups in the cold room. The average weight of the animals at the time treatment was instituted was about 2.2 kg.

The cold room animals were shaved as in the previous experiment, and placed in the cold room four days before the first globulin injection. They were bled the day before being placed in the cold room, four days later (1 hour before being injected), one hour after the first injection, on the 7th day after this injection, before and after the second injection, and on the day of killing, six days later. Treated and control groups were bled at

similar intervals, and the room temperature groups were treated in the same fashion. At each bleeding, serum was separated from clotted blood, and, in addition, a sample of oxalated blood was taken. During the period of treatment, the following studies were made:-

1. Daily urinalysis, as outlined previously,
2. Serum protein determinations, by method described in Appendix,
3. Blood urea nitrogen estimations,
4. Sedimentation velocity, erythrocrit and leucocrit,
5. Ring tests for antiglobulins in serum,
6. All animals were weighed at frequent intervals throughout the experimental period.

All blood studies were made on each of the occasions indicated in the preceding paragraph.

All animals, other than those dying spontaneously, were killed by a blow on the neck, and autopsied immediately. Tissues were fixed in Zenker-formol and histological sections prepared as in the previous experiments. Body weight, thymus, heart, kidney and spleen weights were recorded for all animals at the time of autopsy.

Controls: As indicated, control animals were kept simultaneously in the animal quarters, under as nearly as possible identical circumstances of diet etc., as the treated animals. In addition, most of them were bled at similar intervals, killed and autopsied by the same technique, and at the same time, as the corresponding treated groups.

EXPERIMENT V.

THE EFFECT OF GENERAL DIETARY RESTRICTION UPON EXPERIMENTAL GLOBULIN NEPHRITIS.

Materials and Methods: Twelve animals made up the experimental group. All had been subjected to preliminary nephrectomy as in previous experiments. The series was divided into two groups which were both injected with bovine serum gamma globulin as in Experiment IV. Six of the animals were treated at room temperature, and six in the cold room. The animals were bled at similar intervals to those in Experiment IV, but the only studies carried out consisted of urinalysis and serum precipitin reactions.

The only real difference between the animals in this experiment, and the treated animals in Experiment IV was that the present group was placed on a restricted intake of food and water throughout the period of the experiment. This amounted to 70 gm. of Ogilvie Miracle Chow per day, and 130 c.c. of water. All animals were fed once a day, whereas the water ration was divided into two equal parts, given at an interval of six hours. This latter routine allowed the cold room animals to consume their entire water supply, none being lost to them through freezing. The dietary standard for the experiment was determined during a preliminary observation period, during which account was kept of the amount of food and water consumed by all animals. Then the ration was set at the amount all animals would consume completely, each and every day.

Morphological observations were made in exactly the same manner as in Experiment III.

Group Treatment	No. of Animals	Mean Wt. when killed (kg.)	Mean Wt. gain during experiment (16-17 days) (gm.)	No. with nephritis
Rm. Temp. Controls	10	2.810	439	0
Rm. Temp. Bovine globulin, 2 injections.	6	2.660	403	4
Rm. Temp. Bovine globulin, 2 injections. Restricted diet.	6	2.118	217	0
Cold Room Controls.	10	2.603	342	0
Cold Room Bovine globulin, 2 injections.	15	2.331	403	15
Cold Room Bovine globulin, 2 injections. Restricted diet.	6	1.737	41	0

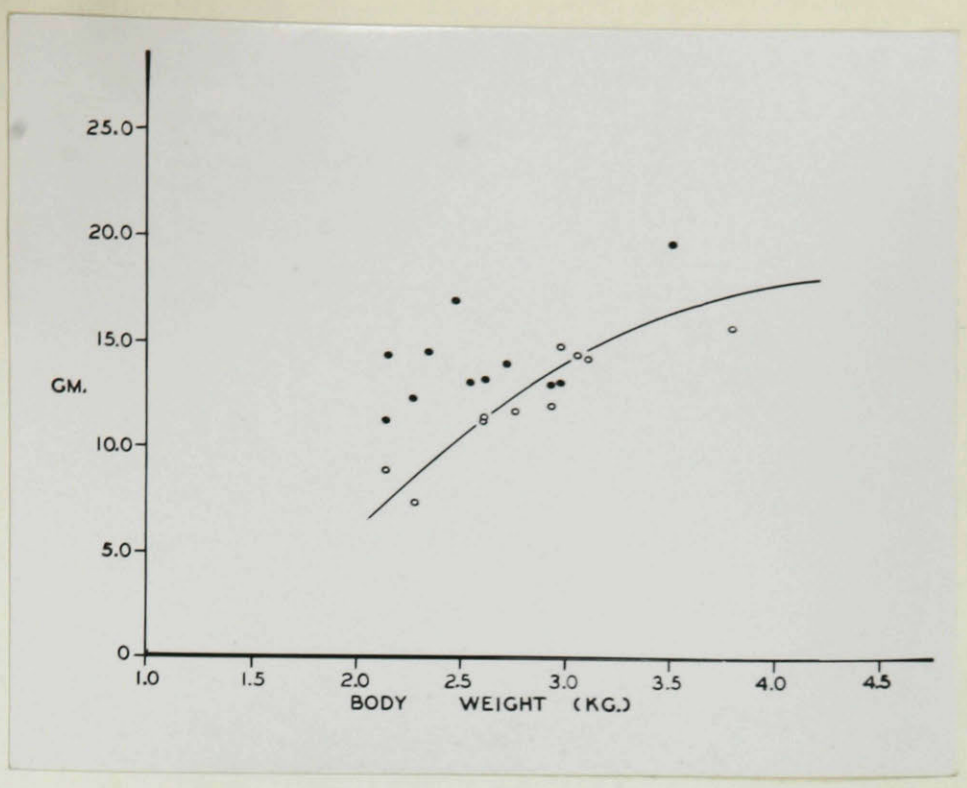
VI. RESULTS.

During the experimental period of sixteen or seventeen days, most animals gained weight. The average unilaterally nephrectomized control animal, kept at room temperature, put on about 440 grams, or about 28 grams per day. Control animals in the cold room gained about 100 grams less in the same period. Weight gain in animals treated with two injections of bovine serum gamma globulin, in both cold and warm rooms (Experiments III and IV), was slightly, but insignificantly, less than that of room temperature controls. Treated animals on restricted food and water intake, kept at room temperature, gained about half as much as control animals on ad libitum intake, but all animals in the group put on some weight. Animals treated with globulin in the cold, while on restricted dietary intake, gained the insignificant amount of about 2.5 grams per day, with some loss of body weight occurring in two of the six animals in this group. In animals of Experiments I, III and IV, there was no apparent relation between amount of weight gain and the severity of nephritis. However, none of the animals of Experiment V, in whom the rate of weight gain was considerably reduced, showed even minimal lesions of nephritis. The data on animal weights for all groups are shown in Table V.

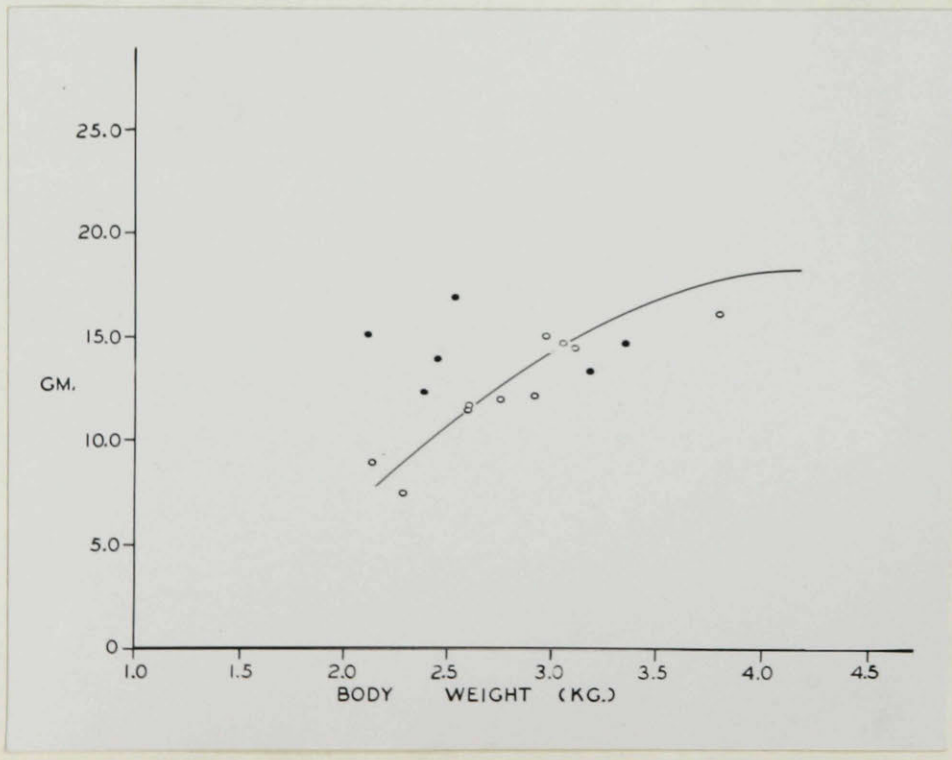
Morphological Studies.

The weights of the remaining kidney, heart, spleen and thymus were plotted graphically against body weights at autopsy for animals of Experiments III, IV and V. Organ weights in control animals generally showed a rough relation to body weight, and disproportionate increases or reductions were readily distinguishable by this method.

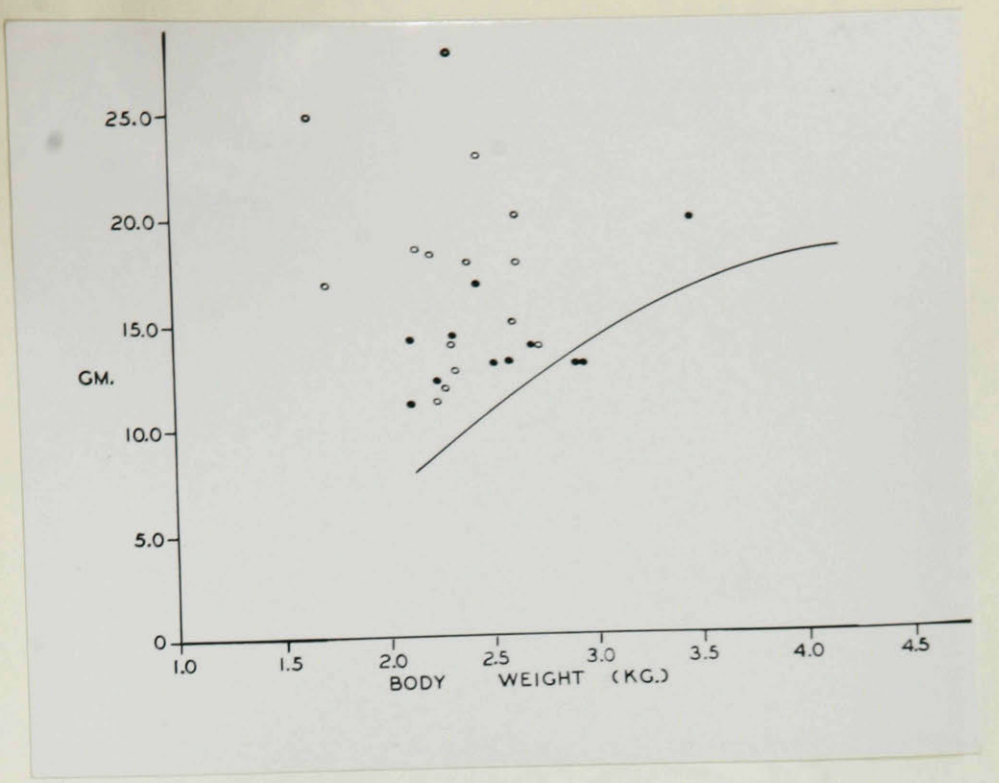
The kidney:body weight relationships in animals of various groups are shown in Text-Figures 1 to 5. Since all animals had been unilaterally nephrectomized at approximately similar periods before autopsy, the weight of the remaining kidney should have borne a more or less constant relation to body weight in the absence of disease. Normal control animals kept at room temperature had kidneys weighing from 7.5 grams in a 2.3 kg. rabbit to 16 grams in a 3.8 kg. animal (Text-figure 1). Nine of eleven untreated animals showed moderate increases in kidney weight after 16 days in the cold room, the most striking instance being a kidney of 17.2 grams in a 2.46 kg. animal (Text-figure 1). Four of six animals given two injections of globulin at room temperature showed moderate increased kidney size. The two animals with kidneys of normal size were the only ones which had no nephritis (Text-figure 2.). There was renal enlargement in all animals treated in the cold, but in only nine of the sixteen animals of the group was this enlargement beyond that found in cold room control animals (Text-figures 3 and 4). The largest kidney encountered in this group was 28.0 grams, in an animal weighing 2.35 kg. On the other hand, treated animals on restricted diet, showed kidneys of normal size, whether treated in the cold or at room temperature (Text-figure 5.).



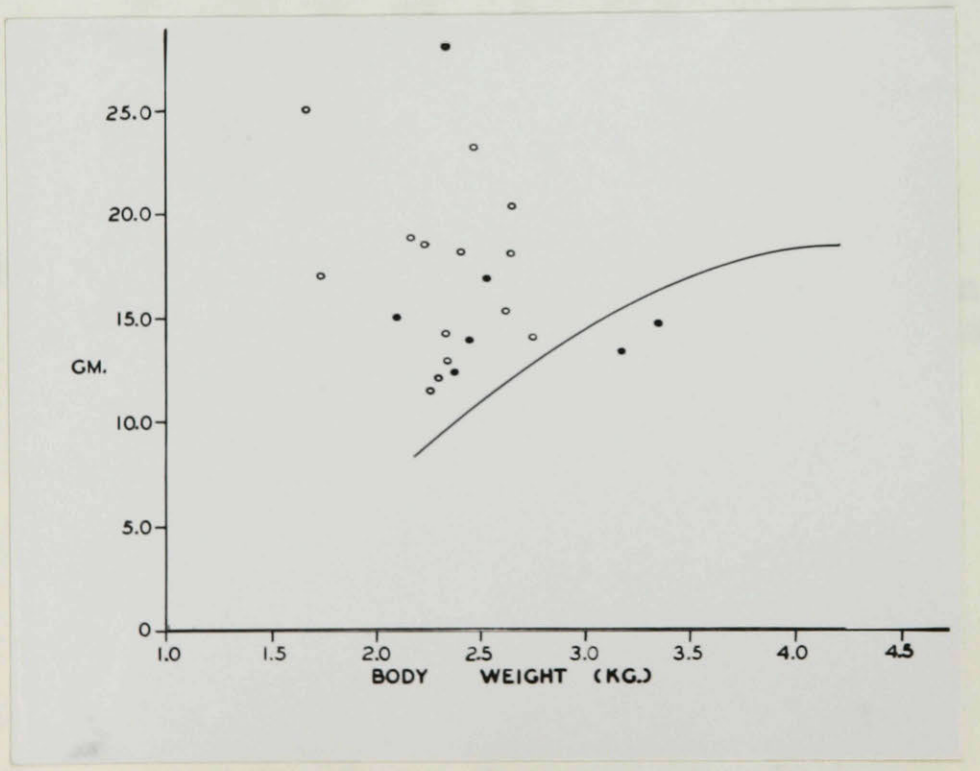
TEXT-FIG. 1. Kidney:body weight relationship in untreated animals kept at room temperature (open points) and in the cold room (solid points). The curve represents the approximate mean kidney:body weight ratio for room temperature untreated animals. (See also Text-figures 2, 3, 4 and 5).



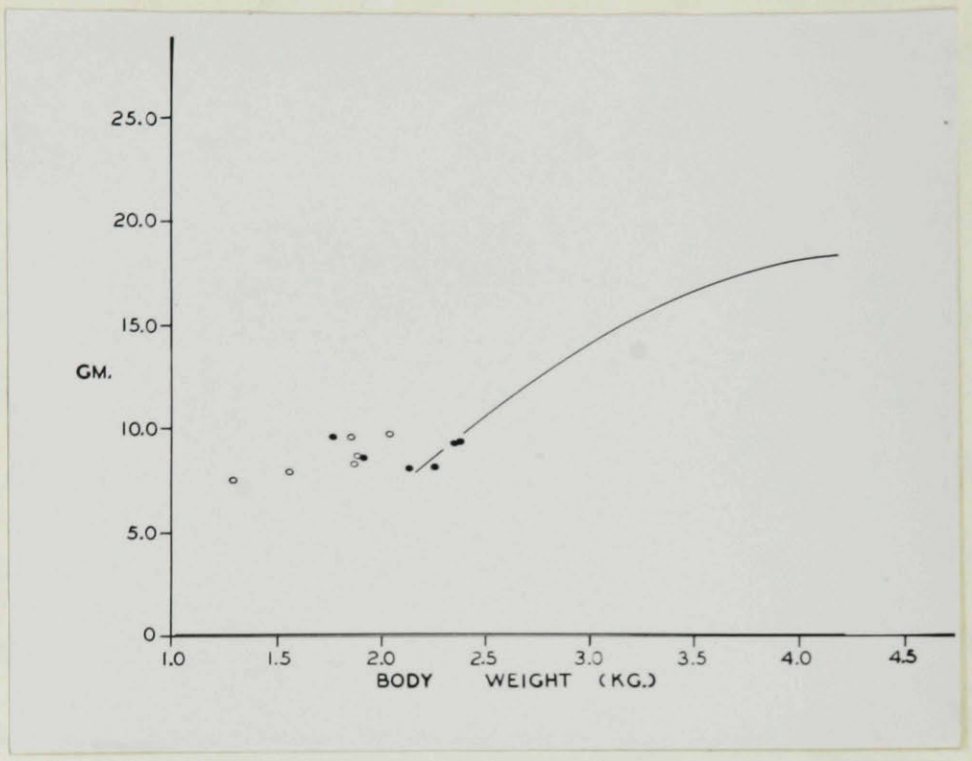
TEXT-FIG. 2. Kidney:body weight relation in room temperature untreated control animals (open points) and room temperature animals given two injections of bovine gamma globulin (solid points).



TEXT-FIG. 3. Kidney:body weight relation in untreated cold room control animals (solid points) and cold room animals given two injections of bovine globulin (open points).



TEXT-FIG. 4. Kidney:body weight relation in cold room globulin treated animals (open points) and room temperature globulin treated animals (solid points).



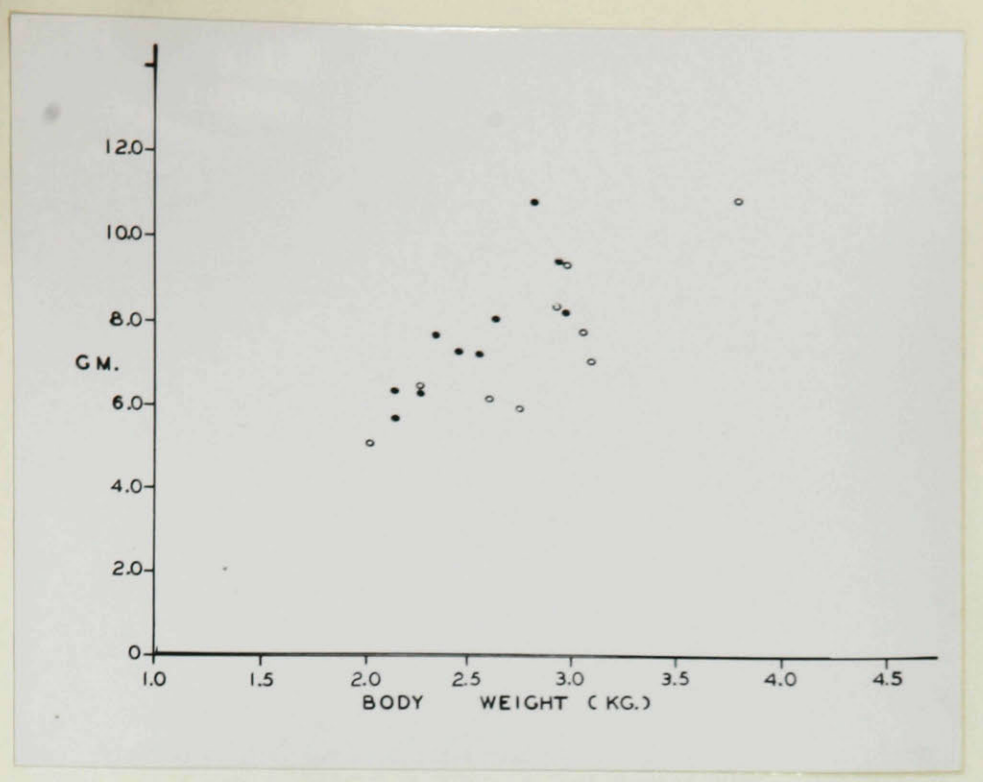
TEXT-FIG. 5. Kidney:body weight relation in cold (open points) and room temperature (solid points) animals given two injections of globulin while on restricted intake of food and water.

The relation between heart and body weights in untreated control animals was less regular than that found with kidneys. This is probably in part referable to the fact that it is more difficult to remove the heart exactly the same way every time, and in part due to the fact that despite efforts to prevent it, blood clot may remain in the heart and alter its apparent weight. Heart weights of control animals ranged from 5.1 grams in a 2.0 kg. rabbit to 11.0 grams in an animal weighing 3.8 kg., with the average cardiac weight for the group being 7.54 grams. Slight cardiac hypertrophy, as shown by increased heart weight, appears to have occurred in untreated animals kept in the cold room (Text-figure 6), with the average heart weight being 8.14 grams. Cardiac enlargement was not apparent in animals treated with two injections of globulin at room temperature (Text-figure 7), but some enlargement is evident in most animals similarly treated in the cold room (Text-figure 8). In animals of Experiment V, injected with globulin while on restricted diet, no alteration of heart weights is apparent (Text-figure 9).

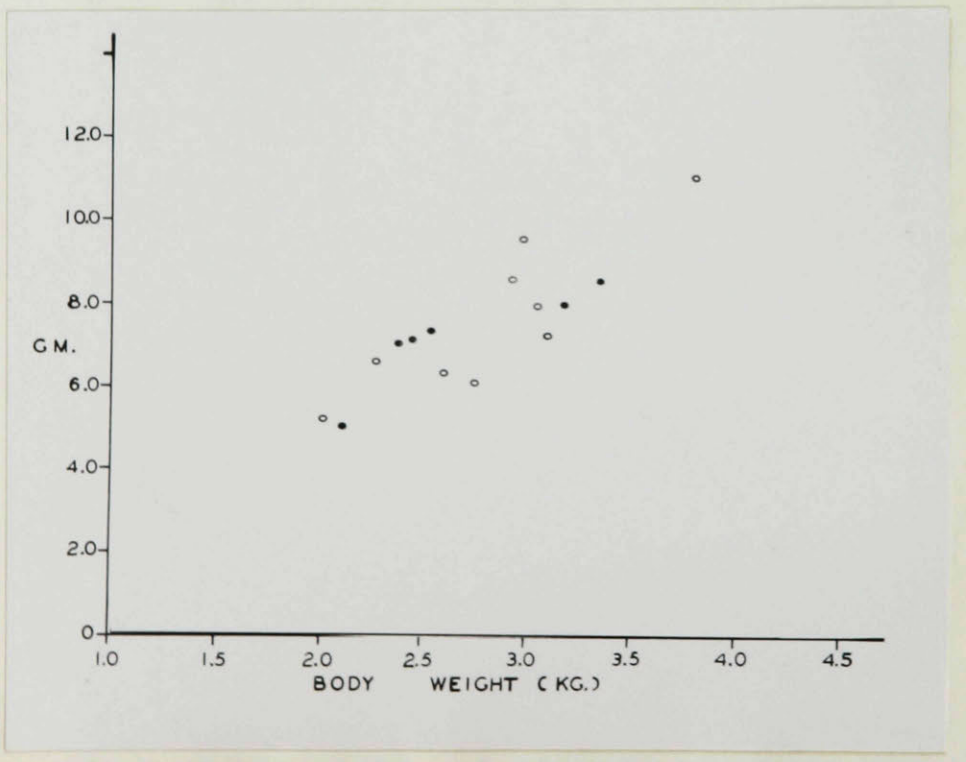
Spleen weights ranged between one and four grams in untreated control animals, and did not appear altered by exposure to cold (Text-figure 10). No increase in splenic weight was evident in globulin treated animals kept at room temperature (Text-figure 11), but comparable animals kept in the cold showed slight increases (Text-figure 12). Spleen weights of animals on restricted diet fell within normal range, whether treated in the cold or at room temperature (Text-figure 13).

Thymus weights showed considerable variation in control animals, averaging 5.63 (3.5 to 7.5) grams, but showed no significant deviation from normal in any of the groups (Text-figures 14 to 17 inclusive).

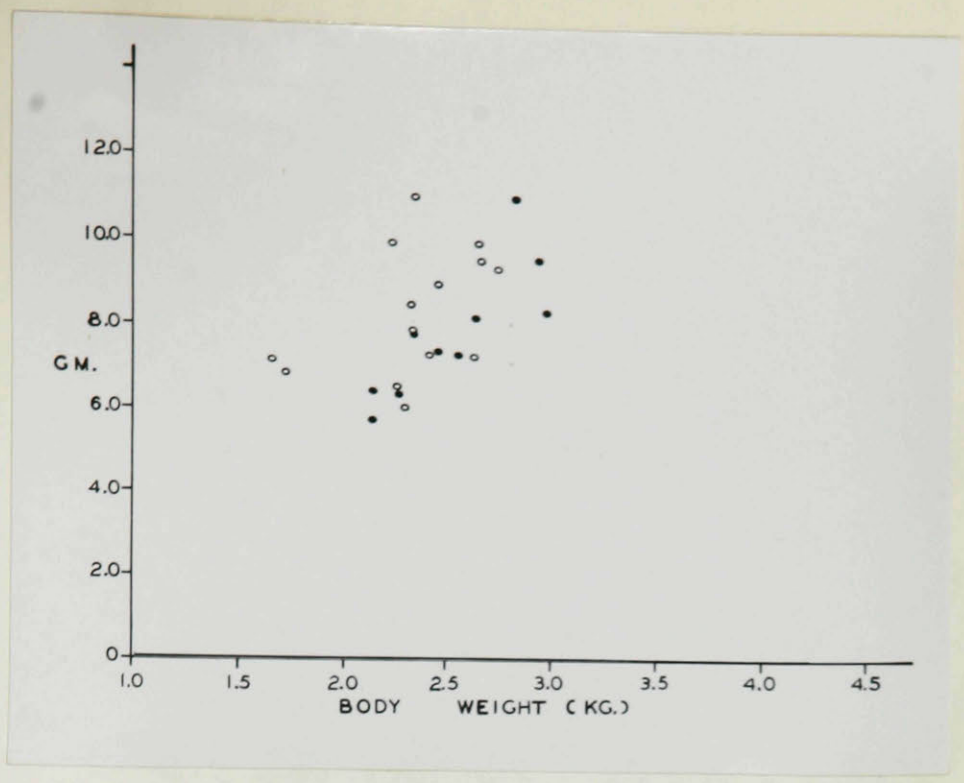
Text continued on page 106.



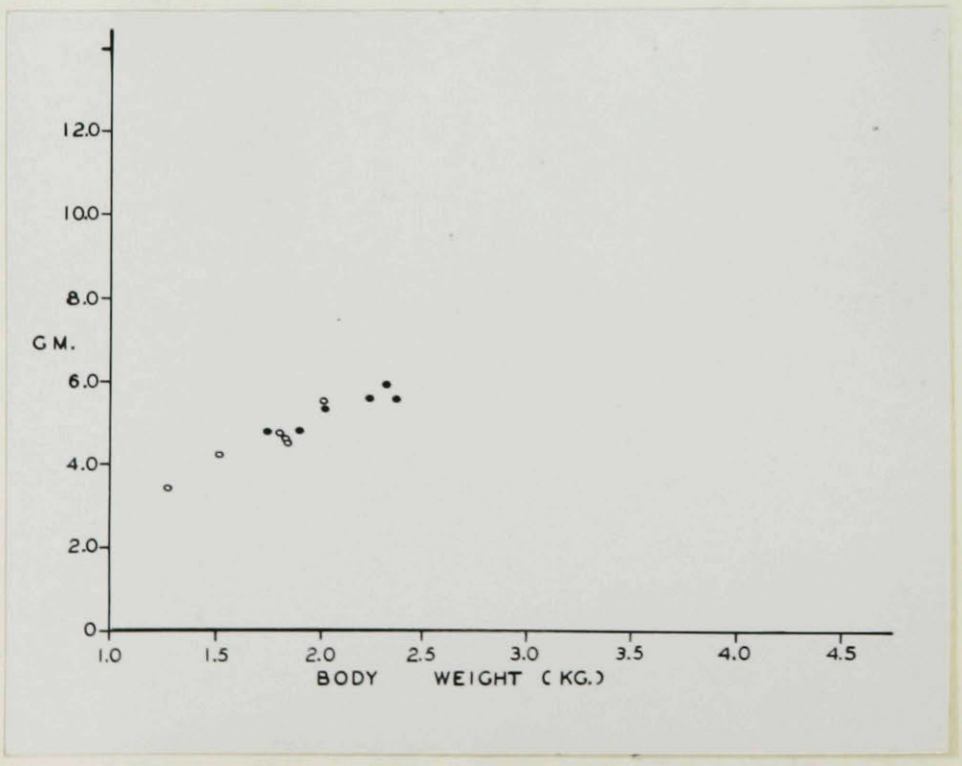
TEXT-FIG. 6. Heart:body weight relation in cold room (solid points) and room temperature (open points) untreated control animals.



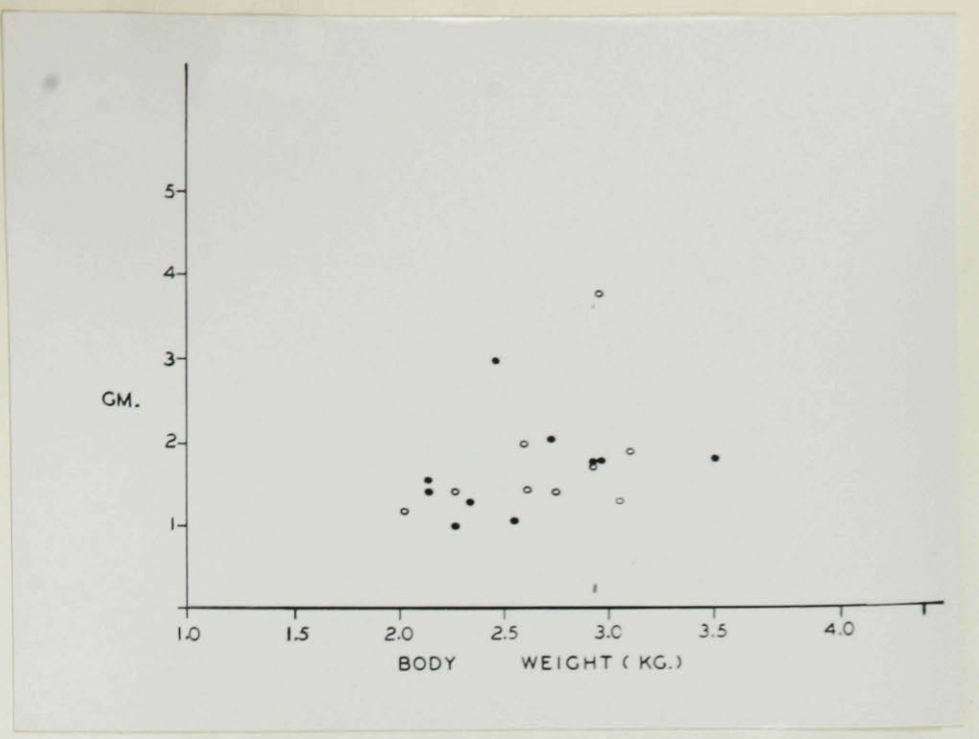
TEXT-FIG. 7. Heart:body weight relation in room temperature control animals (open points) and room temperature globulin treated animals (solid points).



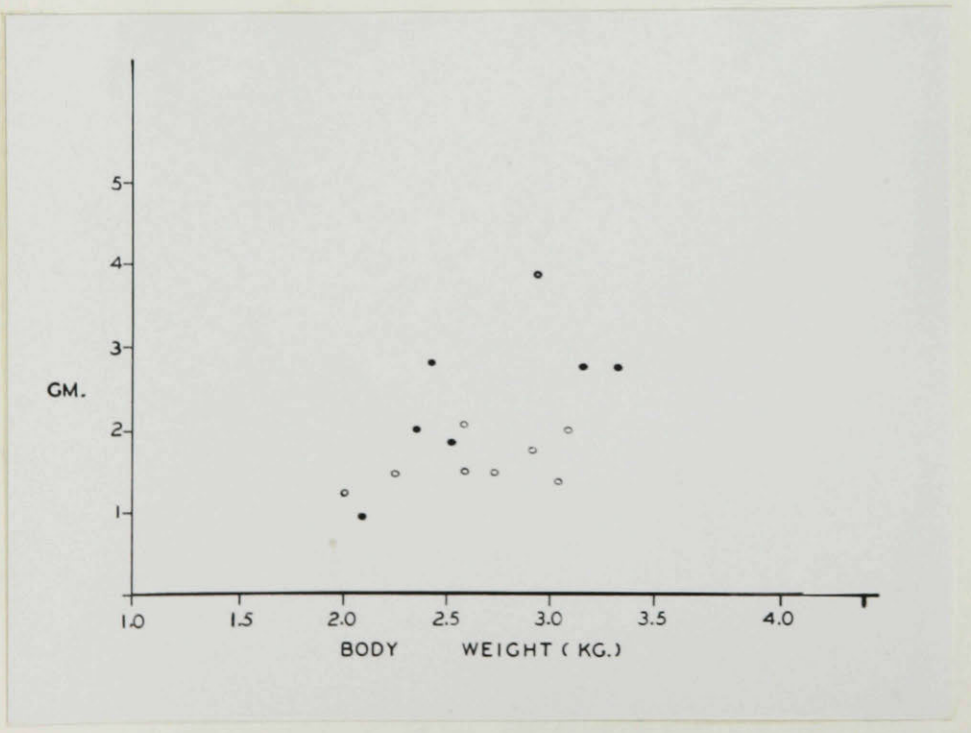
TEXT-FIG. 8. Heart:body weight relation in cold room control animals (open points) and cold room globulin treated animals (solid points).



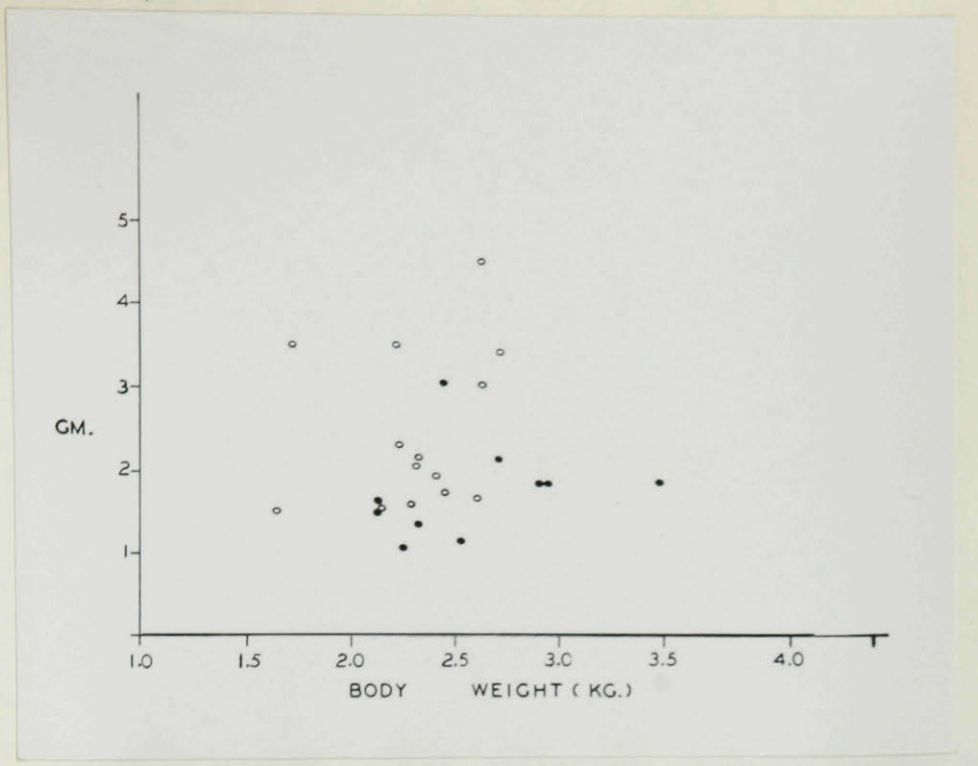
TEXT-FIG. 9. Heart:body weight relation in globulin treated cold room (solid points) and room temperature animals (open points), both on restricted food and water intake.



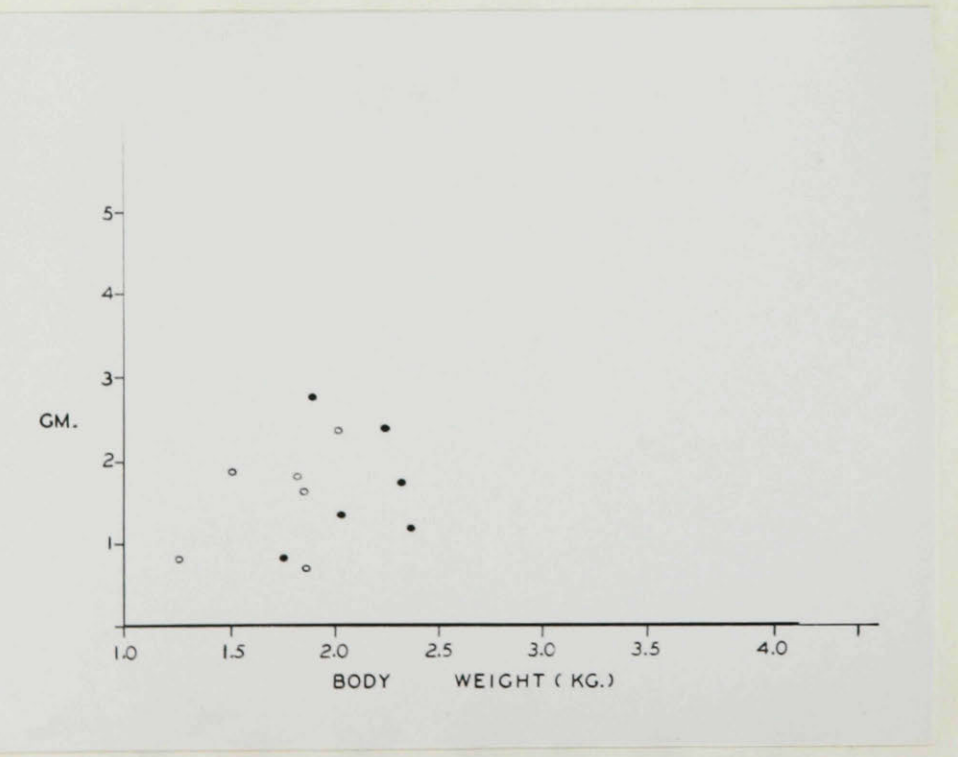
TEXT-FIG. 10. Spleen:body weight relation in room temperature controls (open points) and cold room control animals (solid points).



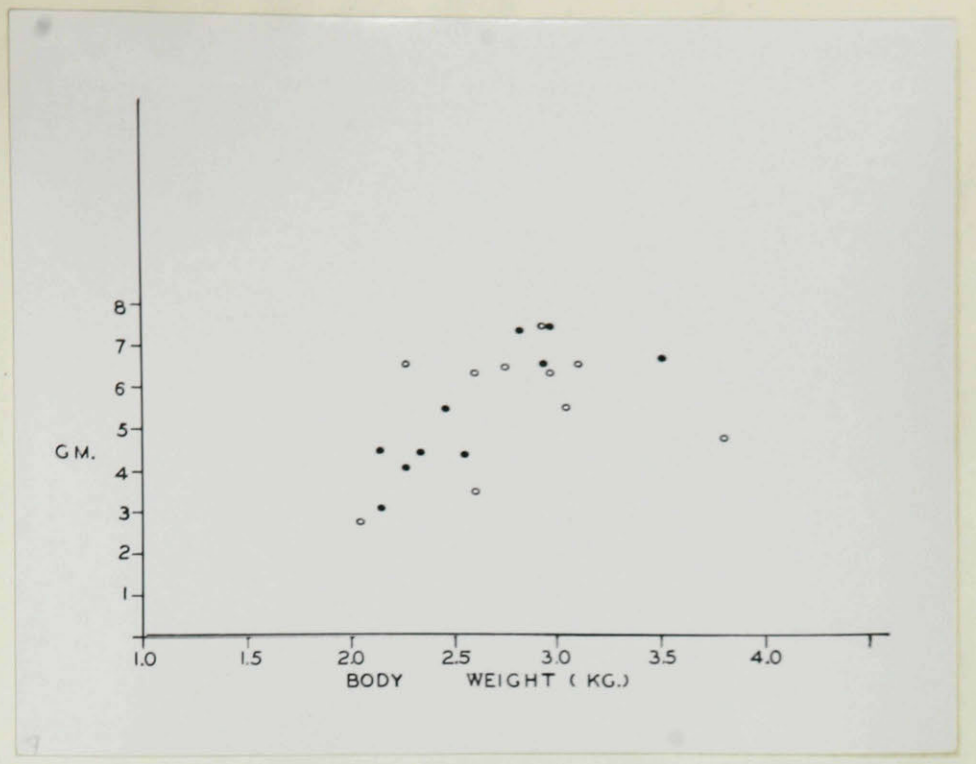
TEXT-FIG. 11. Spleen:body weight relation in room temperature control (open points) and room temperature globulin treated animals (solid points).



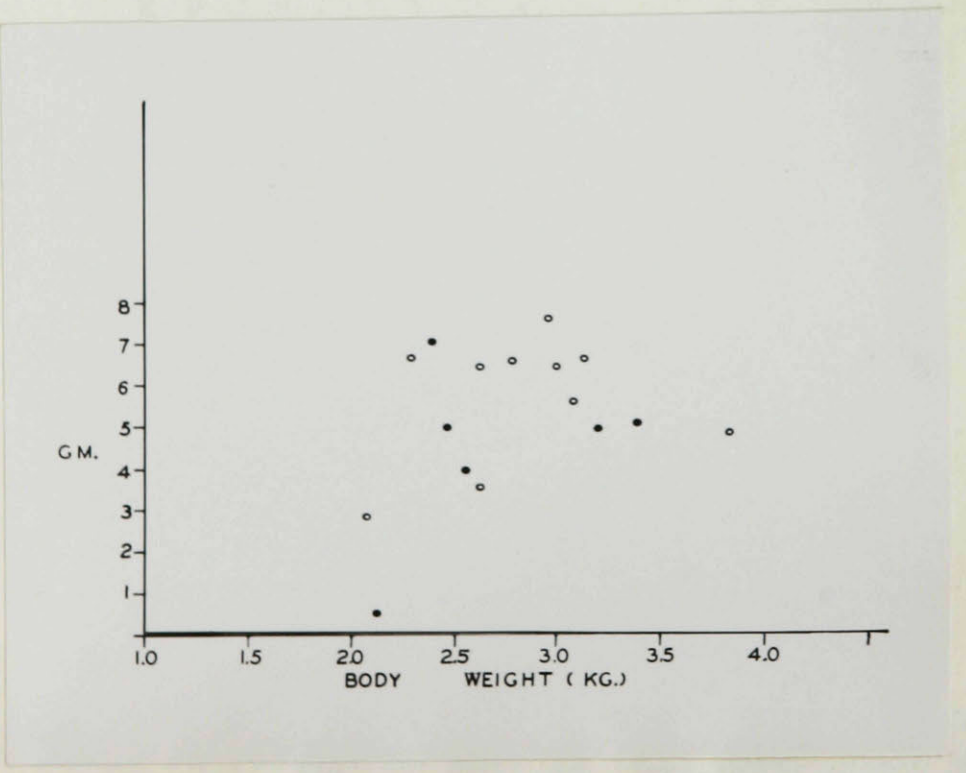
TEXT-FIG. 12. Spleen:body weight relation in cold room control animals (solid points) and cold room globulin treated animals (open points).



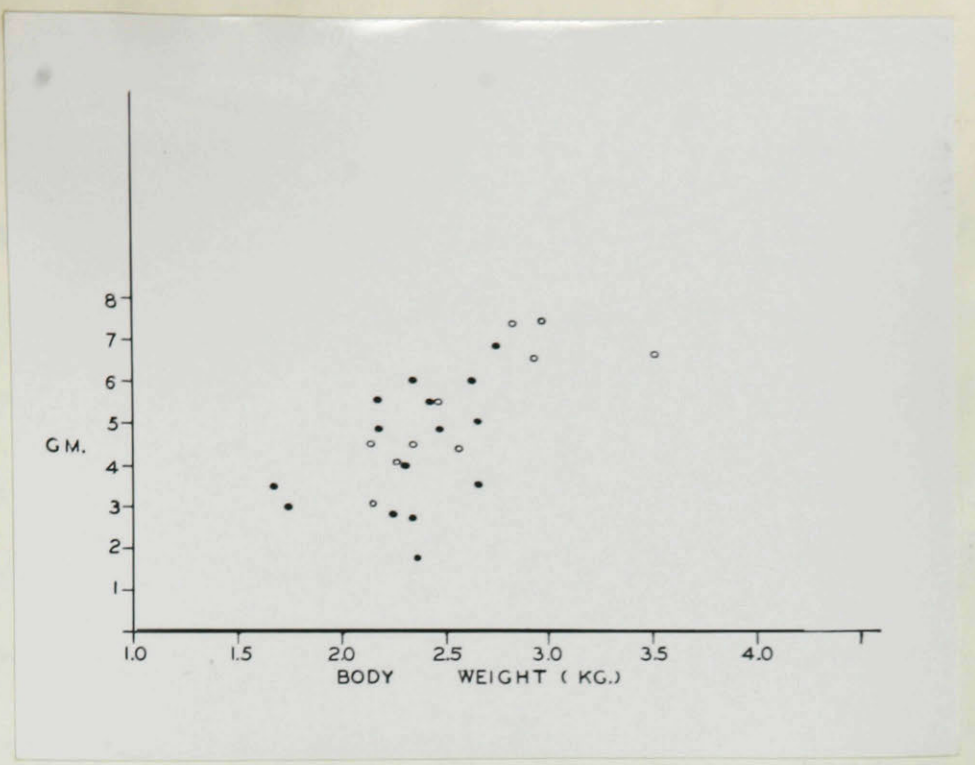
TEXT-FIG. 13. Spleen:body weight relation in globulin treated cold room (open points) and room temperature (solid points) animals, maintained on restricted diet.



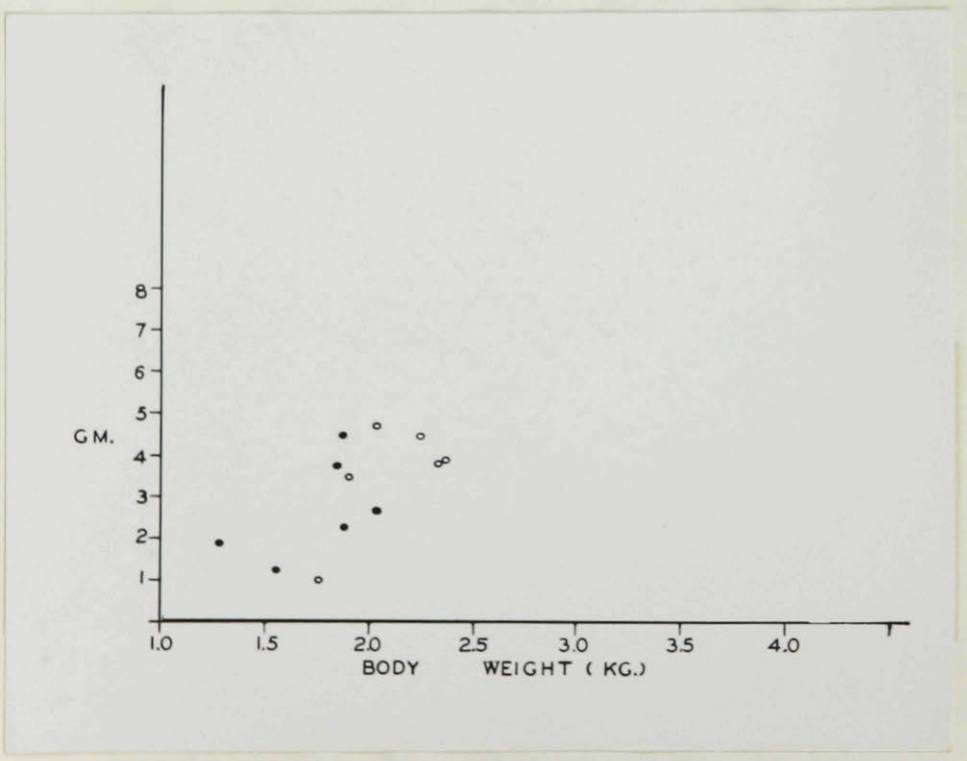
TEXT-FIG. 14. Thymus:body weight relation in room temperature (open points) and cold room (solid points) in control animals.



TEXT-FIG. 15. Thymus:body weight relation in room temperature untreated controls (open points) and room temperature globulin treated animals (solid points).



TEXT-FIG. 16. Thymus:body weight relation in cold room controls (open points) and cold room globulin treated animals (solid points).



TEXT-FIG. 17. Thymus:body weight relation in globulin treated animals on restricted diet in cold room (open points) and at room temperature (solid points).

At autopsy the only striking changes grossly visible were those in the kidney. In most animals of all groups given two injections of globulin, excepting those on restricted diet, the kidneys were yellower and paler than normal (Frontispiece). They were often markedly enlarged. When cut, a distinct cortical pallor was seen. In those kidneys in which the most severe lesions were later found microscopically, it was noted that the fine red peppering of the cortex corresponding to the glomeruli, which is normally visible, was no longer distinguishable. Kidneys from two of these animals (both treated at room temperature), showed fine pitting of their external surfaces.

Microscopically, diffuse glomerulonephritis was found in eighteen of twenty-four (75%) animals given two globulin injections at room temperature (Experiments I and IV), and in all (100%) of sixteen animals treated in the cold room (Experiments II and IV). Diffuse glomerulonephritis did not occur in animals of either group when dietary intake was restricted (Experiment V), nor in any of the control animals. On the basis of histological appearance, the lesions were graded roughly as zero to four plus in severity. The distribution of lesions as to both incidence and severity in animals given two injections of globulin at room temperature and in the cold is shown in Table VI. Since no difference could be noted in the character of the histological alterations in cold room and room temperature animals, the grading criteria are applicable to both.

Table VI.

THE EFFECT OF EXPOSURE TO COLD ON SEVERITY AND INCIDENCE OF ACUTE GLOMERULONEPHRITIS.

Treatment	No. of Animals in Group	Severity*					Incidence	
		0	+	++	+++	++++	% with nephritis.	% with severe nephritis (+++ to ++++)
Bovine Globulin, 2 injections. Rm. Temp.	24	6	4	7	4	3	75 (47 - 93)+	29 (9 - 57)
Bovine Globulin, 2 injections. Cold Room	16	0	2	5	7	2	100	56 (24 - 85)

* See text for grading criteria.

\+ Confidence limits (%) at Probability of 0.005 for number of animals with nephritis in unlimited group similarly treated.

In kidneys showing the mildest degree of damage, there was definite glomerular enlargement, and increase in cellularity. The glomerular tufts were discrete at this stage, but swollen and club-shaped. With basement membrane stains it was possible to distinguish swelling and proliferation of both endothelial and epithelial cells. Even though the basement membrane appeared distended in some instances, endothelial swelling and proliferation had usually resulted in reduction of the capillary lumen. Swelling was particularly prominent in the epithelial cells, the cytoplasm of a single cell sometimes appearing double the normal amount. Polymorphonuclear leucocytes were seen with greater than normal frequency in the capillaries of these glomeruli. Basement membrane changes (other than distention) were usually present, but mild in this group, consisting only of slight nodularity and thickening. Acidophilic protein material was occasionally present in Bowman's spaces and in the tubules, but was inconstant. When found, it was usually of a loose mesh-like texture, but occasionally was homogeneous and deeply staining, resembling thyroid colloid. Deeply acidophilic 'colloid' droplets were sometimes seen in the cytoplasm of the cells lining the proximal convoluted tubules. Casts, when found, occurred in both proximal and distal convoluted tubules, and in the collecting tubules. Changes of this order were graded as +. They occurred in four of 24 animals given two injections of globulin at room temperature, and in two of 16 cold room treated animals. (Fig. 2, compare with normal glomerulus in Fig. 1).

Glomerular changes of the next grade in severity were similar, but of greater intensity than those just referred to. Enlargement of the

glomerular tufts was generally more marked. This appeared to result from more advanced epithelial and endothelial proliferation. In some instances the former was extensive enough to have brought about partial or complete fusion of two or more tufts. Early proliferative changes in the epithelium of Bowman's capsule was often suggested by swelling and thickening of the cells. Protein exudate was seen in the capsular spaces more frequently, and usually was homogeneous and deeply acidophilic. In Mallory-Heidenhain, or periodic acid-Schiff's stains irregular thickening and splitting of the glomerular basement membrane could also be demonstrated. Polymorphonuclear leucocytes were seen in the glomeruli with about the same frequency as in those classed as +. Occlusion of the glomerular capillary lumina by swollen and proliferated endothelial cells was also more marked. In some kidneys in this class, protein casts in the tubules were extremely numerous, whilst in others they were only moderately prominent. Small interstitial infiltrations of lymphocytes, plasma cells and large mononuclear cells were sometimes found in the region of the cortico-medullary junction. These were not prominent however, and some such accumulations had the appearance of being contained within intertubular lymphatic spaces. Kidneys showing lesions of this general severity were graded ++ , and were found in seven of 24 animals given two injections of globulin at room temperature, and in five of 16 animals treated in the cold room (Fig.3.

Lesions classed as severe (+++) were much more marked than the type just described, although of the same general character. In most there was extreme occlusion of glomerular capillaries by endothelial increase,

although in some, a number of patent capillaries could be seen. Fusion of glomerular tufts was present in about half the glomeruli, and more or less marked crescent formation was seen in about the same proportion. Tubular casts were usually prominent, and as a rule, interstitial oedema and cellular infiltrations were more marked. Thickening and shredding of the glomerular basement membrane was usual, and in many instances there was apparent fragmentation of the membrane. Lesions of this type were found in four of 24 animals treated at room temperature, and in seven of 16 animals treated in the cold. (Fig.4). In the kidneys of several animals of this group, where the protein content of the tubules was considerable, excretion of protein material by the tubular epithelial cells appeared to be taking place. Rounded, spherical masses of homogeneous or spongy, deeply acidophilic protein could be seen in the epithelial cytoplasm, bulging it into the lumen. These blobs of protein appeared to be extruded from the cell and discharged into the lumen of the tubule, in which large numbers of such formations were seen (Fig.5). The cells did not appear otherwise abnormal, and did not contain the hyaline droplets common in other tubule cells.

In the most severely damaged kidneys most of the changes alluded to above were found, but in much more marked degree. (Figs. 6, 7, and 8). Proliferation of the epithelium of Bowman's capsule had led to partial or complete encirclement of the glomerulus in most cases, and more or less complete epithelial fusion of glomerulus to capsule was common. Proliferative changes in the glomeruli were usually so marked as to render distinction

of individual tufts impossible. Occasional capillary lumina which could be distinguished were often plugged with acidophilic protein material. Many glomeruli appeared reduced in size by the compression of excessive crescent formation. There was invariably a marked increase in basement membrane material, which appeared as a complex, laminated structure of great irregularity. Alterations of this degree were graded ++++, and were seen in three of 24 room temperature treated animals, and in two of 16 cold room treated animals.

The character of the glomerulonephritis in these experimental animals is similar in all essential respects to that of the human extra-capillary glomerulonephritis of Fahr(96), or Type I nephritis of Ellis(6). The degree of this similarity is readily apparent if the lesions from experimental animals (Fig. 2 to 8) are compared with those of human Type I nephritis (Fig.9), as shown in the publication by Davson and Platt(19).

In the kidney of one animal treated in the cold room (Experiment III) a medium sized artery in the cortico-medullary junction showed acute inflammatory changes through the full depth of its wall. The wall was thickened by oedema, and infiltrated with lymphocytes, plasma cells, and large mononuclear cells. The muscle fibres of the media were granular and partially necrotic. The inflammatory reaction appeared to be of greatest intensity in zone of junction between the media and adventitia (Fig.10).

Changes of a well developed, easily distinguishable diffuse glomerulonephritis were not found in the animals of Experiment II, which were treated with only a single globulin injection, at room temperature, and killed eight days later. Some alterations did occur, however, and may well represent the earliest features of this type of diffuse glomerulonephritis. In general, there was a slight, but definite, glomerular enlargement, as determined by comparison with control kidneys from animals killed at the same time. The glomerular tufts were prominent, and in some instances definitely swollen and club-shaped. Basement membrane stains showed moderate distention of the capillary lumina, most of which were widely patent. A slight degree of swelling of the epithelium covering the glomerular capillaries was sometimes evident, but endothelial swelling could not be clearly distinguished. The glomerular capillaries in several instances, in addition to red blood cells, contained plasmatic masses of protein material. The inflammatory nature of the glomerular changes was suggested by the finding of a six- to ten-fold increase in the numbers of polymorphonuclear leucocytes in the glomeruli. This was estimated by enumerating the leucocytes seen in fifty consecutive glomeruli under high magnification. In control animals killed at the same time, the leucocyte count varied between three and ten per 50 glomeruli, whereas in treated animals the number was between 30 and 60 for every animal in the group. It seemed unlikely that this increase could be accounted for solely on the basis of a generalized leucocytosis, or variation in the thickness of the sections examined. Casts and loose protein coagula were occasionally found in the proximal and distal convoluted tubules. Accumulations of lymphocytes and plasma cells in the

intertubular spaces were found in some instances. The glomerular changes are illustrated in Figure 11.

It should be emphasized that in no instance was a diagnosis of glomerulonephritis made in this study unless the glomerular damage was diffusely distributed, involving all glomeruli to a greater or lesser extent. Focal interstitial nephritis and pyelonephritis were encountered from time to time in both treated and control animals, but usually were easily distinguished from the diffuse lesions described. Where doubt existed, it was considered that if present, glomerulonephritis was of slight enough degree to warrant discard of the diagnosis. Diffuse glomerulonephritis was not seen in any of the control animals, nor in any of the kidneys surgically removed from animals subsequently injected with globulin.

Focal granulomatous lesions of the heart valves and valve rings were found in about half the animals treated with globulin, and have been the subject of a published report(216). The exact incidence and detailed morphology of these lesions in all groups of animals has not been completely studied at the present time, but it can be stated that their occurrence does not appear to have been influenced appreciably by exposing the animals to cold during the period of treatment. In addition, a small proportion of the animals showed lesions of an acute arteritis such as that described, or, or more commonly, a proliferative arteritis, both lesions of the type commonly associated with experimental foreign protein sensitization.

No renal lesion whatever could be distinguished in either of the groups of animals in Experiment V, which were maintained throughout on a reduced dietary intake. A glomerulus from one of these animals is illustrated in Figure 12.

Foot Note: Text continued on page 120.

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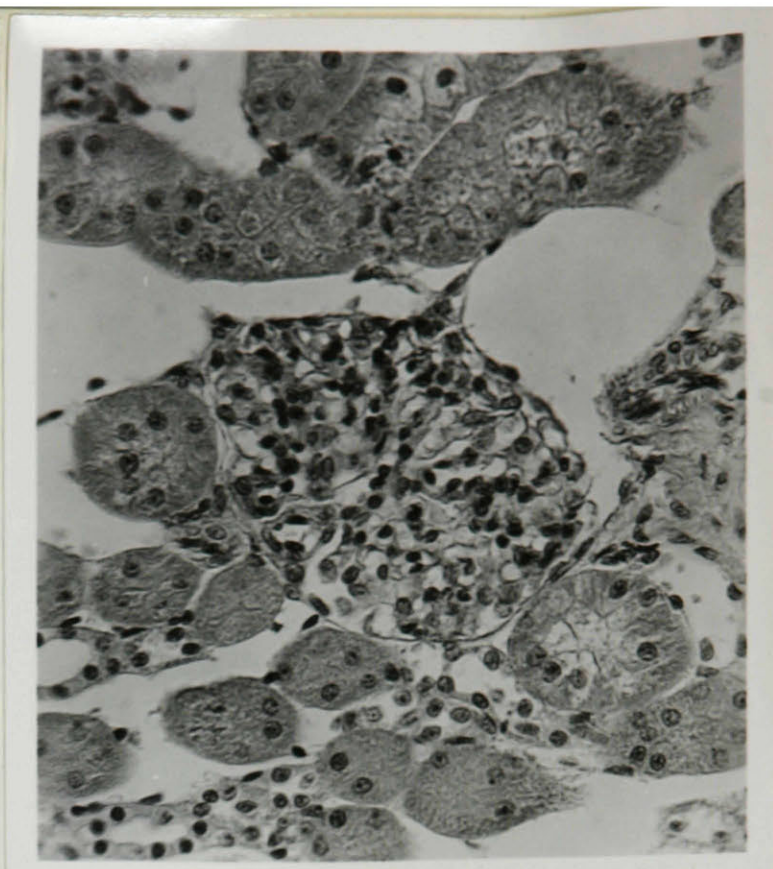
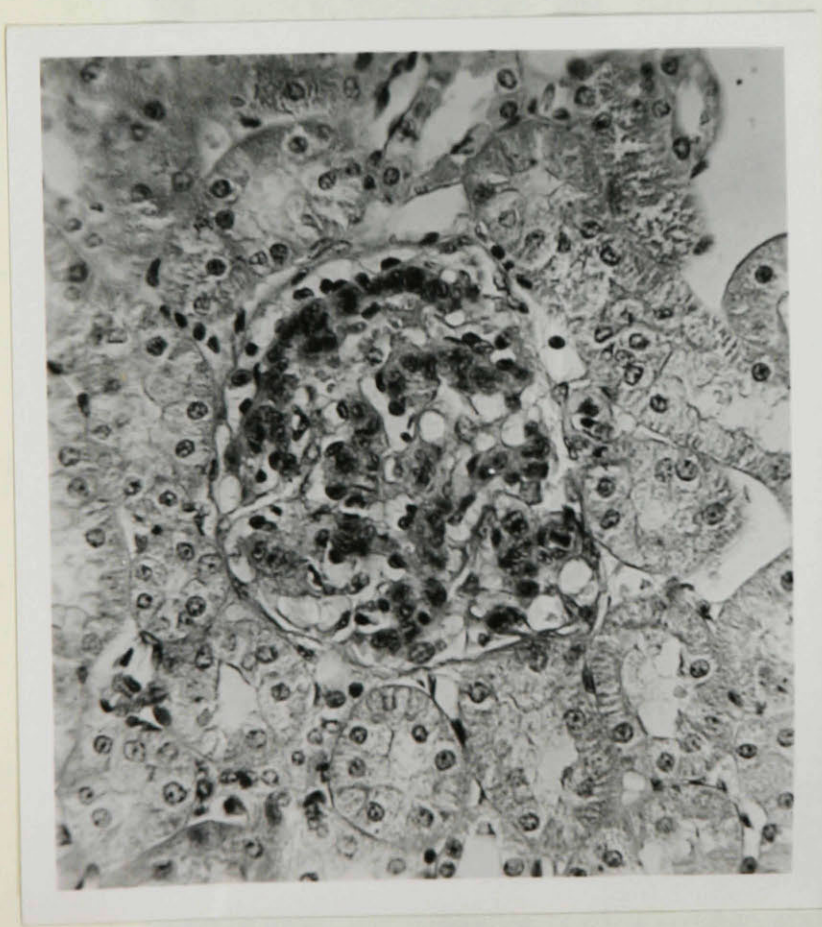


2.

Fig. 1. Normal glomerulus from unilaterally nephrectomized control animal. Glomerular tufts are discrete and capillary loops delicate and patent. Haematoxylin and eosin. X 300.

Fig. 2. Diffuse glomerulonephritis, grade +. There is conspicuous increase in glomerular cellularity and reduction in capillary lumina. The more darkly staining nuclei are endothelial. Haematoxylin and eosin. X 300.



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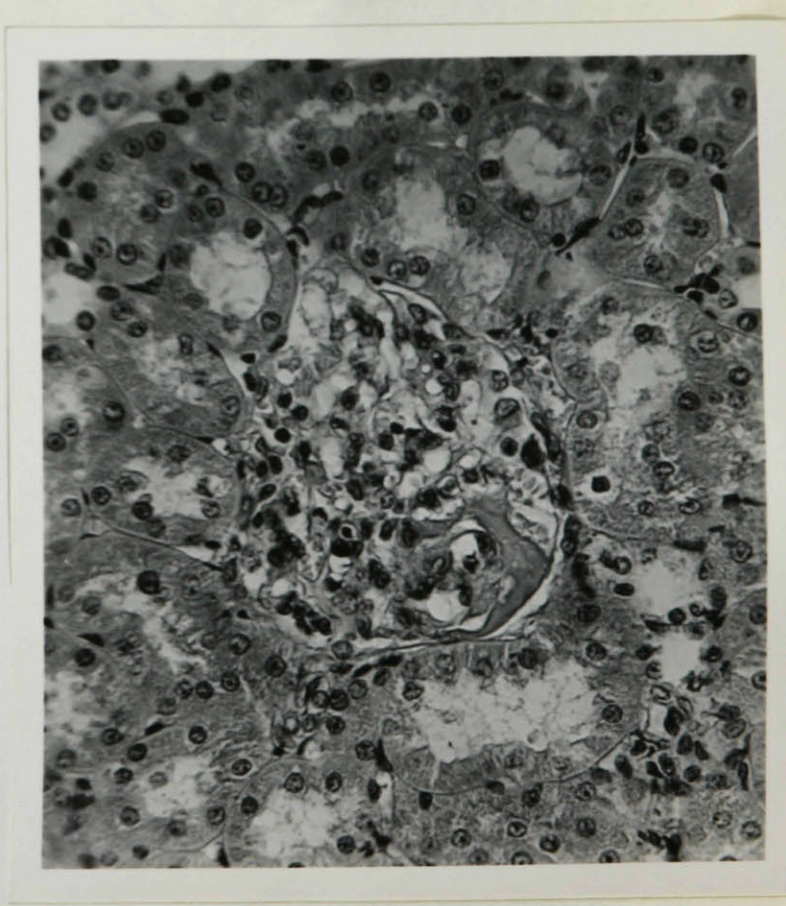


4.

Fig. 3. Diffuse glomerulonephritis, grade ++. More marked reduction in capillarity, with proliferation and swelling of endothelial cells. Thickening of capsular epithelium suggests early crescent formation. Haematoxylin and eosin. X 300.

Fig. 4. Diffuse glomerulonephritis, grade +++. Early crescent formation, hyaline protein exudate in Bowman's space. Haematoxylin and eosin. X 300.

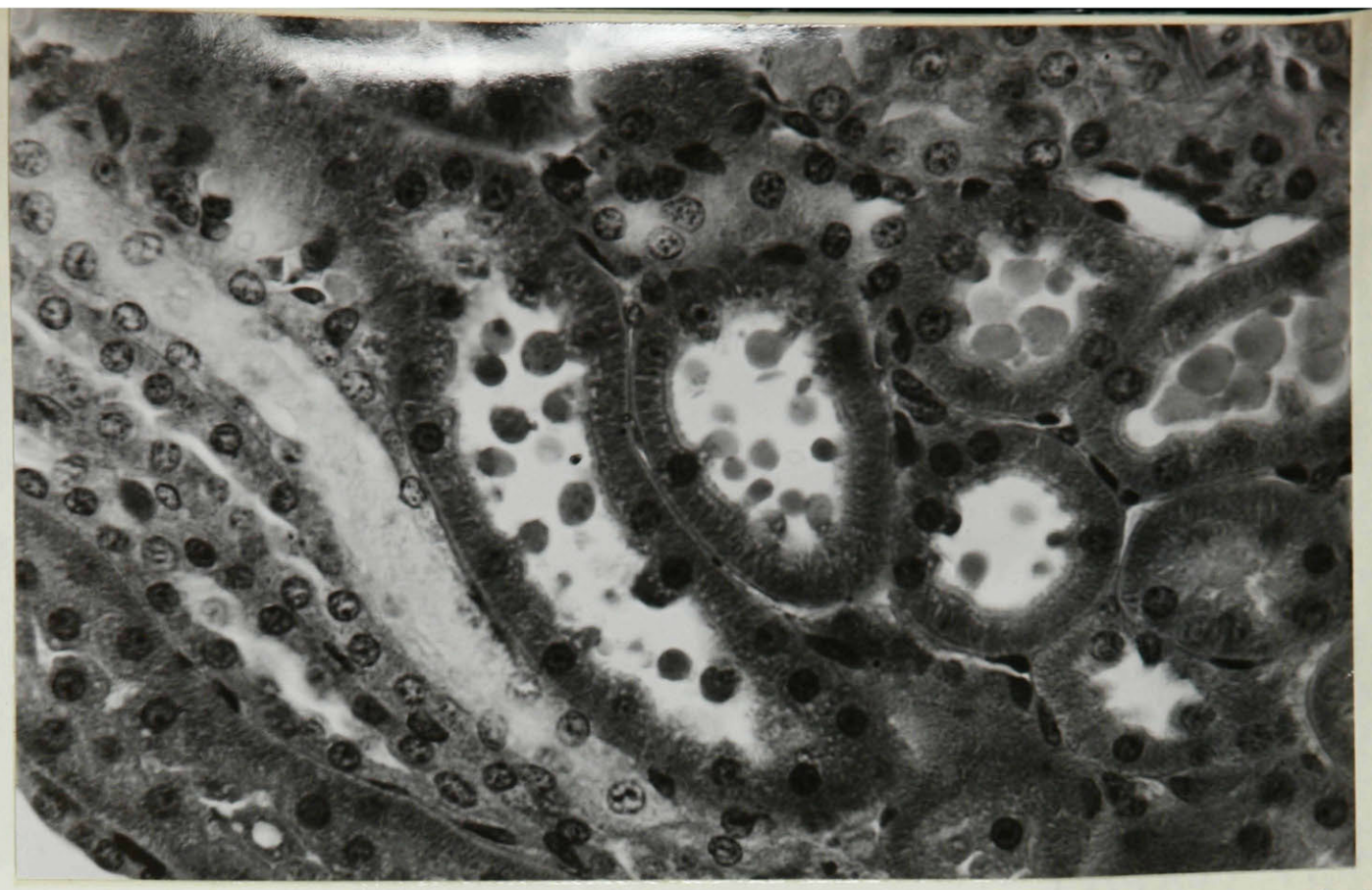


Fig. 5. Protein extrusion by epithelial cells of proximal convoluted tubule. Large blob of homogeneous protein material appears to form in cytoplasm of epithelial cell, then be extruded into lumen. Note otherwise normal appearance of cells. Hemalum, phloxin, saffron. X 550.

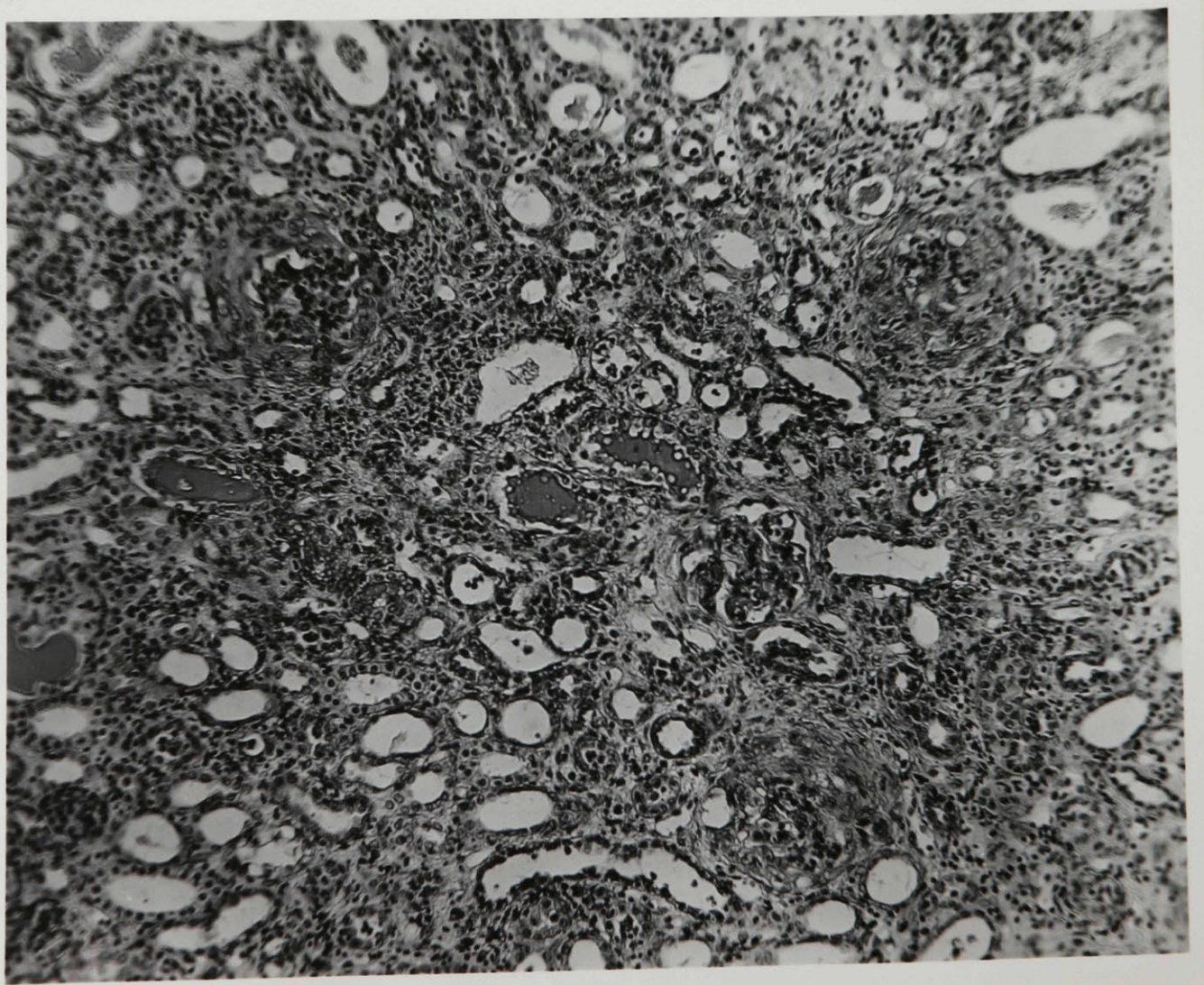
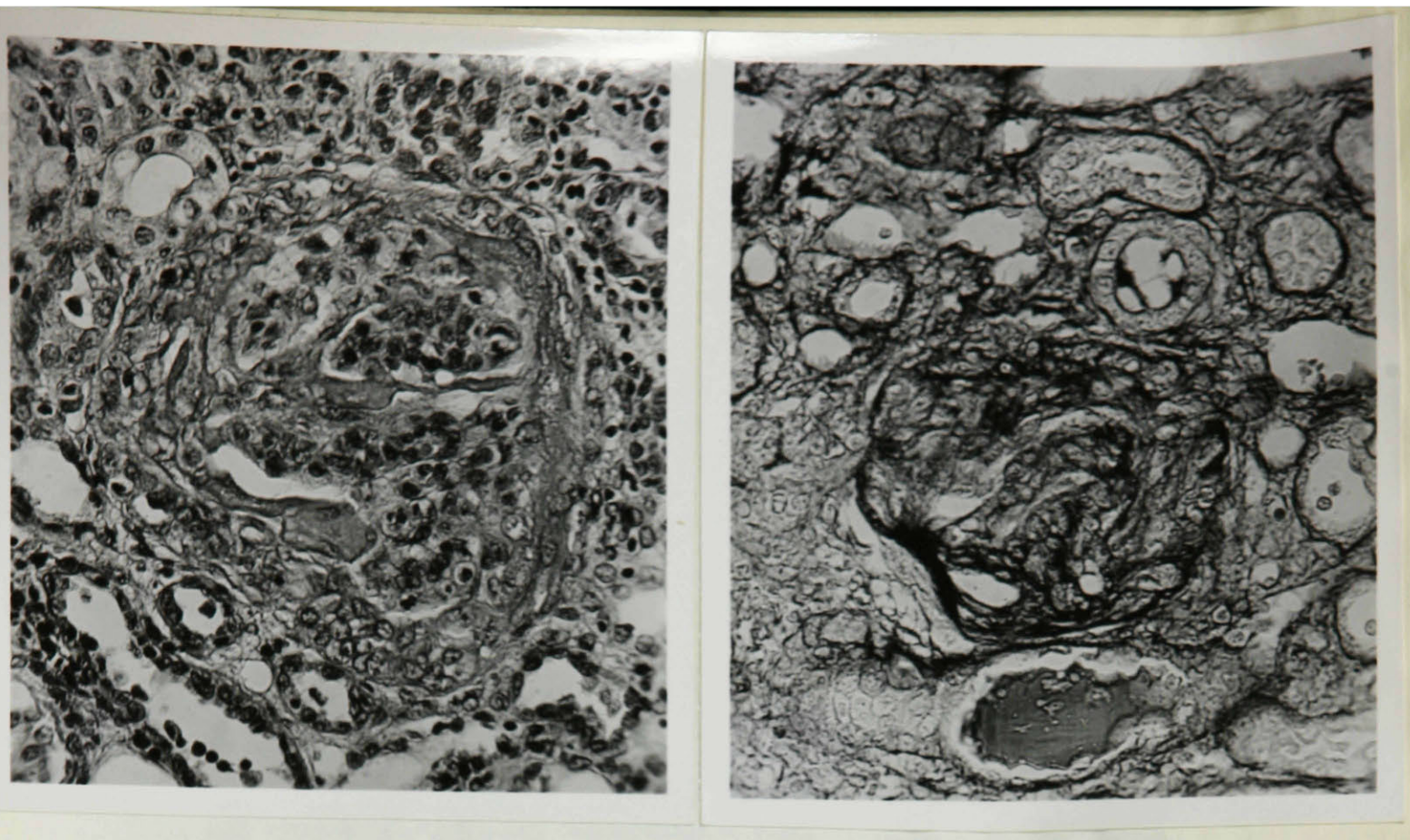


Fig. 6. Diffuse glomerulonephritis, grade ++++ from animal in Experiment I which had uraemia. Note diffuse distribution of lesions. In addition to glomerular changes, there is diffuse increase of interstitial connective tissue with cellular infiltration. Note casts and tubular atrophy. Haematoxylin and eosin. X 137.



7. 8.

Fig. 7. Diffuse glomerulonephritis, grade ++++; glomerulus from same kidney as shown in Fig.6. Marked cellular proliferation, forming almost complete synnechia between glomerulus and capsule, compressing remnants of tuft at center. Haematoxylin and eosin. X 300.

Fig. 8. Diffuse glomerulonephritis, grade ++++; same kidney as Figs. 6 and 7. Irregular shredding and thickening of glomerular and capsular basement membranes. Mallory-Heidenhain. X 300.

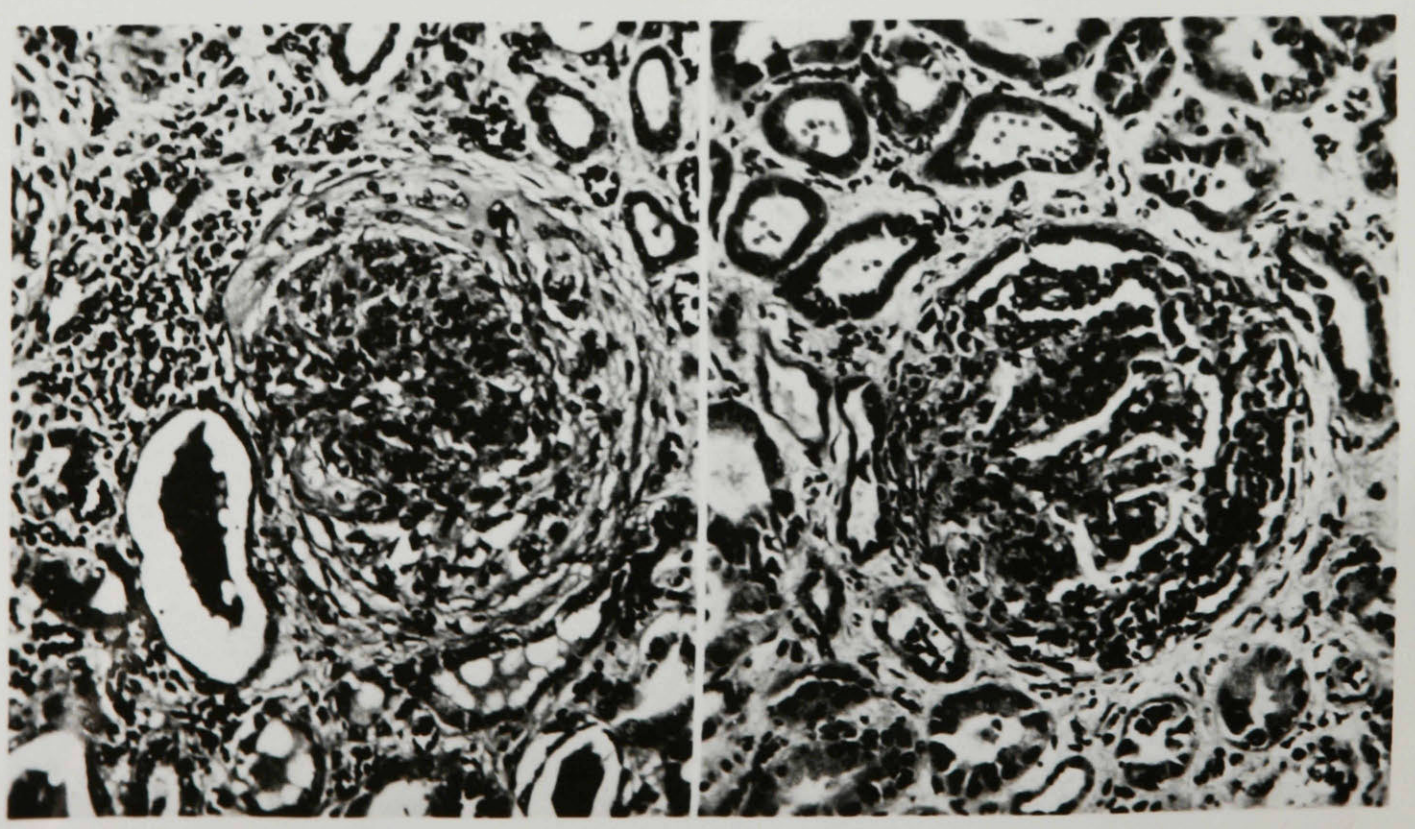


Fig. 9. Human nephritis, Type I (after Davson and Platt). Note similarity to experimental lesions in previous figures. There is swelling and clubbing of tufts, crescent formation, and, in glomerulus at right, complete fusion of glomerulus and capsule.

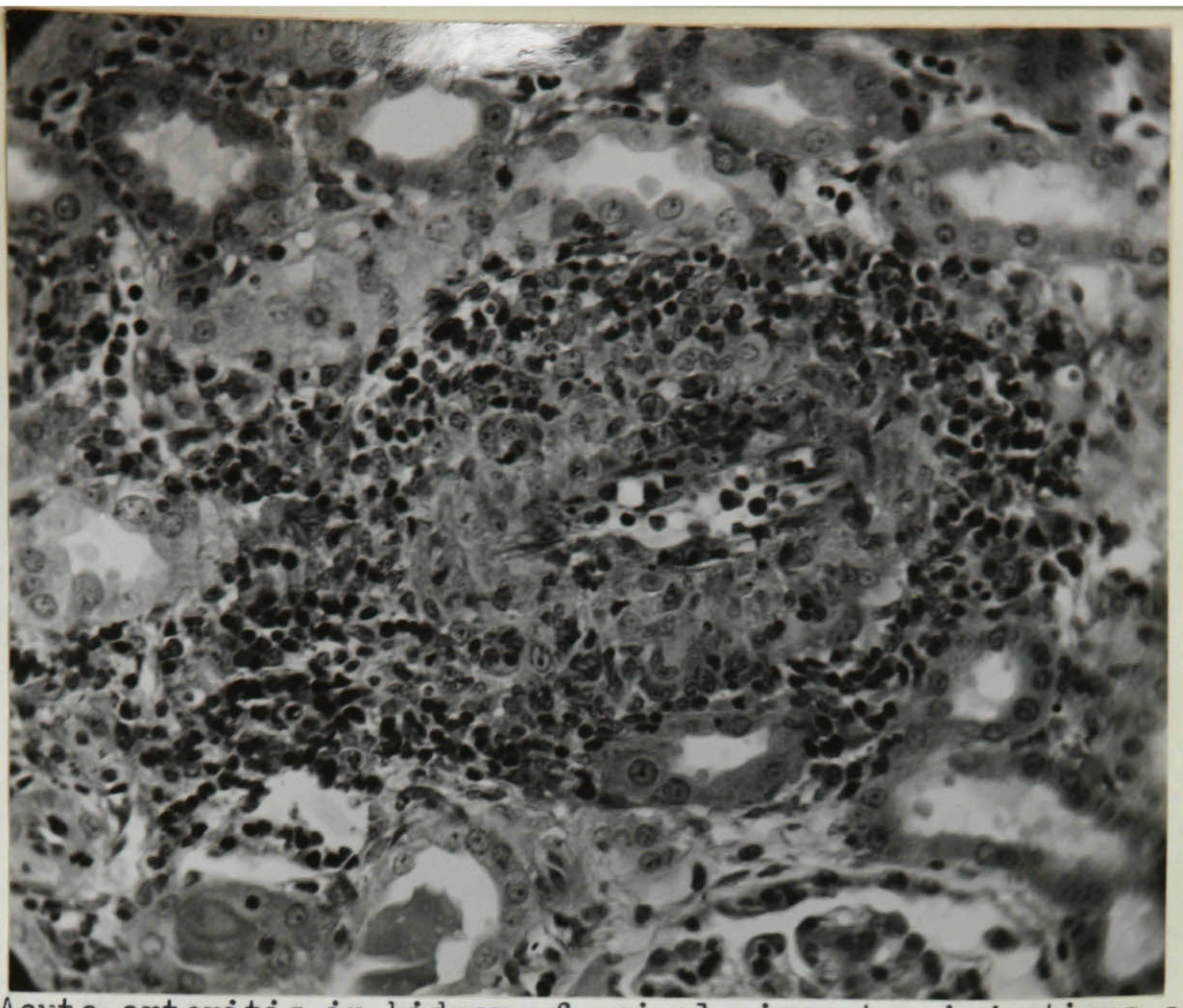


Fig. 10. Acute arteritis in kidney of animal given two injections of bovine gamma globulin while in cold environment. Note disruption of entire thickness of wall, lymphoid and plasma cell infiltration, which is most marked in zone of junction of media and adventitia. Hemalum, phloxin, saffron. X 330.

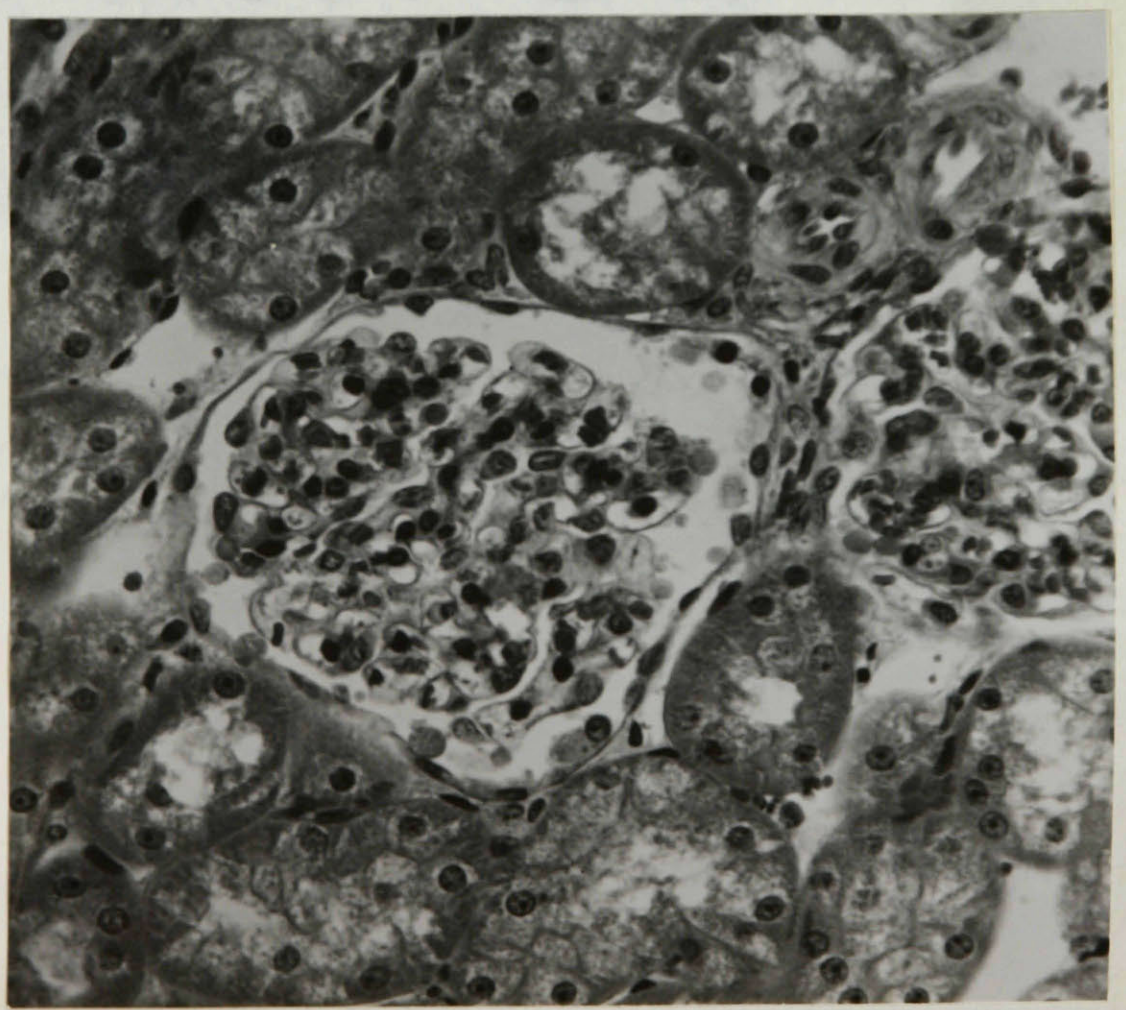


Fig. 11. Glomerulus from animal killed 8 days after single injection of bovine gamma globulin. Note capillary patency and increase in cytoplasm. Some loose protein in Bowman's space. Hemalum, phloxin and saffron. X 410.

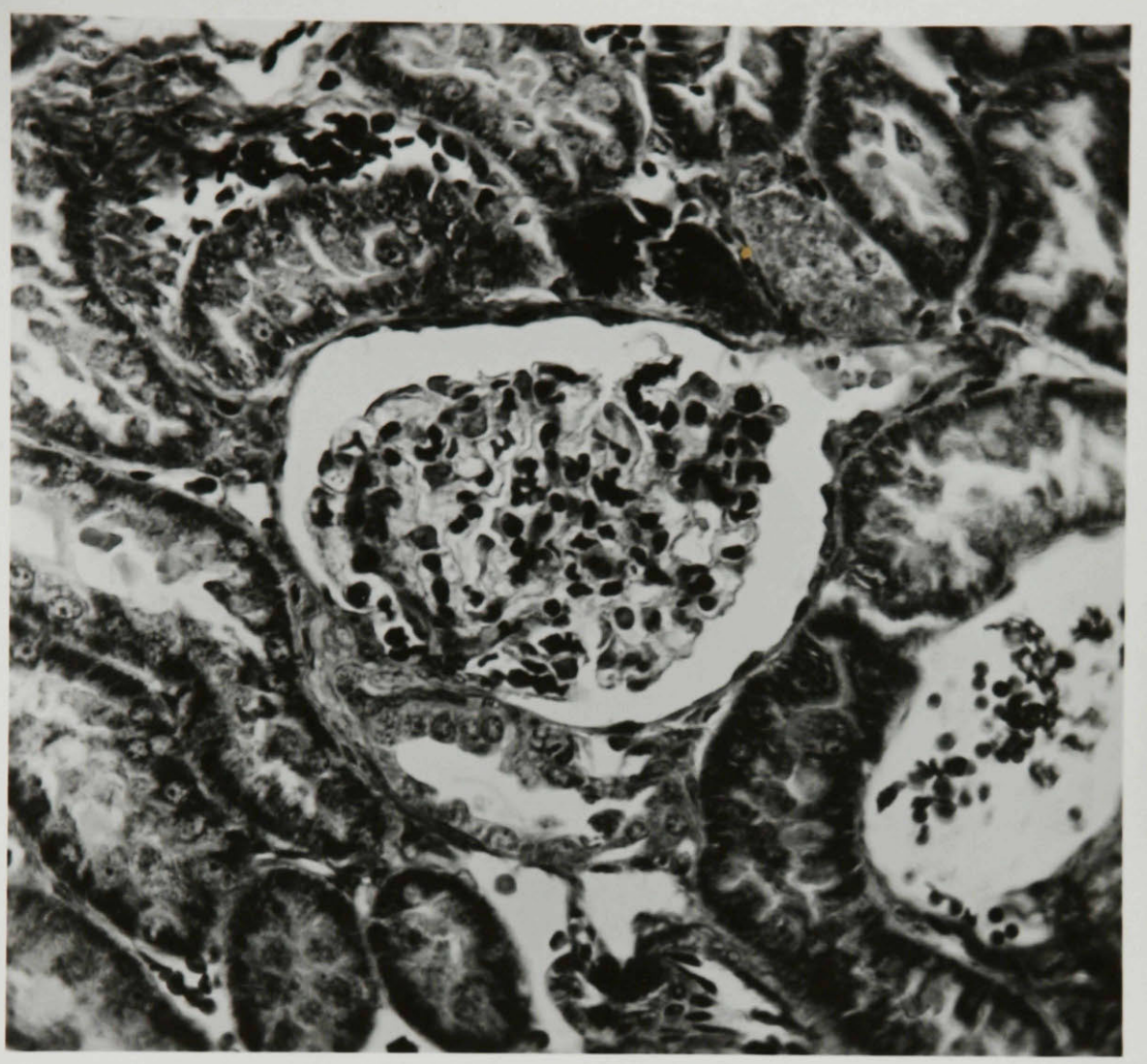


Fig. 12. Glomerulus from animal given two injections of bovine gamma globulin while on restricted diet in cold room (Experiment V). This completely normal appearance was found in all animals on restricted diet, Compare with normal glomerulus in Fig. 1. Masson's trichrome stain. X 410.

Urinary Findings.

Urinalyses were not carried out in Experiment I. In Experiment II and in Group A of Experiment III, measurement of urinary protein was done by boiling only and recording in the conventional zero to four plus grading, which does not lend itself well to averaging. However, since the findings in Group A of Experiment III corresponded closely with those of other groups of animals treated in the cold room, the findings in the remaining eleven animals can be taken as representative of the group as a whole.

The animals of Experiment II, given a single injection of globulin at room temperature and killed eight days later, showed considerable variation in urinary output, as did other animals treated at room temperature. All five animals in the treated group showed albuminuria at some time between the fifth and eighth day, but this did not exceed ++. Occasional red blood cells were found in urines of some of these animals, but gross haematuria did not occur. Granular and hyaline casts were also irregularly seen.

Average urinary output and urinary protein in control and treated animals are shown in Table VII (page 124) (animals of Experiment IV). In untreated control animals the 24 hour quantity of urine varied considerable from day to day, and from animal to animal, ranging from 75 c.c. to 222 c.c. in the average figures, with the mean daily output throughout the period being 164 c.c. A small amount of protein was frequently present in the urine of control animals, the highest level encountered was 60 mg./100 c.c. All animals in the control group showed some urinary protein at one time or

another. Red blood cells and casts were not found in urine of control animals. The quantity of urine produced by treated animals kept at room temperature did not differ significantly from that of control animals, and small amounts of protein were excreted by these animals before treatment, and for the first seven days after the first globulin injection. Massive proteinuria was found in four of these six animals on the eighth, ninth and tenth days after the first globulin injection. The heaviest concentration of urinary protein being 3.2 gm./100 c.c. Following the second injection significant, but considerably smaller, amounts of protein were excreted by these same animals. There was rough correspondence between the amount of protein excreted during treatment and the severity of the lesions in the kidney at autopsy. The urine of almost all animals in all groups had a strongly alkaline reaction, with the result that when casts were present, they were swollen and difficult to distinguish microscopically. However, in one animal of this group whose urine one day was acid, the urine was loaded with clearly visible hyaline and waxy casts. The four animals in the group who developed nephritis showed between 5 and 10 r.b.c. per high power field on one or more occasions between the seventh and tenth days.

Urinary quantities and protein concentrations for control animals and groups treated with two injections of bovine globulin in the cold room are shown in Table VIII (page 125) (animals from Experiments III-B and IV). The quantities of urine produced by animals in the cold room tended to be much more constant than in room temperature animals, and somewhat less. The average daily output for control animals was 123 c.c., within a range

of 87 to 174 c.c. Control animals in the cold showed similar slight amounts of protein in the urine to that seen in room temperature control animals, with perhaps a slight increase between the ninth and thirteenth days. Globulin treated animals kept in the cold showed some reduction in daily urine output, but there was no significant oliguria which could be related to symptoms of nephritis. As with animals treated at room temperature, slight proteinuria occurred up to the eighth day after globulin injection, with conspicuous and massive proteinuria between the eighth and tenth days. Urinary protein excretion fell for three days after the second globulin injection, but for the last three days of the experiment, massive proteinuria again occurred. Proteinuria between the eighth and tenth days is less in the average figures for these animals, than between the fourteenth and sixteenth days. This is due to the fact that not all cold room animals showed proteinuria between the eighth and tenth days, whereas every cold room treated animal was excreting considerable amounts of protein during the last three days of the experiment. Casts and red blood cells occurred in the cold room treated animals, and were usually seen between the eighth and tenth days. Two animals in this group exhibited gross haematuria during this period.

The urinary findings in animals of Experiment V are shown in Table IX (page 126). Conspicuous reduction in urine output throughout the experimental period is apparent in both cold and at room temperature groups. It is also seen that urine output tended to be much more constant. Occasional very faint traces of protein were found in the urines of the animals treated at room temperature, but proteinuria was completely and consistently lacking

in the cold room group. These findings corresponded with the normal gross and histological appearances of the kidneys of these animals at autopsy.

In summary then, it may be said that in animals on ad libitum diet, given two injections of bovine gamma globulin, urinary changes of acute nephritis (proteinuria, cylindruria and haematuria) first appeared between seven and ten days after the initial injection of globulin. All animals treated at room temperature, and subsequently found to have nephritis, showed maximal urinary changes at this time. In animals treated in a cold environment, a secondary, and more marked rise in urinary protein output appeared between three and six days after the second injection of globulin, a phenomenon which did not occur in animals treated at room temperature. Furthermore, animals treated in the cold did not all develop urinary symptoms after the first injection, but these were consistently present in all animals after the second injection. Finally, restriction of food and water intake completely inhibited the urinary changes of acute nephritis in all animals treated in the cold, and at room temperature.

TABLE VII.

AVERAGE URINARY OUTPUT AND URINARY PROTEIN

ROOM TEMPERATURE ANIMALS.

Day	6 animals Rm. Temp. Controls		6 animals Rm. Temp., 2 injections Globulin	
	Quantity (c.c.)	Protein (mg/100 c.c.)	Quantity (c.c.)	Protein (mg/100 c.c.)
-7	108	2	140	0
-6	125	0	154	0
-5	116	0	161	0
-4	116	0	161	0
-3	94	5	140	7
-2	180	2	193	0
-1	201	2	153	0
0	187	9	154	2
	- Saline Injection -		- Globulin Injection -	
1	139	10	125	11
2	145	2	144	4
3	151	8	177	5
4	184	4	195	6
5	209	1	165	1
6	222	4	193	2
7	214	2	204	1
8	160	3	139	588
9	160	3	139	590
10	75	1	67	793
			- Globulin Injection -	
11	156	14	280	89
12	215	12	203	18
13	218	10	244	10
14	218	10	244	10
15	177	5	160	52
16	177	5	160	40

TABLE VIII.

AVERAGE URINARY OUTPUT AND URINARY PROTEIN

COLD ROOM ANIMALS.

Day	11 animals Cold Controls		11 animals Cold Rm., 2 injections Globulin	
	Quantity (c.c.)	Protein (mg/100 c.c.)	Quantity (c.c.)	Protein (mg/100 c.c.)
-7	118	17	189	1
-6	92	6	119	2
-5	92	6	119	3
-4	123	1	188	11
		- Placed in Cold Room -		
-3	101	2	156	16
-2	103	9	134	8
-1	87	9	137	0
0	104	4	139	0
	- Saline Injection -		- Globulin Injection -	
1	112	0	129	0
2	106	2	119	6
3	117	8	140	3
4	129	3	150	2
5	130	10	120	6
6	120	7	123	8
7	122	7	113	18
8	124	6	129	285
9	125	30	136	276
10	139	22	163	100
			- Globulin Injection -	
11	122	18	138	100
12	127	14	149	40
13	138	14	138	30
14	142	8	105	407
15	152	5	111	331
16	174	0	153	429

TABLE IX.

URINE AND URINE-PROTEIN OUTPUT OF ANIMALS GIVEN 2 INJECTIONS OF GLOBULIN WHILE ON LIMITED FOOD AND WATER INTAKE.

Day	6 Rm. Temp. Animals		6 Cold Rm. Animals	
	Quantity (c.c.)	Protein (mg.%)	Quantity (c.c.)	Protein (mg.%)
-4	64	0	46	0
			- Placed in Cold Room -	
-3	66	0	79	0
-2	76	0	112	0
-1	87	0	89	0
0	46	0	56	0
	- Globulin Injection -			
1	54	0	63	0
2	50	0	57	0
3	49	0	52	0
4	47	0	87	0
5	45	0	78	0
6	41	0	75	0
7	77	0	81	0
8	47	0	74	0
9	67	0	81	0
10	78	0	82	0
	- Globulin Injection -			
11	64	0	82	0
12	73	0	83	0
13	77	0	83	0
14	52	0	101	0
15	81	0	111	0
16	59	0	57	0

Immunologic Studies.

Complex quantitative immunologic studies have not been carried out in this work. In Experiment I, qualitative ring tests for anti-globulin precipitins, and for anti-kidney antibodies were carried out in conjunction with intradermal skin sensitivity tests. The results of these tests, and their relation to the presence of glomerulonephritis, are shown in Table X.

TABLE X.

Relation of Immunologic Reactions to Presence of Renal Lesions

Animal No.	Time of test	Serologic reaction to				Intradermal test			Glomerulonephritis
		Own kidney extract	Pooled kidney extract	Liver extract	Globulin	Kidney extract	Liver extract	Globulin	
2	Control*	0	0	0	0				
	11 days	0	+	0	++				
	17 days	0	0	0	++	+	0	++	0
4	Control	0	0	0	0				
	11 days	++	+	0	++				
	17 days	0	0	0	++	0	0	+++	++++
5	Control	0	0	0	0				
	11 days	++	0	0	++				
	17 days	0	0	0	++	0	0	++	Focal interstitial nephritis only
7	Control	0	0	0	0				
	11 days	0	0	0	++				
	17 days	0	0	0	++++	+	0	++	++
8	Control	0	0	0	0				
	11 days	0	+	0	++				
	17 days	0	0	0	++++	0	0	++	++
9	Control	0	0	0	0				
	11 days	0	0	0	+				
	17 days	0	0	0	++	0	0	+	++
11	Control	0	0	0	0				
	11 days	0	0	0	++				
	17 days	0	0	0	++	0	0	+	Spontaneous pyelonephritis only
12	Control	0	0	0	0				
	11 days	0	0	0	++				
	17 days	0	0	0	++	0	0	++++	+++
13	Control	0	0	0	0				
	11 days	0	0	0	±				
	17 days	0	0	0	++	+	0	+	0
14	Control	0	0	0	0				
	11 days	0	0	0	++				
	17 days	0	0	0	++++	0	0	+	++++

* Sera obtained 24 hours before initial globulin injection.

Preliminary tests showed no demonstrable anti-rabbit-kidney, or anti-rabbit-liver antibodies in any of the lots of bovine serum gamma globulin used. Sera from eight of ten animals in Experiment I (Room temperature, two injections of globulin) gave moderately strong reactions with bovine globulin 11 days after the initial injection. The reactions of the remaining two animals were weak or doubtful at this time. All animals showed anti-globulin antibodies in sera drawn just before killing.

The sera of two animals gave positive reactions with extracts of the animals' own kidney 11 days after the first globulin injection. One of these animals showed diffuse glomerulitis of severe degree at autopsy (Figs. 6, 7 and 8). The other showed rather severe focal pyelonephritis of the type found also in control animals. Less marked reactions were obtained between sera of three animals and the pooled kidney extract in tests made 11 days after the initial globulin injection. However, in only one of these did a corresponding reaction with extract of the rabbit's own kidney appear. All reactions with both liver and kidney extracts were negative in sera drawn just before the animals were killed.

All animals tested in Experiment I showed some degree of skin sensitivity to bovine gamma globulin. The tests were graded according to severity. The most marked reactions were classed as ++++, and consisted of well marked areas of oedema, erythema, and central necrosis. Reactions graded as + consisted of a zone of oedema only. Some slight reaction was noted at the site of injection with kidney extract in three animals. These

consisted of oedema only. Two of these animals had normal kidneys, whilst the third showed lesions of diffuse glomerulonephritis.

The results of ring tests for anti-globulins in other groups of animals are shown in Table XI (page 130). In animals treated at room temperature, anti-globulins were not demonstrable on the seventh day after the first injection, but were present in all animals on the tenth day. In another group of animals treated in the cold room, and tested daily for antibodies, all tests became positive on the eighth day after the first injection. Similar tests in the same group, after the second injection, showed antibodies to appear on the third day after injection. These days were also those on which urinary manifestations of acute nephritis made their most florid appearance. A second group treated in the cold room showed anti-globulins in half the animals on the seventh day after injection, and in all animals on the tenth day. Animals treated at room temperature while on restricted diet showed somewhat less marked development of anti-globulins than their counterparts on ad libitum diet. After the first injection, animals treated in the cold while on restricted diet, showed almost complete absence of anti-globulins on the tenth day, at which time animals on ad libitum diet had all developed antibodies. Indeed, the only animal in this group which did show antibodies at this time was one which had developed severe diarrhoea during the course of treatment.

TABLE XI.

TIME OF APPEARANCE OF ANTIBODIES TO BOVINE GAMMA GLOBULIN AFTER FIRST INJECTION.

Group	No. of Animals	Days after Injection. (No. of animals showing Positive Ring Test.)											Nephritis.
		0	1	2	3	4	5	6	7	8	9	10	
Room Temperature	6	0*							0			6 (+ to +++)	4
Cold Room	6	0	0	0	0	0	0	0	0	6 (+ to +++)			6
Cold Room	6	0							3 (+)			6 (+ to +++)	6
Room Temperature Restricted Diet	6	0							1 (+)			5 (+ to ++)	0
Cold Room Restricted Diet	6	0							2 (+)			1≠ (+)	0

* Boxes containing figures indicate days on which antibody tests were made.

≠ This animal differed from rest of group in having severe diarrhoea at this time.

Haematologic Findings.

(a) Blood Coagulation Studies: Coagulation time determinations were made on animals of Experiment I, treated at room temperature, and on those of Experiment III-B, treated in the cold room. The results of these studies in Experiment I are shown in Table XII.

Changes in the Blood Coagulation Time during the First 7 Days after a Massive Bovine Gamma Globulin Injection

	No. of readings	Mean coagulation time	Standard deviation
		min.	
18 globulin-treated animals	168	3.90 ± 0.104	1.35
18 nephrectomized controls*	34	5.42 ± 0.215	1.25
5 saline-injected controls‡	50	5.68 ± 0.16	1.16

* These observations were made on the same 18 animals that were subsequently injected with globulin. They were recorded 3 to 6 weeks after unilateral nephrectomy, within the 5 days preceeding the initial globulin injection.

‡ These observations were made during 7 days following injection with 10 cc. of normal saline per kilo of body weight.

The individual variation in the readings was such that there was no uniformity of coagulation time in one particular animal from day to day, nor in all animals on any particular day of the experiment. The results do show, however, that during the first week after the initial globulin injection there is an apparent increase in blood coagulability, as shown by a shortening of the mean coagulation time during this period. This change was not noted during the six days following the second globulin injection.

The normal mean coagulation time of the 18 animals in Experiment I before the initial globulin injection was 5.43 minutes, with a Standard Deviation of 1.25. The mean coagulation time of the same animals during the first seven days after injection was only 3.90 minutes, with a Standard Deviation of 1.35. Analysis of this difference in mean coagulation times shows that the increase in coagulability is probably significant. During the six days following the second globulin injection the mean coagulation time did not differ significantly from the mean normal time, being 5.39 minutes. No significant alteration in blood coagulability was observed in five control animals on which coagulation studies were done following injection with normal saline (Table XII).

On the eighth day after the initial globulin injection, eight of eighteen animals in Experiment I showed a slight prolongation of coagulation time (7.5 to 12.0 minutes). Although it was considered that this might be a phenomenon associated with the development of antibodies, this phenomenon has not been studied further up to this point. Several animals also showed marked prolongation of coagulation time within one-half hour of the desensitizing injection of globulin on the eleventh day. Absence of this well-known transient feature(231) of anaphylaxis in the remaining animals was probably due to the fact that all observations on this date were not made at exactly the same interval after globulin injection.

In similar coagulation studies made upon five animals treated with globulin in the cold room (Experiment III), no significant alteration in blood coagulability could be determined at any point in the experiment.

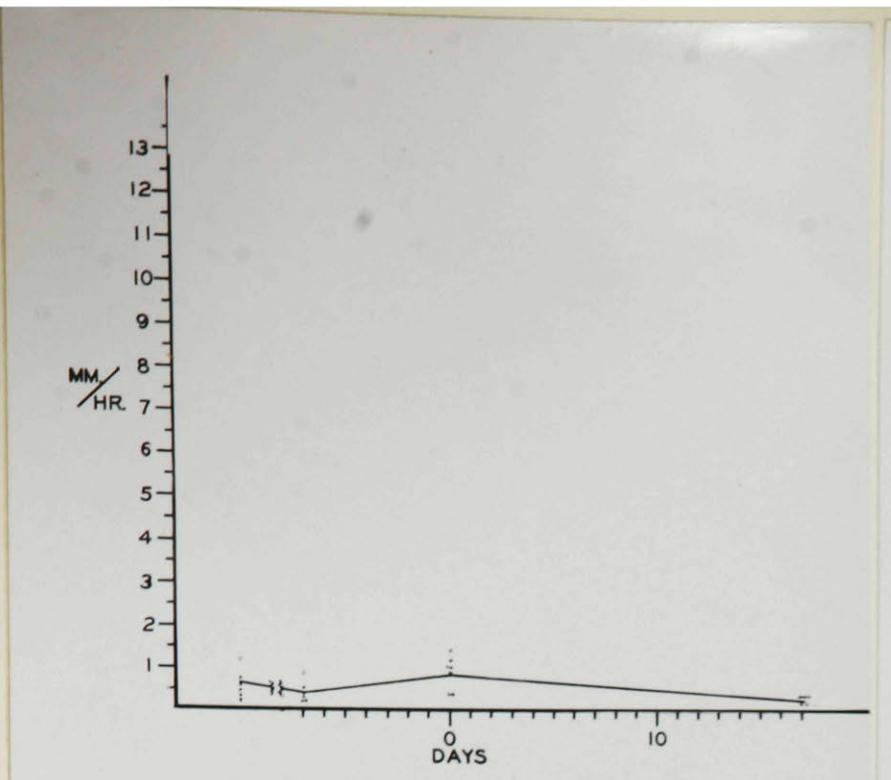
Before treatment, the mean coagulation time for these animals was 4.99 minutes, during the first three days in the cold room it was 4.66 minutes, and during the first week after treatment it was 5.81 minutes. The amount of variation indicates that the differences between these values is probably not significant.

(b) Sedimentation Velocities: Most of the data concerning sedimentation velocities come from Experiment IV. A total of 46 control determinations were made in animals at room temperature. These fell within the narrow range of 0.2 to 1.3 mm./hr., with a mean value of 0.56 mm. The amount of variation found in control animals, bled on different occasions was slight (Tex-figure 18). In untreated animals kept in the cold room, there was a slight, but insignificant, tendency for the sedimentation rate to rise after about ten days in the cold. The highest value recorded for any of these animals was not above 2.0 mm. (Text-figure 19). In globulin treated animals kept at room temperature, the mean sedimentation velocity was elevated on all occasions after the first injection (Text-figure 20). The most marked increase was found immediately before the second globulin injection, when two animals had sedimentation rates of 23.0 and 42.0 mm. Nevertheless, two of the six animals in this group failed to show any significant elevation of sedimentation rate; these same animals were the only members of the group which also failed to develop nephritis. Globulin-treated animals kept in the cold room showed much less marked alterations in sedimentation rate (Text-figure 21). The greatest increase occurred seven days after the initial injection, with only three of six animals showing significant elevation on this occasion. All animals in the group, however, had lesions of diffuse glomerulonephritis when killed.

(c) Erythrocrits: Forty-six determinations made on untreated room temperature animals of Experiment IV showed the mean value for packed red blood cells to be 41.8%, between extremes of 33.5 and 48.0%. Untreated control animals kept at room temperature showed only slight variations from this figure when bled at about the same intervals as the various treated groups. (Text-figure 22). A slightly downward trend occurred in animals given two injections of bovine globulin while kept at room temperature, with the lowest mean value being 35.0 on the last day of the experiment (Text-figure 23). A somewhat more marked haemodilution appeared in cold room control animals, and was most marked after ten days in the cold room, tending to rise during the next week. (Text-figure 24). A similar series of readings was obtained from globulin treated animals kept in the cold (Text-figure 25). In all groups of animals treated with bovine globulin, no relationship was apparent between the degree of haemodilution or anaemia, and the severity of nephritis, and it was felt that the fall in erythrocrit was in most instances the result of repeated bleedings.

(d) Leucocrits: Rough estimates of the volume of packed white blood cells were made in all animals of Experiment IV. The mean value for 48 control readings was 0.91 mm. There was considerable variation among individual animals, but in general repeated bleedings of control animals was associated with a rising volume of packed white blood cells (Text-figures 26 and 27). This rise was slightly more pronounced in globulin treated animals (Text-figures 28 and 29), reaching its highest level on the tenth day after injection in the group treated at room temperature (2.3 mm.)

No correspondence could be noted between the amount of this rise on any particular occasion, and the severity of nephritis when it developed.



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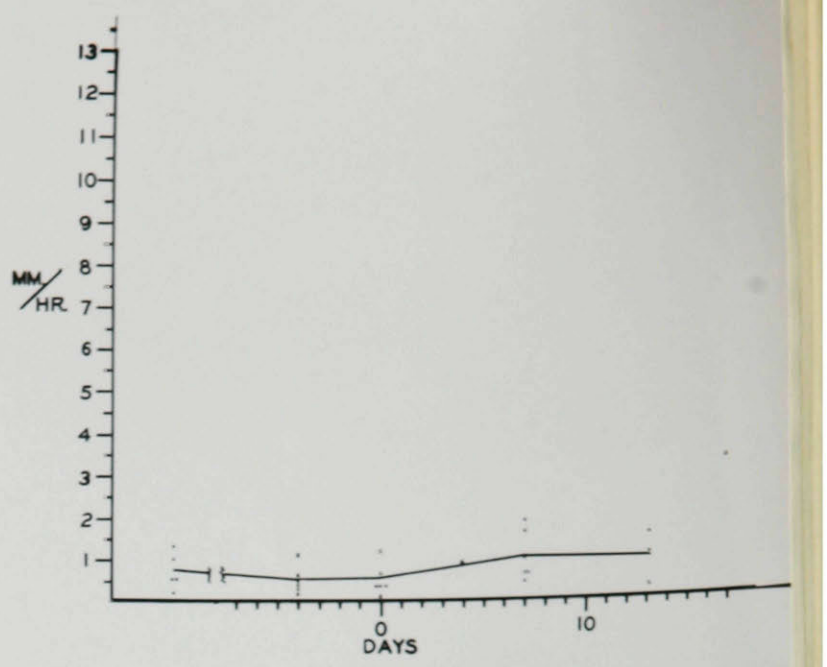
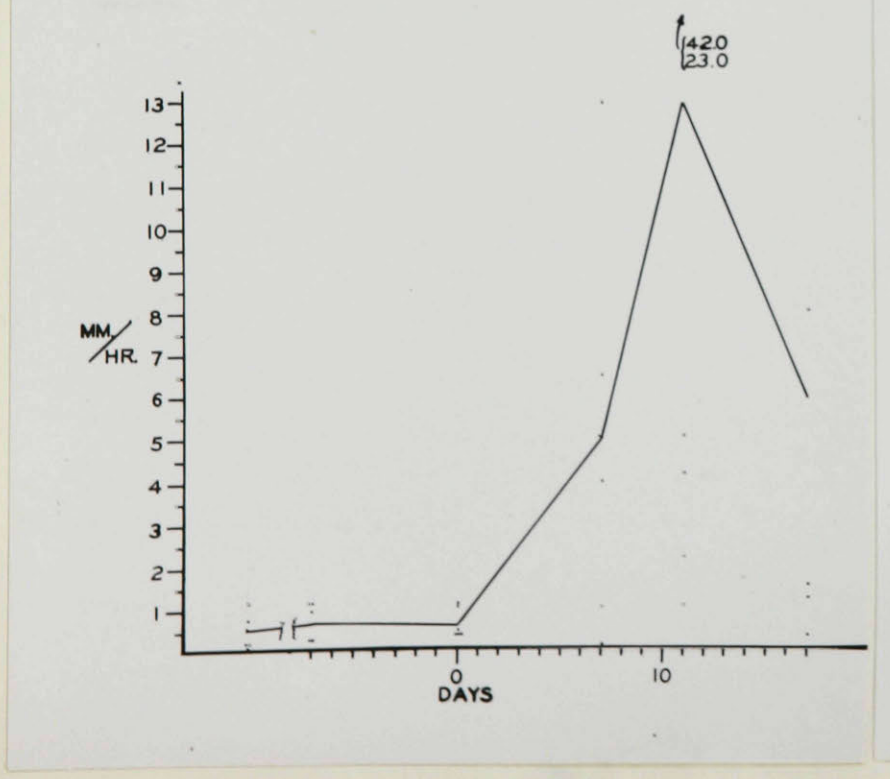


19.

TEXT-FIG. 18. Sedimentation velocities in room temperature control animals, injected with normal saline Day 0.

TEXT-FIG. 19. Sedimentation velocities in cold room control animals, injected with normal saline Day 0.

Animals in cold room from Day -3 to end of Experiment.



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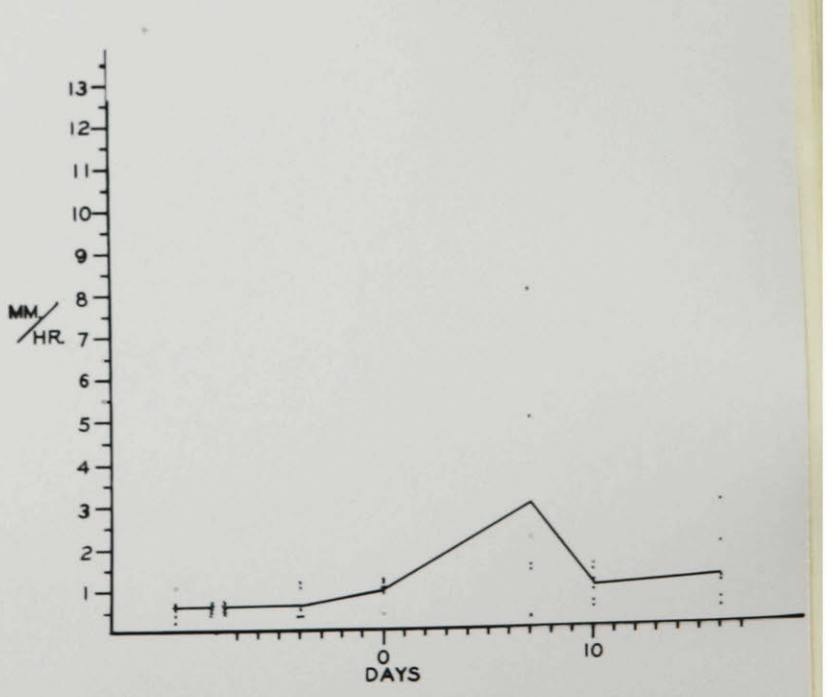
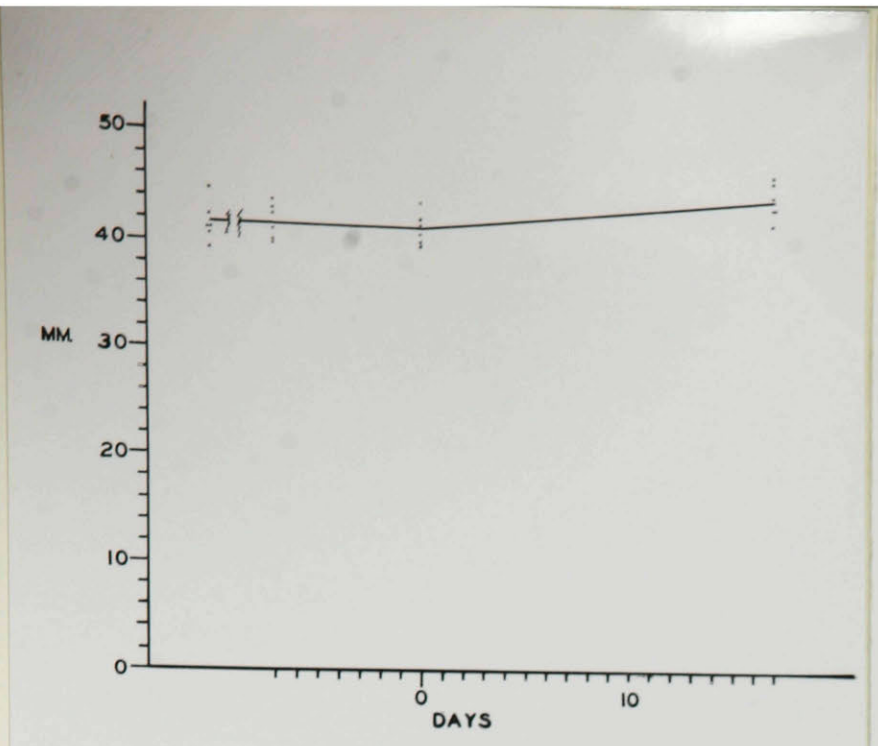


21.

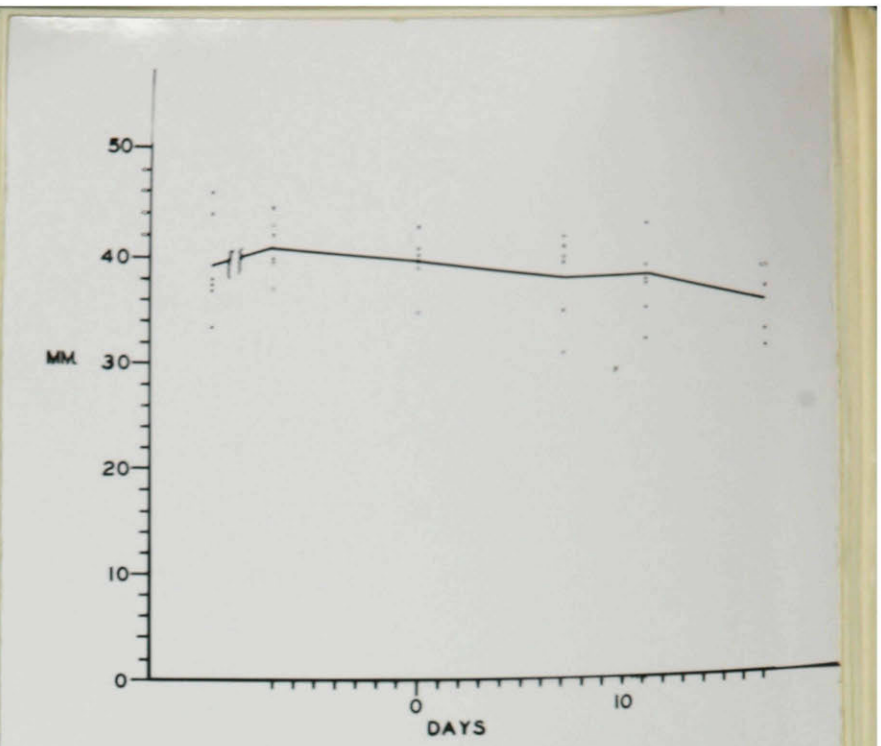
TEXT-FIG. 20. Sedimentation velocities in room temperature globulin-treated animals, injected Day 0 and Day 11.

TEXT-FIG. 21. Sedimentation velocities in cold room globulin-treated animals, injected Day 0 and Day 10.



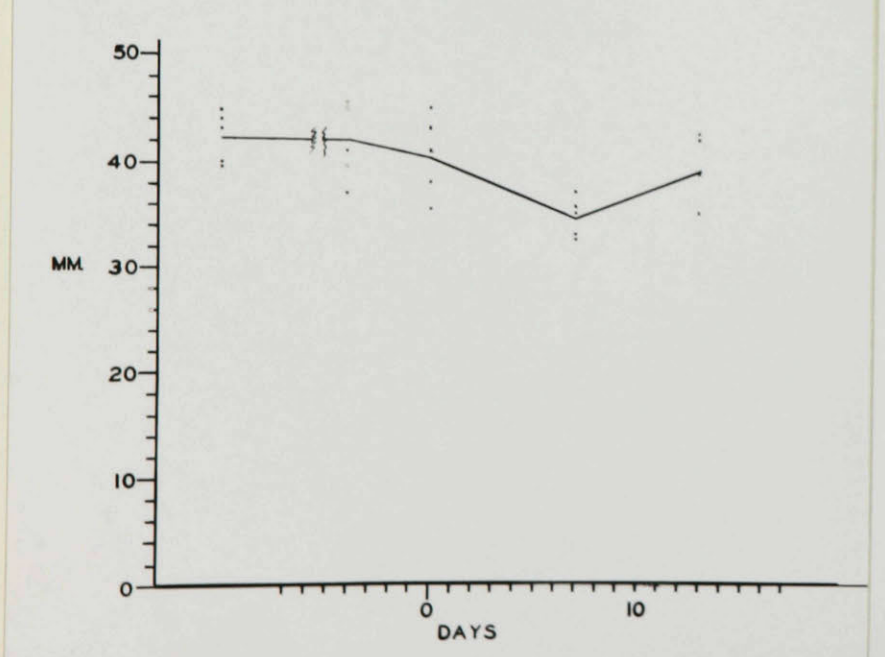
22.

TEXT-FIG. 22. Erythrocrits of room temperature control animals.



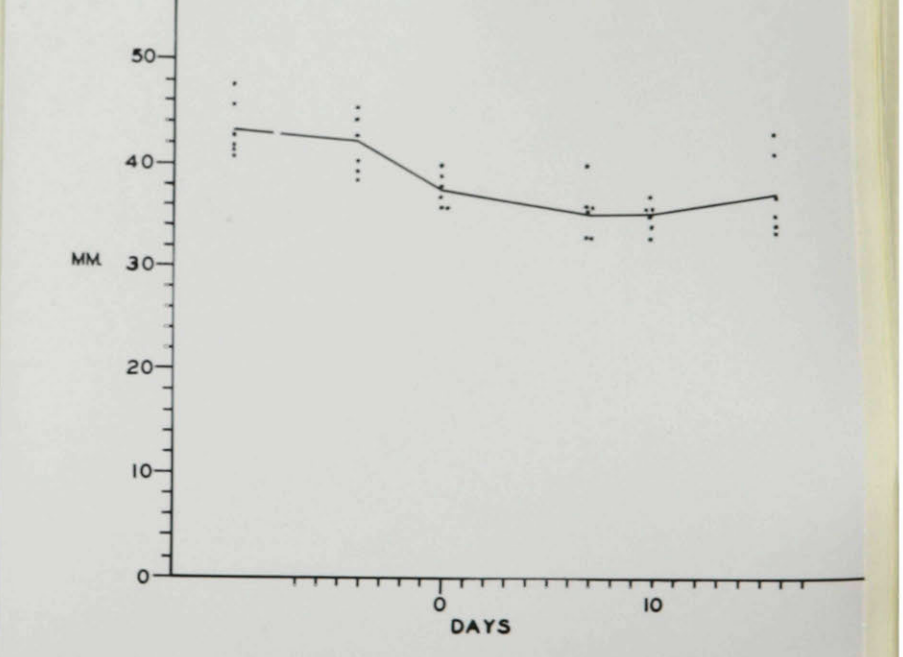
23.

TEXT-FIG. 23. Erythrocrits of room temperature globulin-treated animals.



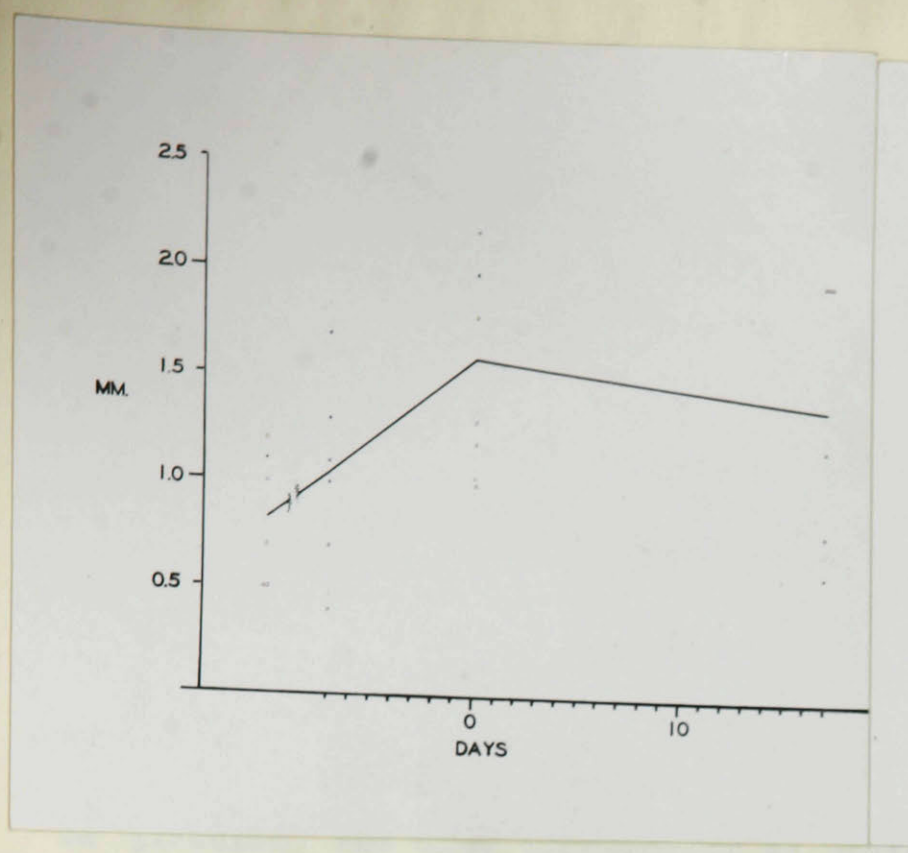
24.

TEXT-FIG. 24. Erythrocrits of cold room control animals.



25.

TEXT-FIG. 25. Erythrocrits of cold room globulin-treated animals.



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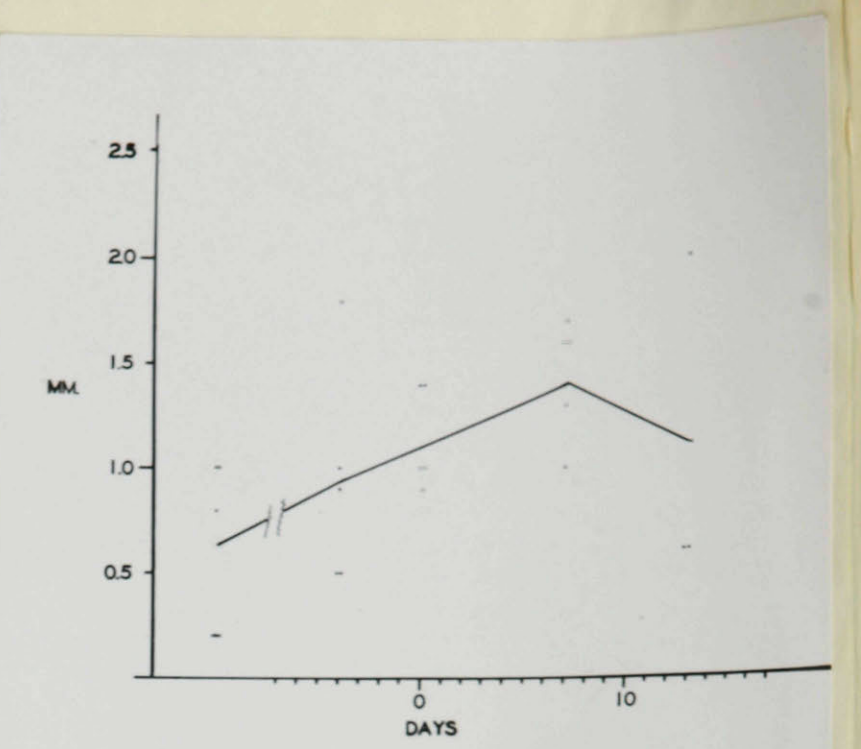
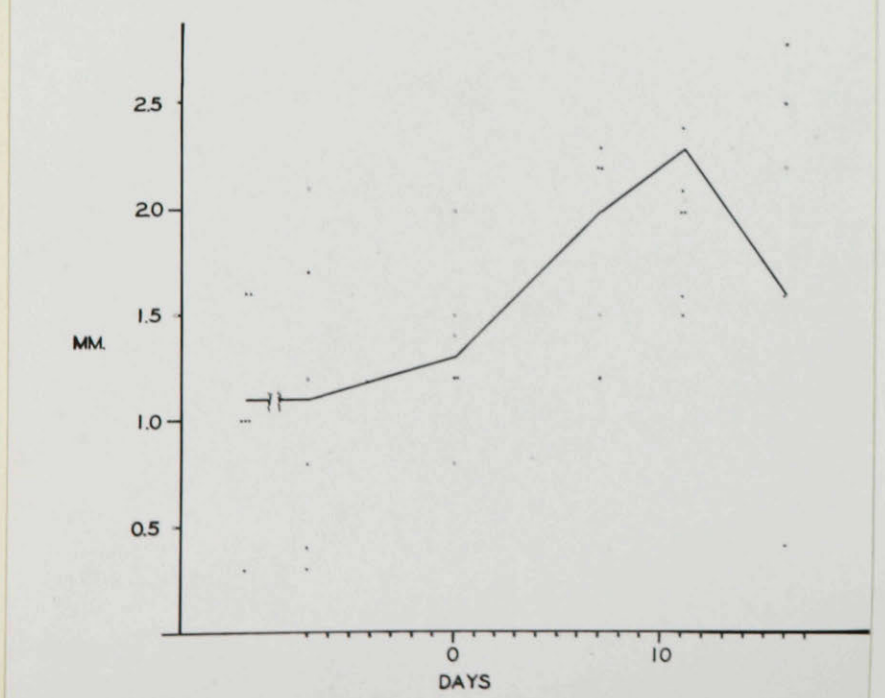


27.

TEXT-FIG. 26. Leucocrits, room temperature control animals.

TEXT-FIG. 27. Leucocrits, cold room control animals.



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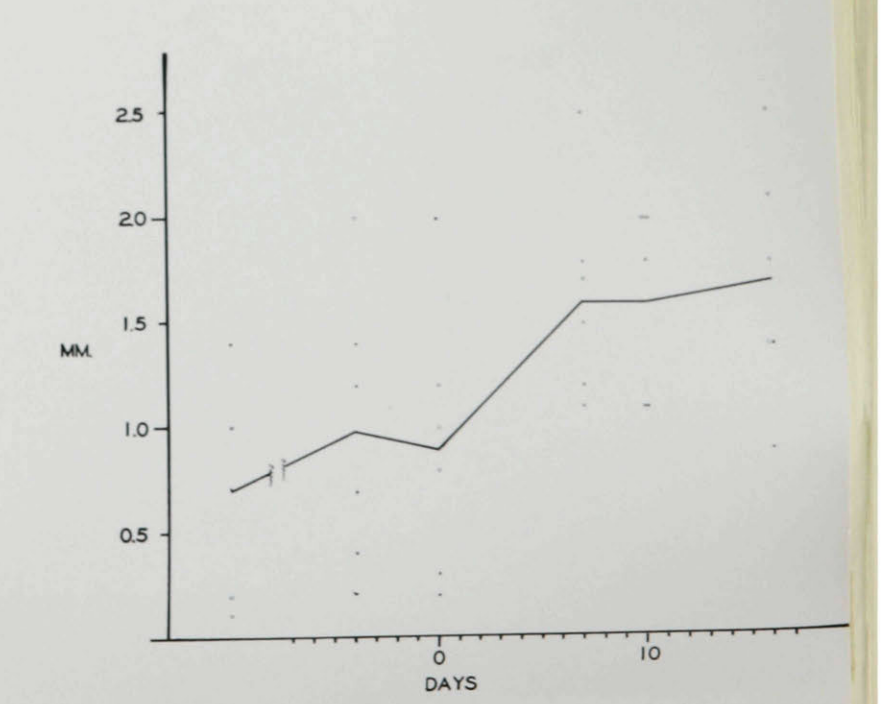

29.

TEXT-FIG. 28. Leucocrits, room temperature globulin-treated animals.

TEXT-FIG. 29. Leucocrits, cold room globulin-treated animals.

Blood Chemistry.

(a) Blood Urea Nitrogen: Of a total of forty animals receiving two massive injections of bovine serum gamma globulin, three developed uraemia. One of these was an animal from Experiment I, which on the day of killing had a blood urea nitrogen level of 189.0 mg.%. Another animal in Experiment III had a urea nitrogen level of 125.0 mg.% three days after the second injection of globulin, and died (presumably of uraemia) a day and a half later. The third was an animal in Experiment IV, treated at room temperature, and who developed severe diarrhoea toward the end of the experiment. The urea nitrogen level in this animal reached 209.4 mg.% by the tenth day, and it died three days later. The first two animals showed the most severe degree of nephritis at autopsy (++++), whereas the last showed changes graded only as ++.

The mean normal blood urea nitrogen for all untreated animals of Experiment IV was 18.5 mg.%, with variations between 8.0 and 28.7 mg.% (Text-figures 30 - 33 inclusive). The values tended to be slightly higher (19.9 mg.%) in young animals, than in older ones (16.5 mg.%). Slight elevations of urea nitrogen values were found in both room temperature and cold room treated animals in blood samples taken the day the animals were killed. The mean level this day for the cold room group was 34.6 mg.% (20.5 - 52.5 mg.%). Room temperature treated animals (excluding the animal with diarrhoea referred to above) had an average level of 25.1 mg.% (17.8 - 30.5 mg.%). In these groups there was a rough degree of correspondence between the level of blood urea nitrogen at the end of the experiment, and the severity of the nephritis.

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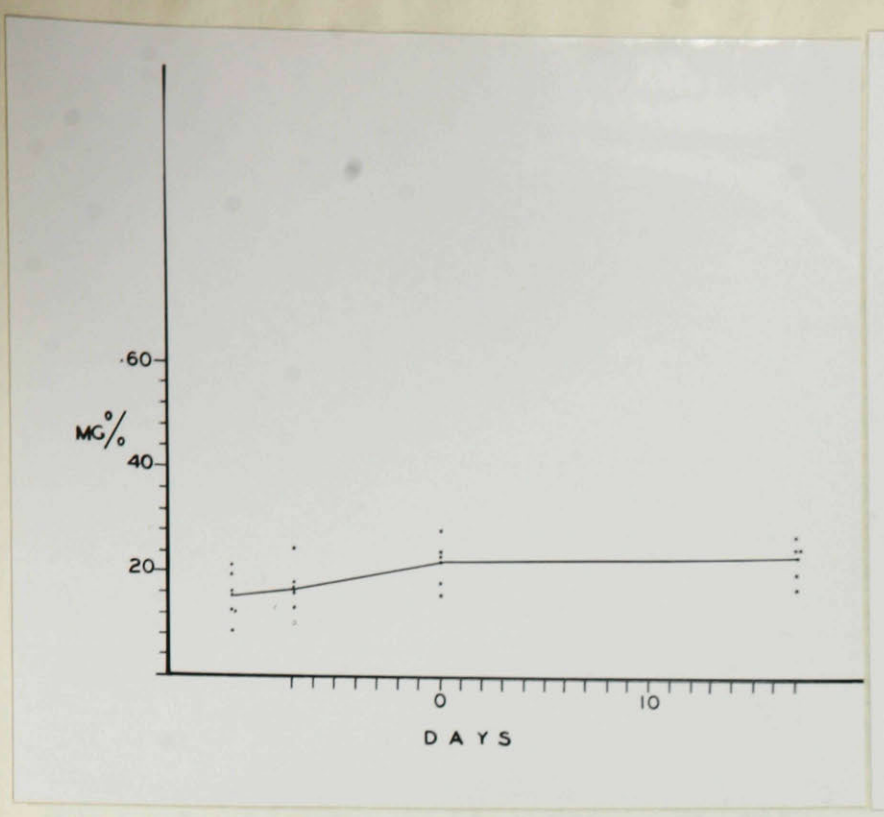

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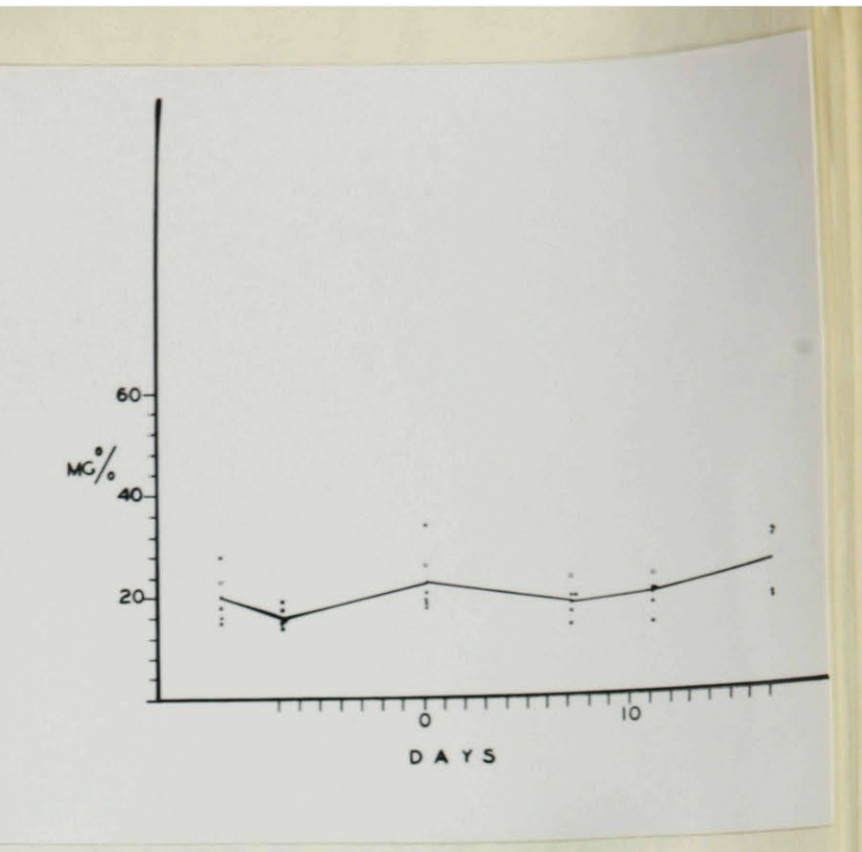
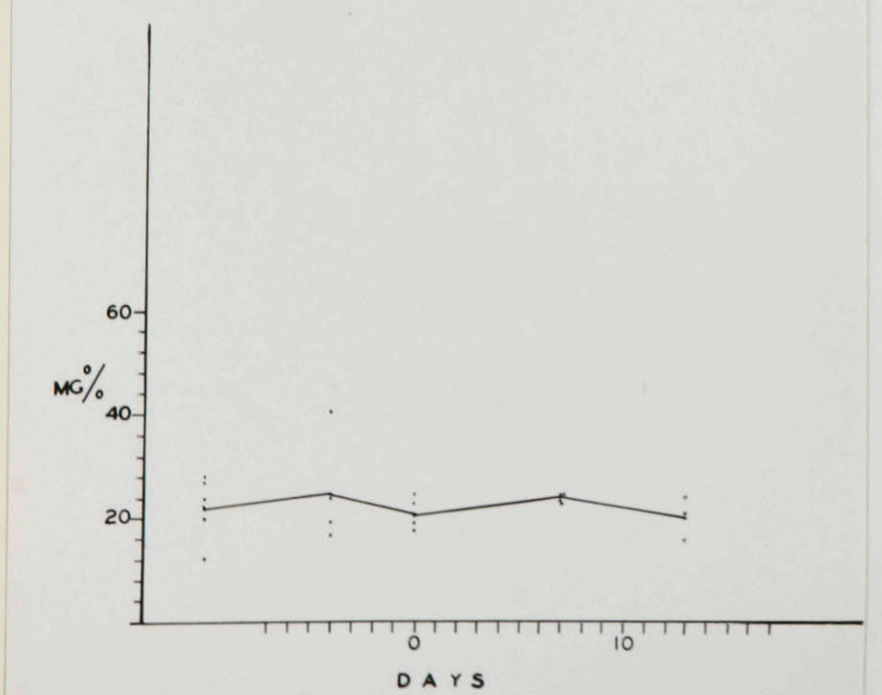


31.

TEXT-FIG. 30. Blood urea nitrogen values for 6 room temperature untreated control animals, injected with normal saline Day 0.

TEXT-FIG. 31. Blood urea nitrogen values for 6 room temperature globulin-treated animals, injected Days 0 and 11.



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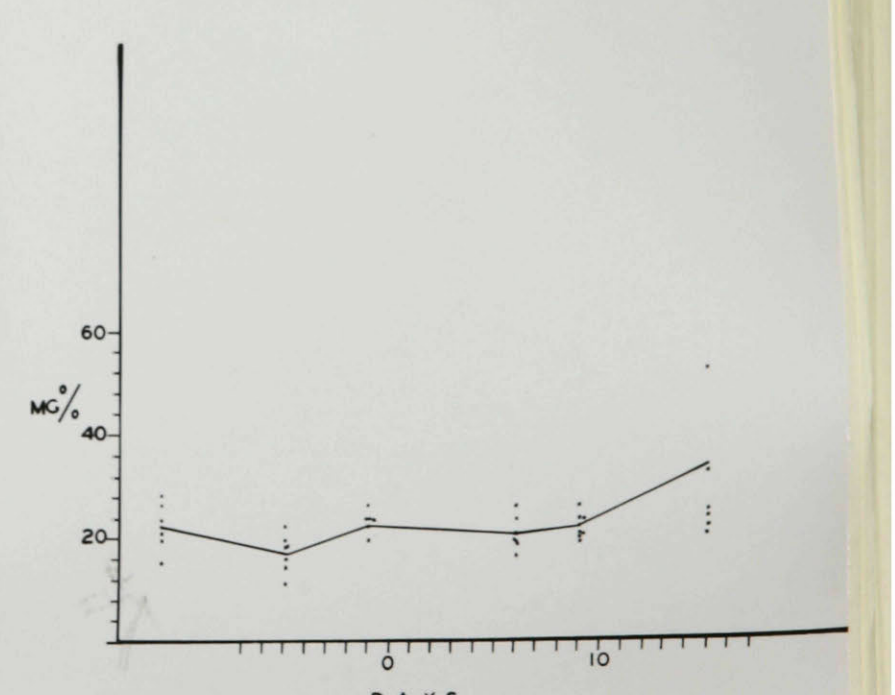

33.

TEXT-FIG. 32. Blood urea nitrogen values for 5 cold room untreated control animals, injected with normal normal saline Day 0.

TEXT-FIG. 33. Blood urea nitrogen values for 6 cold room globulin-treated animals, injected Days 0 and 10.

(b) Serum Proteins: A modification of the technique described by Wolfson et al.(232) was used in all serum protein determinations (see Appendix). As originally reported, this technique was put forward as a rapid salting-out method by which fractional serum protein analyses could be made which gave results closely approximating those obtainable by the more complex technique of electrophoresis. It was considered that if this technique could be adapted to analysis of rabbit serum, the data obtained would be considerably more valuable than simple albumin and globulin determinations. It was thought especially desirable to determine fluctuations in the gamma globulin fraction during the course of development of experimental nephritis, since it is in this fraction that antibodies normally appear.

To this end, electrophoretic analyses of two serum samples were obtained through the kindness of Dr. Lena Lewis and Dr. Kenneth Savard at the Cleveland Clinic. The samples analysed were pooled sera of two animals in each case, one was from control animals of Experiment III, and the other from animals which had been given two injections of bovine gamma globulin (also in Experiment III). The sera from the immunized animals were obtained by cardiac puncture, sixteen days after the first globulin injection, and six days after the second, and were known to contain anti-globulins. A comparison of the results obtained by electrophoresis and by fractional chemical analysis (as it was used in all subsequent determinations) is shown in Table XIII.

TABLE XIII.

Comparison of Electrophoretic and Fractional Salting-out Analyses of Rabbit Serum Proteins.

Sample	Method	Albumin	Alpha Glob.	Beta Glob.	Gamma Glob.
Normal	Electro-phoretic	66.5*	6.7	16.8	10.0
	Chemical	68.1	6.0	15.8	10.1
Immune	Electro-phoretic	40.3	10.9	28.2	20.6
	Chemical	43.0	11.8	28.2	17.0

* Figures represent per cent of total protein.

These results suggested that with increasing concentrations of serum gamma globulin, progressively decreasing proportions of it might be detected by the chemical method. However, the results did show that within the apparent range of variation, a certain correlation could be anticipated between the chemically estimated fractions and the electrophoretic composition of the sera. Since the terms alpha, beta and gamma globulin are intended only to designate serum protein fractions showing more or less specific patterns of migration through an electrical field, there appears to be little justification for applying them to fractions separated by the method used here. For this reason, in all fractional analyses recorded, the various globulin components will be referred to simply as A-globulin, B-globulin and G-globulin.

Even though these fractions appear to correspond more or less quantitatively with the alpha, beta and gamma fractions, in the present work it has not been determined that these were the substances actually being estimated.

Despite these technical shortcomings, certain information has been obtained which may be of value, and which warrants future investigation by more refined methods.

The principal variations in serum protein patterns determined by this technique will be summarized below. Data from all determinations made are graphically presented in Text-figures 34 to 56 inclusive.

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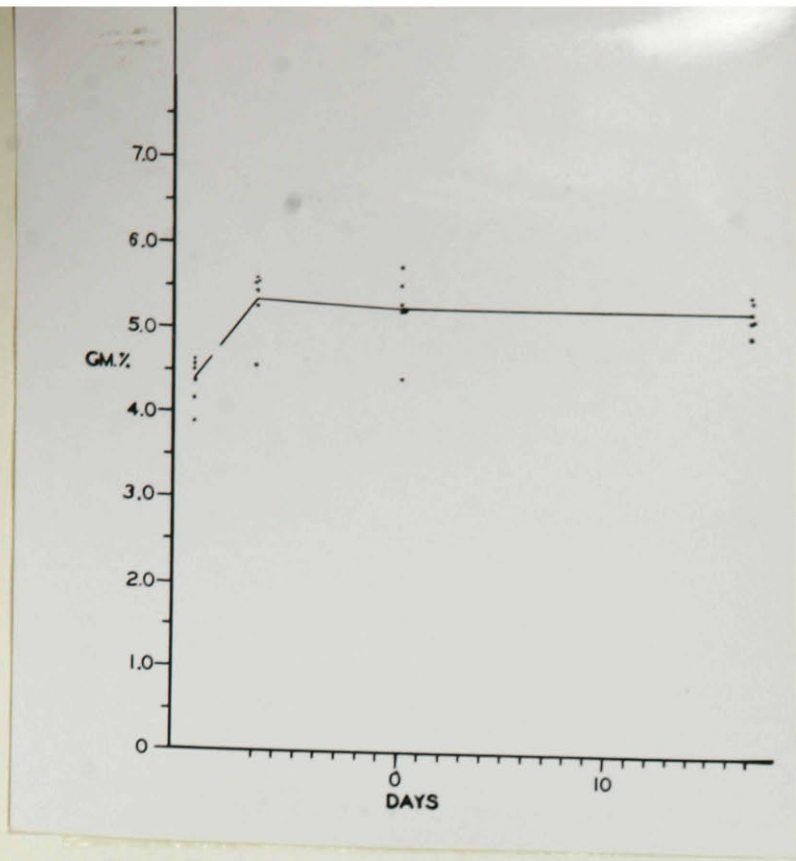

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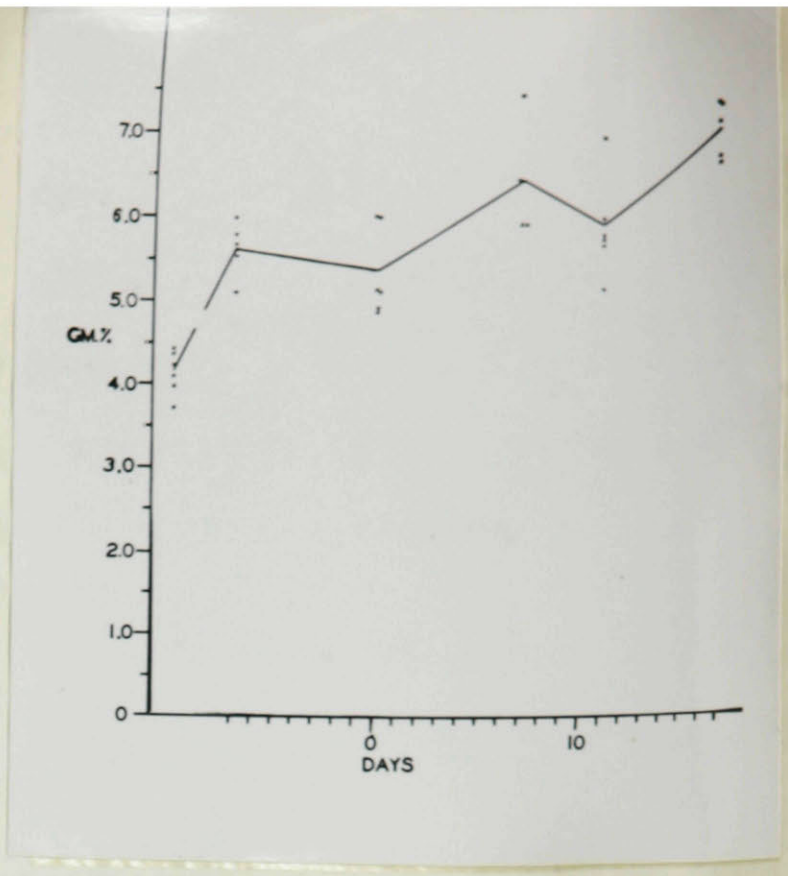
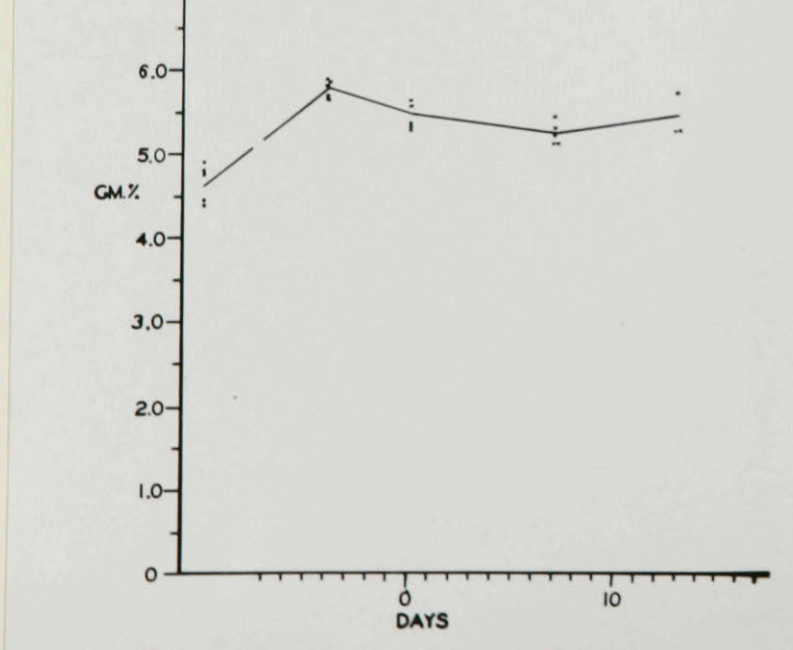


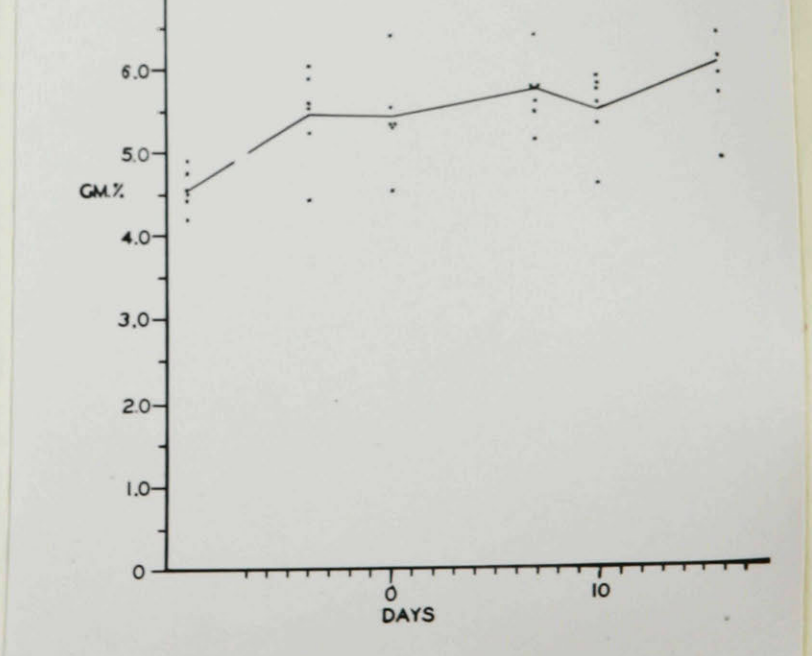
35.

TEXT-FIG. 34. Total serum protein levels for 6 room temperature untreated control animals, injected with normal saline, Day 0.

TEXT-FIG. 35. Total serum protein levels for 6 room temperature globulin treated animals, injected Days 0 and 11.



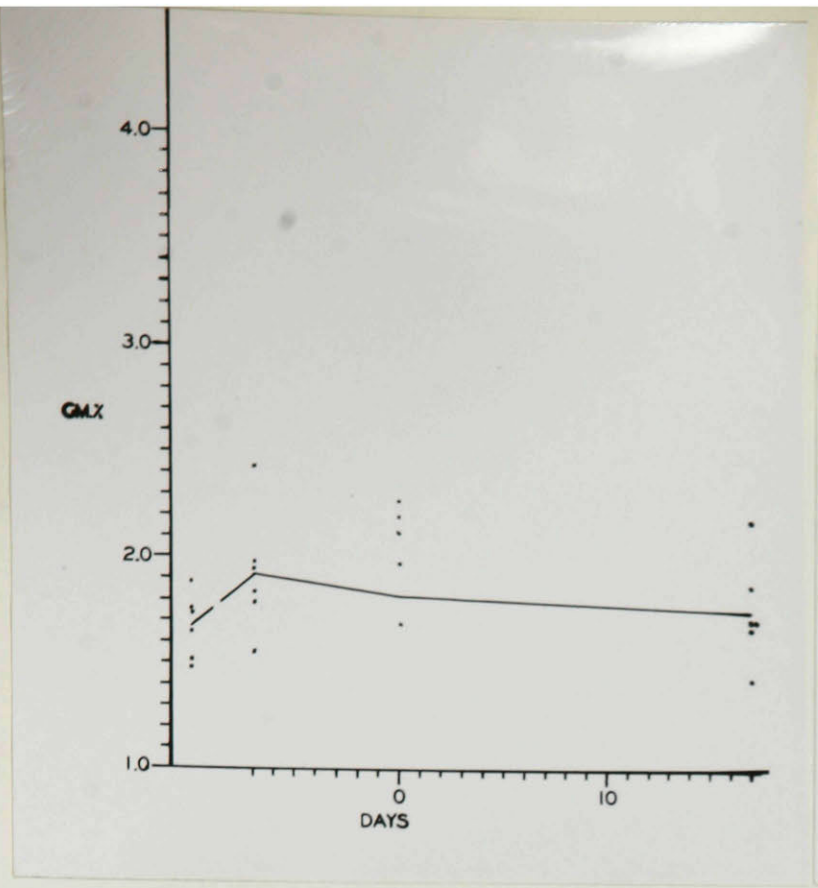
36.



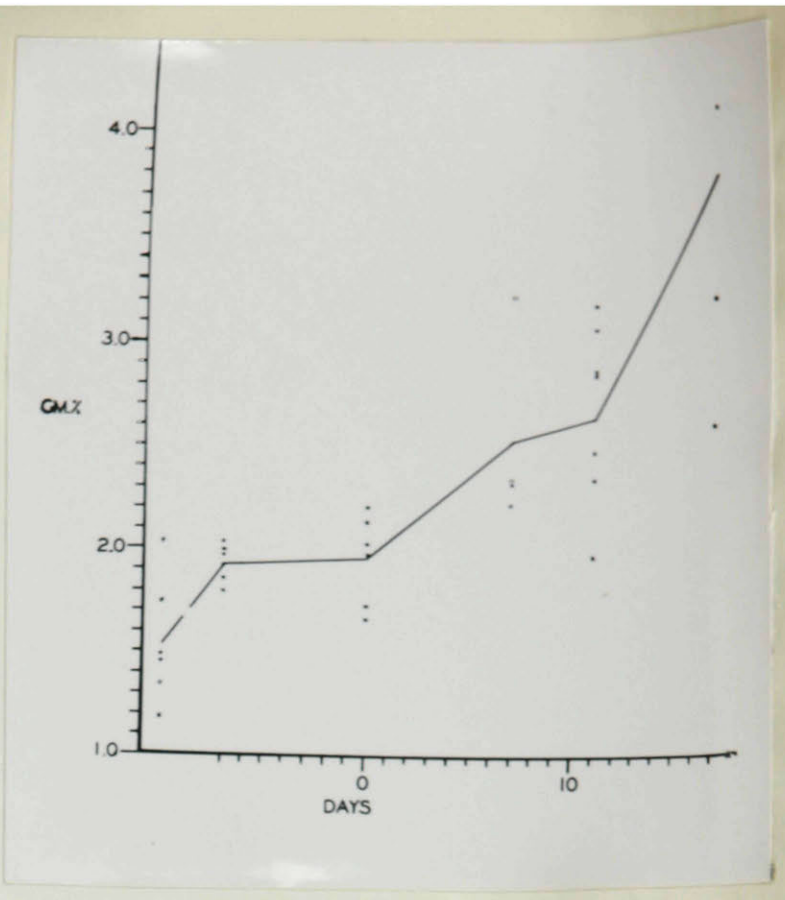
37.

TEXT-FIG. 36. Total serum protein levels for 5 cold room untreated control animals, injected with normal saline Day 0.

TEXT-FIG. 37. Total serum protein levels for 6 cold room globulin treated animals injected Days 0 and 10.



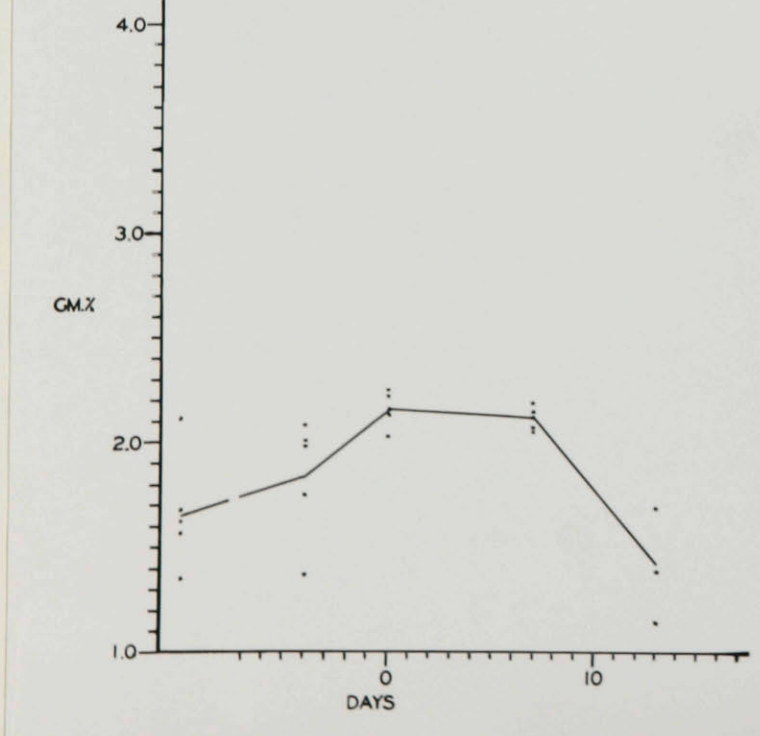
38.



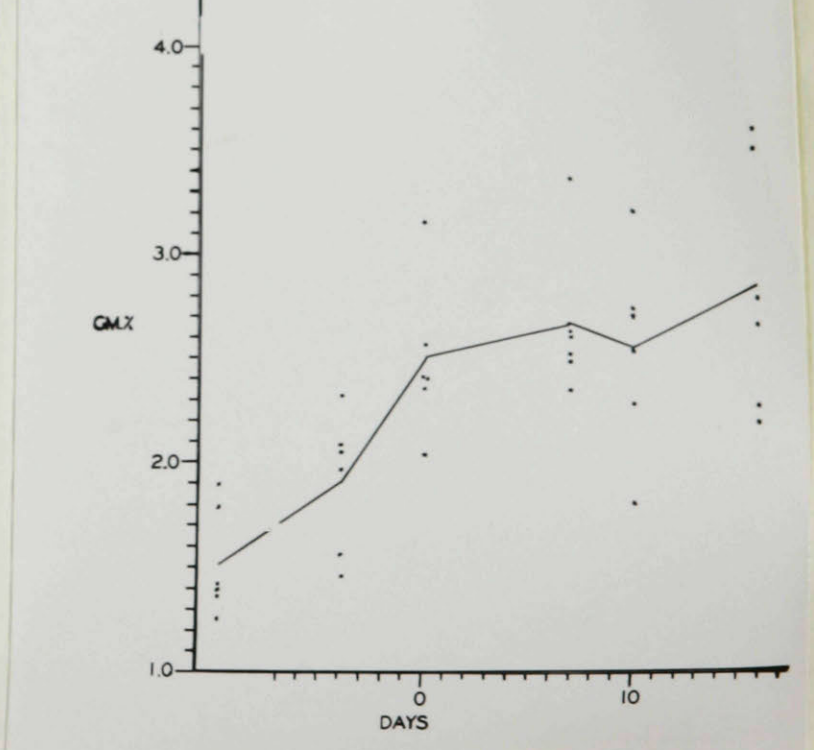
39.

TEXT-FIG. 38. Total serum globulin levels for 6 room temperature untreated control animals, injected with normal saline Day 0.

TEXT-FIG. 39. Total serum globulin levels for 6 room temperature globulin treated animals, injected on Days 0 and 11.



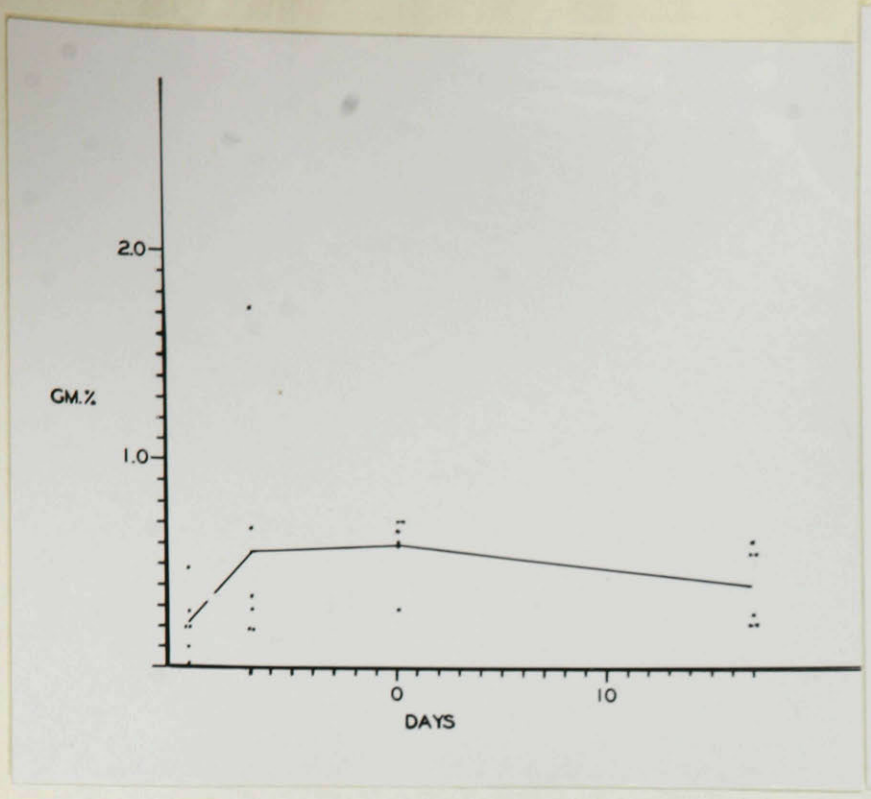
40.



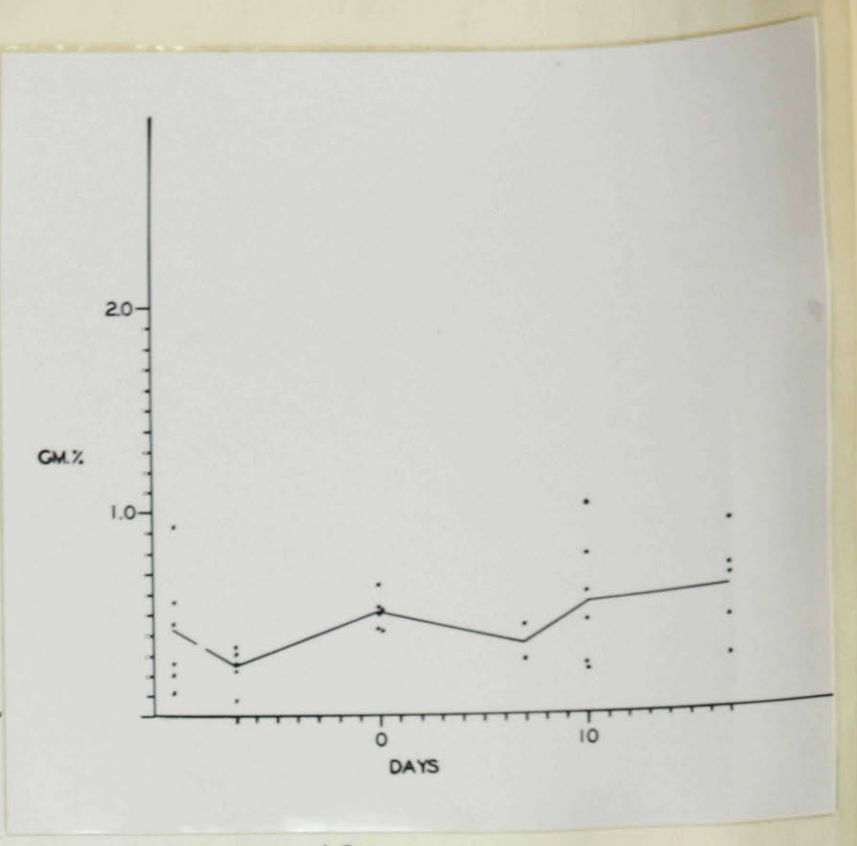
41.

TEXT-FIG. 40. Total serum globulin levels for 5 cold room untreated control animals, injected with normal saline Day 0.

TEXT-FIG. 41. Total serum globulin levels for 6 cold room globulin treated animals, injected Days 0 and 10.



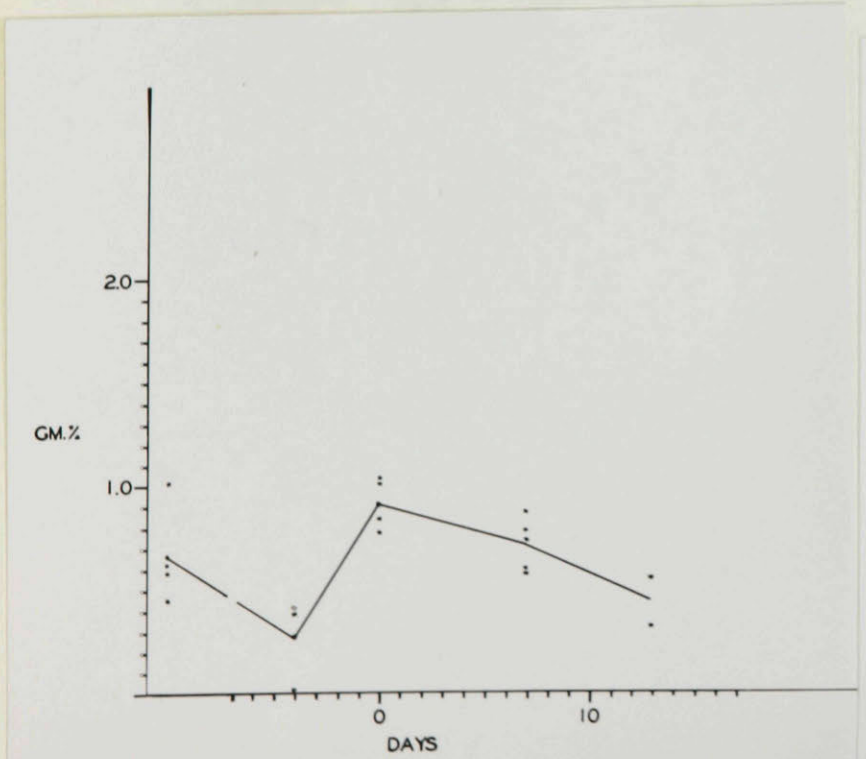
42.



43.

TEXT-FIG. 42. Serum A-globulins, 6 room temperature control animals, injected with normal saline Day 0.

TEXT-FIG. 43. Serum A-globulins, 6 room temperature globulin treated animals, injected Days 0 and 11.



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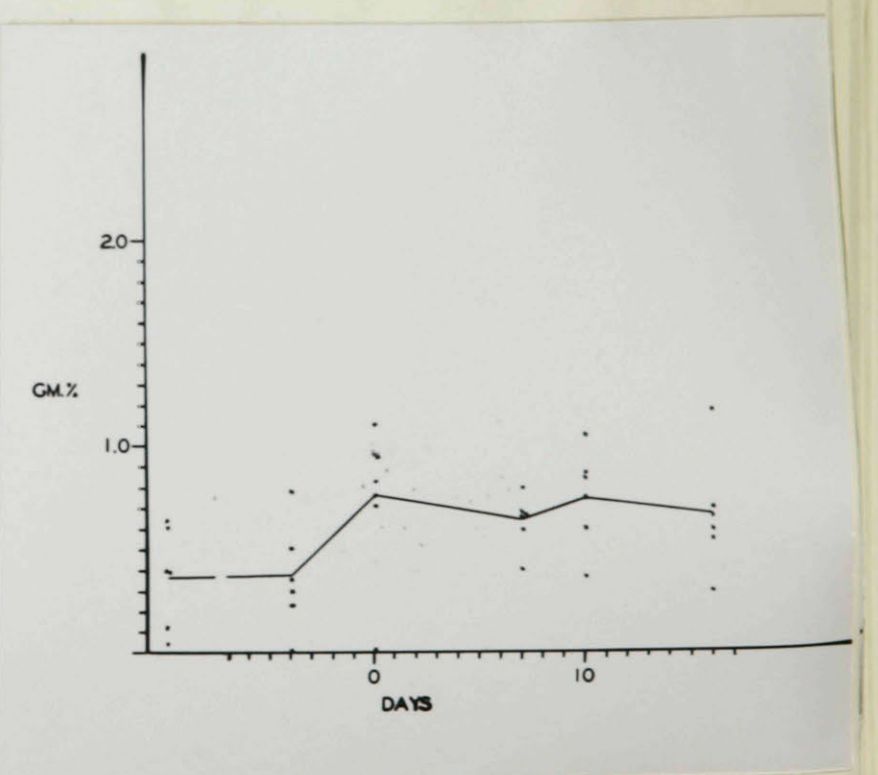
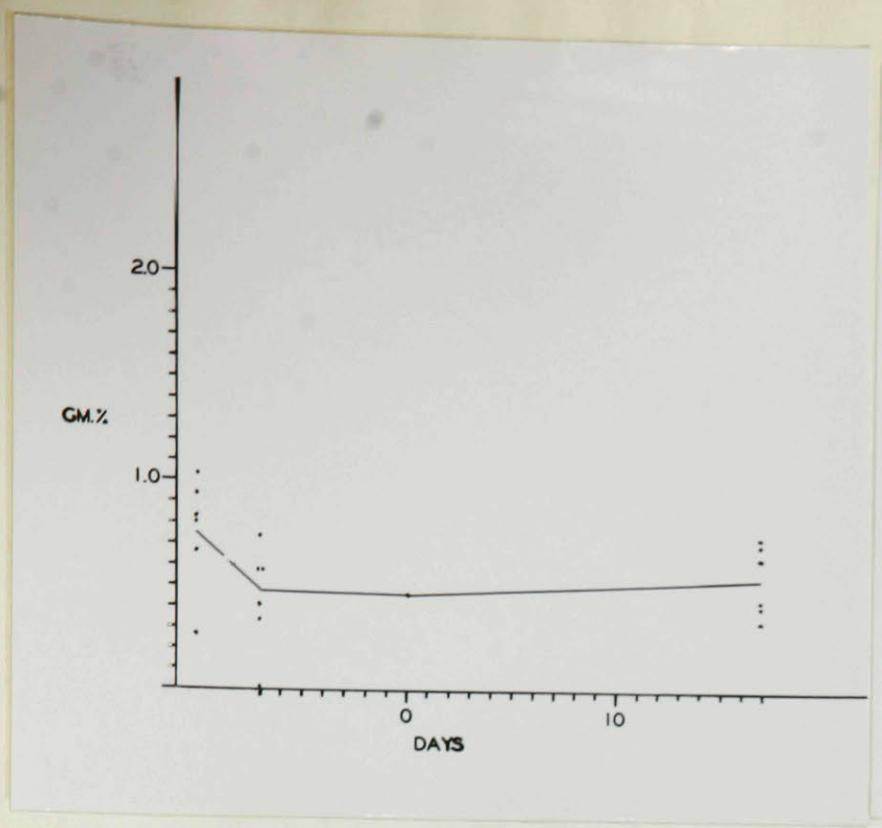


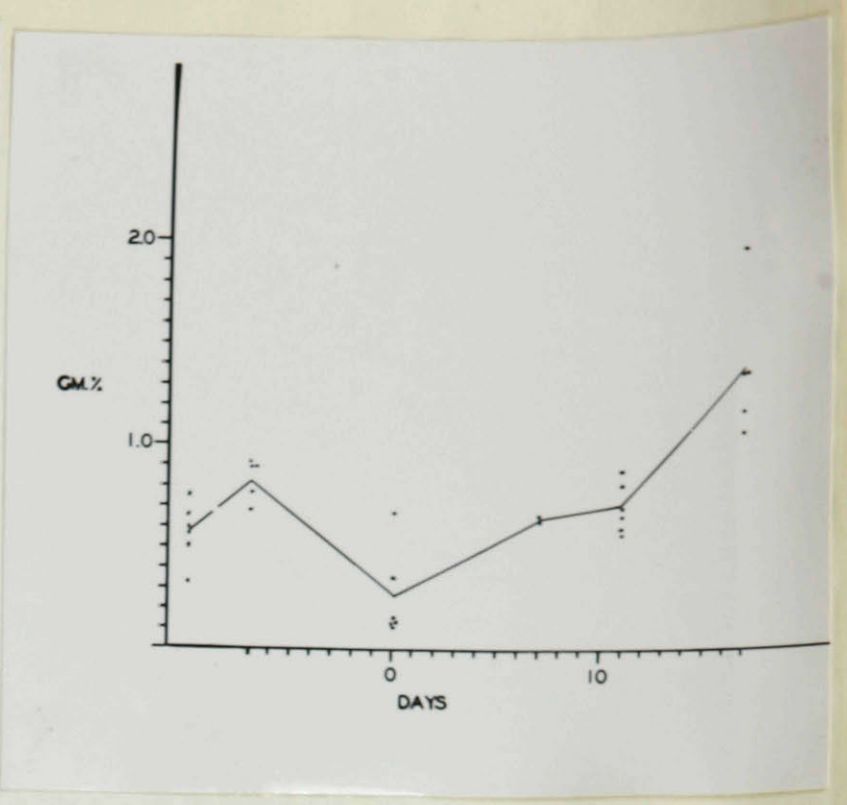
45.

TEXT-FIG. 44. Serum A-globulins, 5 cold room control animals, injected with normal saline Day 0.

TEXT-FIG. 45. Serum A-globulins, 6 cold room globulin treated animals, injected Days 0 and 10.



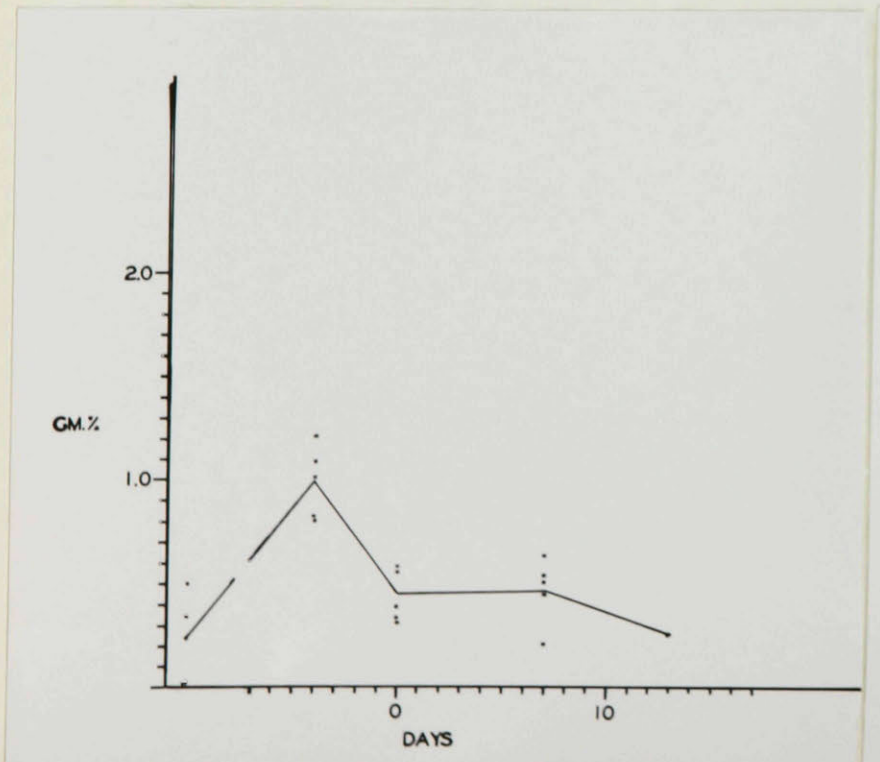
46.



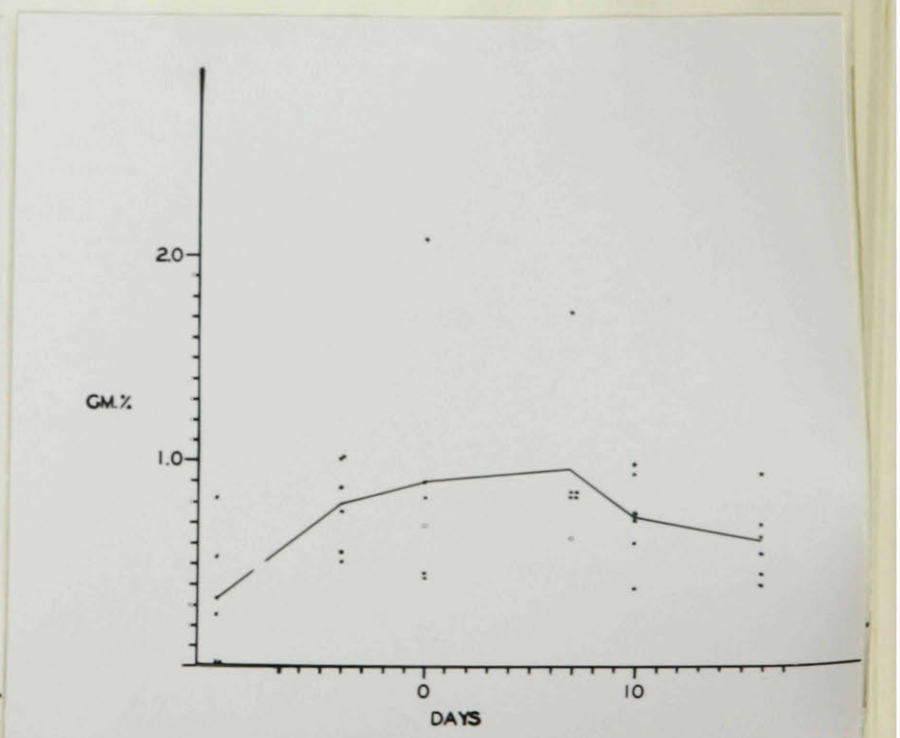
47.

TEXT-FIG. 46. B-globulins, 6 room temperature control animals injected with normal saline Day 0.

TEXT-FIG. 47. B-globulins, 6 room temperature globulin treated animals, injected Days 0 and 11.



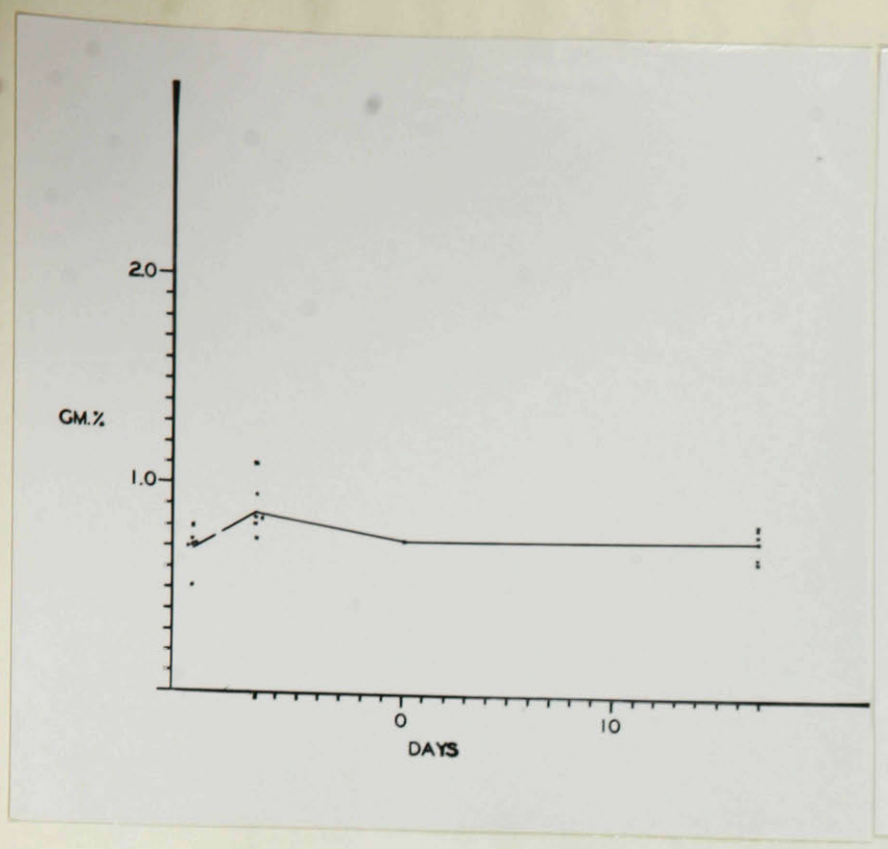
48.



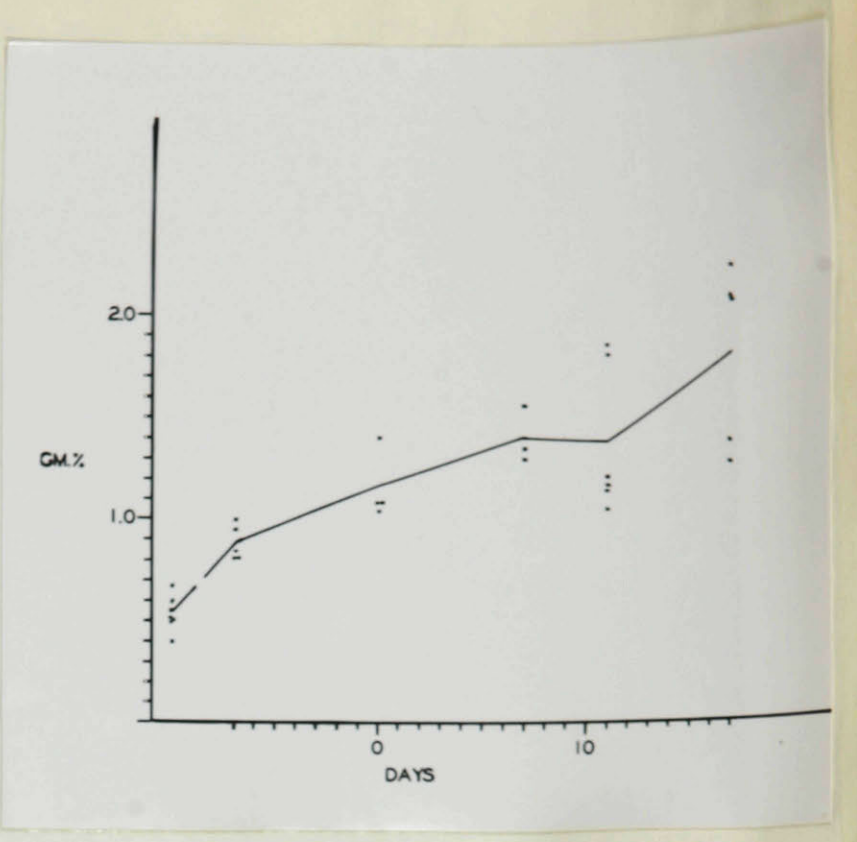
49.

TEXT-FIG. 48. B-globulins, 5 cold room control animals, injected with normal saline Day 0.

TEXT-FIG. 49. B-globulins, 6 cold room treated animals, injected with bovine globulin Days 0 and 10.



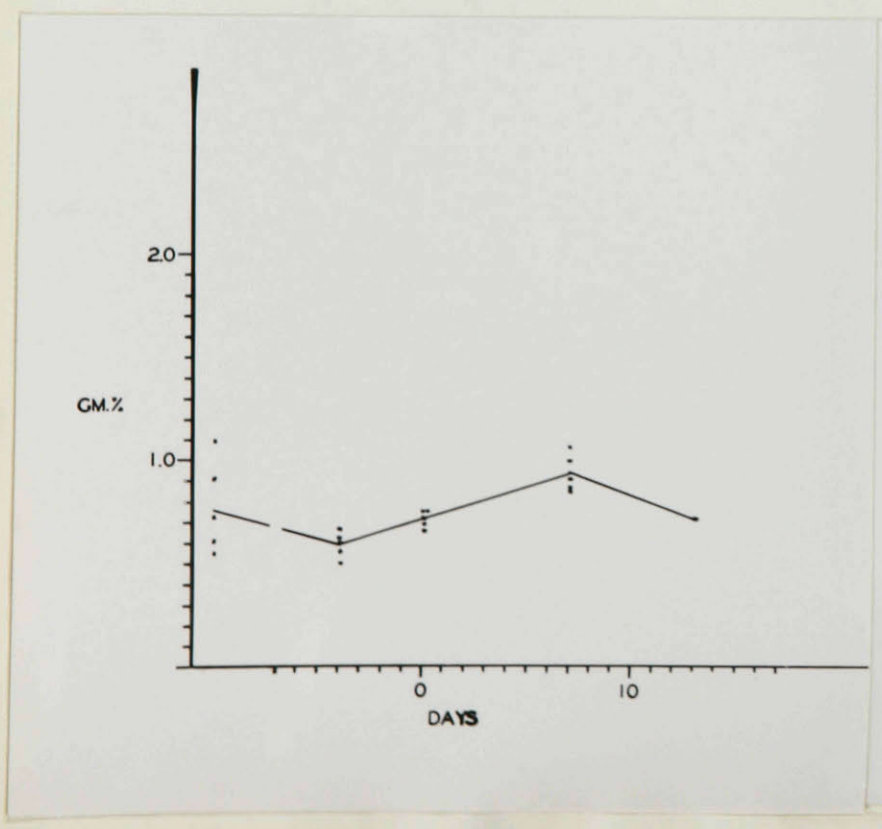
50.



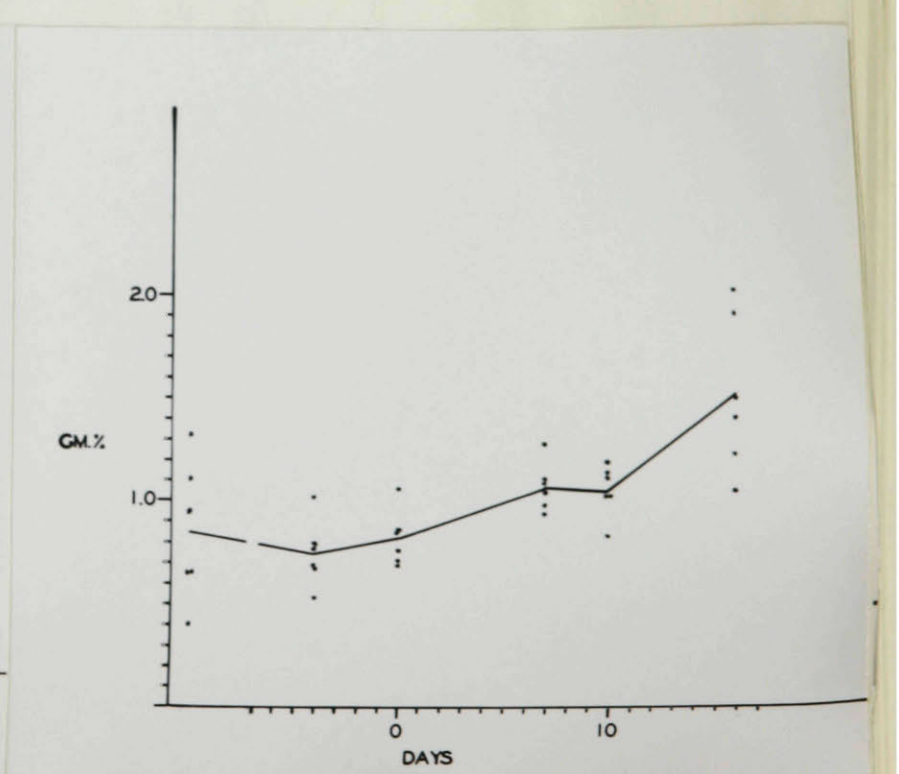
51.

TEXT-FIG. 50. G-globulins, 6 room temperature control animals, injected with normal saline Day 0. Samples spoiled for all but one animal Day 0.

TEXT-FIG. 51. G-globuline, 6 room temperature treated animals, injected Days 0 and 11.



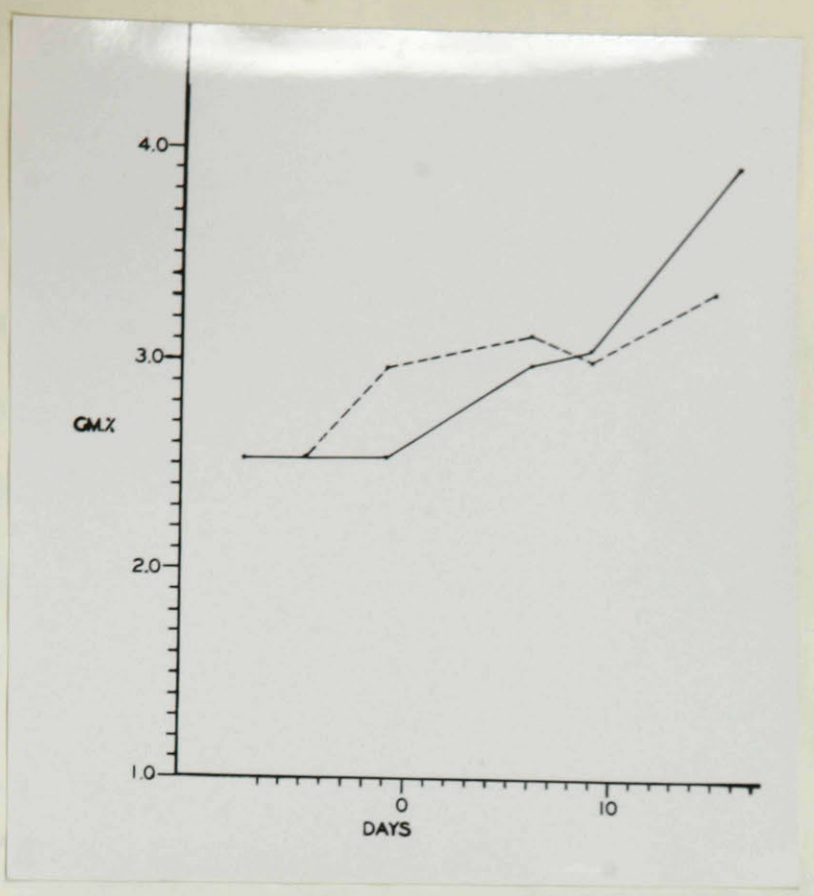
52.



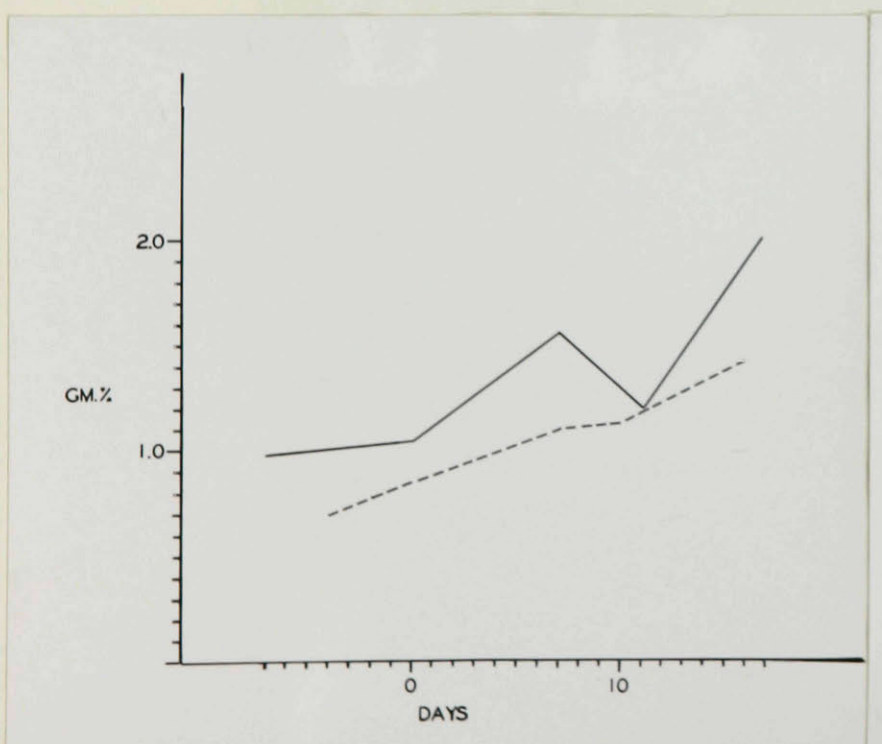
53.

TEXT-FIG. 52. G-globulins, 5 cold room control animals, injected with normal saline Day 0.

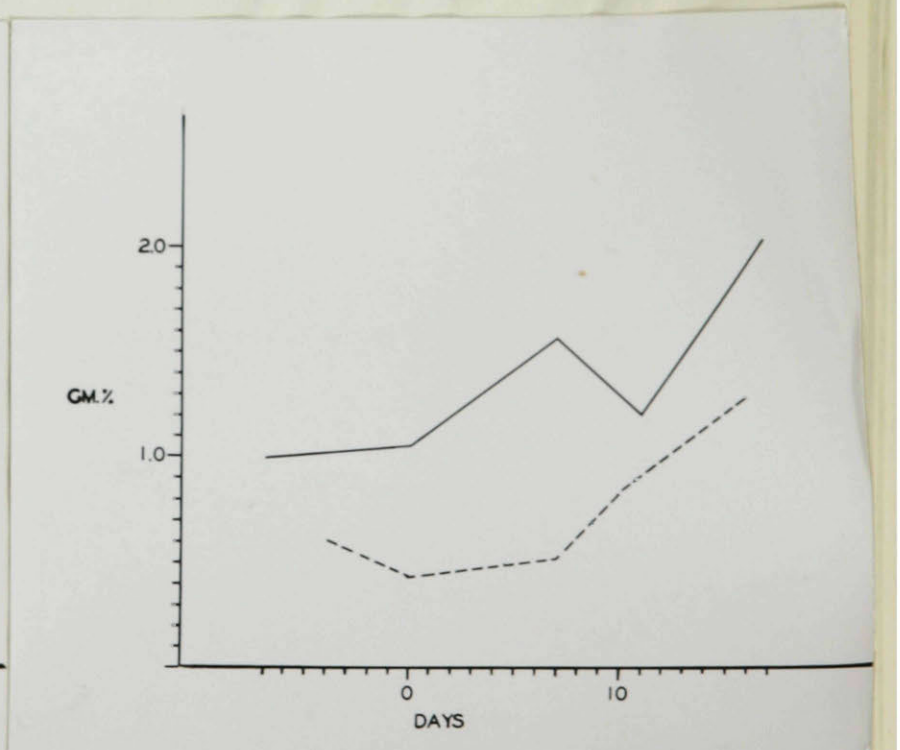
TEXT-FIG. 53. G-globulins, 6 cold room treated animals, injected Days 0 and 10.



TEXT-FIG. 54. Comparison of average total serum globulin levels for room temperature (solid line) and cold room treated animals (broken line). Latter group was placed in cold room Day -4.



55.



56.

TEXT-FIG. 55. Comparison of G-globulin levels in two animals with severe (+++) nephritis. Cold room animal is represented by broken line, room temperature animal by solid line.

TEXT-FIG. 56. Comparison of G-globulin levels of two animals, one with severe nephritis (+++) and one with very mild (+) nephritis. Animal with severe nephritis represented by solid line.

A total of 23 fractional serum protein analyses were made prior to nephrectomy on the animals of Experiment IV, when they were young (average weight 1.32 kg.) and had just been received in the animal quarters. The following average values were obtained:-

Total serum protein	4.42 gm.%	5.53 gm.%
Serum albumin	2.83 gm.%	3.64 gm.%
Serum globulin	1.59 gm.%	1.89 gm.%
A-globulin	0.42 gm.%	0.36 gm.%
B-globulin	0.47 gm.%	0.77 gm.%
G-globulin	0.71 gm.%	0.75 gm.%

The values for the young animals are those on the left, the values on the right being those for the same animals about one month later (average weight 2.09 kg.),just before they were divided into groups for treatment. During this period, there was an increase in serum concentration of albumin and globulin, the main increase in the latter appearing in the B-fraction.

Average values for the eleven animals of the cold room group, after four days in the cold, in the absence of other treatment were as follows:-

Total serum protein	5.41 gm.%
Serum albumin	3.09 gm.%
Serum globulin	2.32 gm.%
A-globulin	0.84 gm.%
B-globulin	0.66 gm.%
G-globulin	0.82 gm.%

The principal changes taking place in this period were a fall in serum albumin, coupled with a rise in globulin. Most of the globulin rise was found in the A-fraction.

That these changes were probably an effect of exposure to cold, and not of blood-taking (the only other procedure to which the animals were subjected) was indicated by values at a corresponding interval from eleven room temperature control animals:-

Total protein	5.32 gm.%
Albumin	3.43 gm.%
Globulin	1.89 gm.%
A-globulin	0.55 gm.%
B-globulin	0.37 gm.%
G-globulin	0.95 gm.%

Seven days after the first globulin injection, five animals kept at room temperature showed the following average pattern:-

Total protein	6.42 gm.%	5.69 gm.%
Albumin	3.91 gm.%	3.02 gm.%
Globulin	2.51 gm.%	2.67 gm.%
A-globulin	0.36 gm.%	0.63 gm.%
B-globulin	0.64 gm.%	0.96 gm.%
G-globulin	1.39 gm.%	1.08 gm.%

with corresponding values for animals treated in the cold on the right. In room temperature animals, the increase in total protein was due to an increase

in both albumin and globulin, but principally the latter. The globulin increase was almost entirely in the G-fraction. In cold room animals there was a fall in albumin, with an even more marked rise in globulin, in which both B- and G- fractions participated. The A- fraction was slightly lower than that found earlier in cold room animals, though still almost double that of room temperature animals.

In determinations made immediately before the second injection of bovine globulin, the following values were obtained (room temperature animals in left-hand column, cold room animals in the center, and cold room controls on right):-

Total protein.....	5.84 gm.%	5.46 gm.%	5.23 gm.%
Albumin	3.20 gm.%	2.91 gm.%	3.12 gm.%
Globulin	2.60 gm.%	2.55 gm.%	2.11 gm.%
A-globulin	0.56 gm.%	0.75 gm.%	0.72 gm.%
B-globulin	0.71 gm.%	0.73 gm.%	0.46 gm.%
G-globulin	1.36 gm.%	1.06 gm.%	0.93 gm.%

Both groups showed a fall in serum albumin, perhaps related to proteinuria, which was considerable in both groups at this time. The principal difference between the two groups was in the A- and G- fractions. The former is lower in the room temperature animals, while the latter is higher. However, the A- globulin in the cold room control animals at this time was practically the same as in the treated group, so the effect on this fraction may be referable to the exposure to cold, rather than treatment.

Analyses of sera drawn just before the animals were killed gave the following results for room temperature and cold room globulin-treated animals (left and right columns, respectively):-

Total protein.....	6.88gm.%	6.03 gm.%
Albumin	3.15 gm.%	3.19 gm.%
Globulin	3.73 gm.%	2.84 gm.%
A-globulin	0.60 gm.%	0.68 gm.%
B-globulin	1.36 gm.%	0.62 gm.%
G-globulin	1.77 gm.%	1.54 gm.%

At this point, there was a markedly increased serum globulin level in animals treated at room temperature (Text-fig. 54), as compared with similar animals kept in the cold. Both B- and G-globulins participate in this increase in room temperature animals, whereas, the G-globulin fraction is the only one increased in the cold room animals.

Since the number of animals in both globulin-treated groups on which serum protein studies were done is small, it is difficult to be certain of any relation between changes observed, and the development of nephritis. During the course of treatment, the G-globulin fraction showed a greater elevation than other fractions, and this was more marked in animals treated at room temperature. It may be of some significance that three of the six animals in this group showed severe nephritis (+++ to ++++), whereas only two of the six animals treated in the cold showed comparable lesions. G-globulin values for two pairs of animals on which complete sets of analyses

are available are shown in Text-figs. 55 and 56. In the two animals which had lesions of about equal severity (+++), the levels run fairly close together (Text-fig.55). On the other hand, G-globulin values for an animal with severe nephritis (+++) are considerably higher than those of an animal with very mild (+) nephritis (Text-fig.56).

VII. DISCUSSION.

In these experiments, the primary task has been that of producing acute diffuse glomerulonephritis in rabbits. After this had been done, the clinical course of the experimental disease was plotted. Other experiments were designed to evaluate the effect of continuous exposure to cold upon the incidence and severity of experimental nephritis. Preliminary studies were then made of the effect of general, moderate dietary restriction upon the disease, and a separate study was carried out in an attempt to delineate some of the early morphological manifestations of acute experimental nephritis.

There seems little doubt that the diffuse glomerulonephritis which appeared in unilaterally nephrectomized rabbits given two large intravenous injections of bovine serum gamma globulin, was the result of this treatment. Similar lesions were not found in a single one of 34 unilaterally nephrectomized control animals, some of which were allowed to survive for long periods. Nor were lesions found in any of the kidneys surgically removed before treatment was begun. Focal interstitial infiltrations and pyelonephritis were encountered in some animals, similar to that reported by others(117). Such lesions are completely lacking in the diffuse distribution which is an essential feature of the experimentally induced nephritis, and was never a cause of diagnostic confusion.

Acute diffuse glomerulonephritis occurred in 18 out of 24 animals treated at normal room temperature (65° to 70°F.). This represents an

incidence of 75%, within confidence limits of 47 - 93%, at a Probability of 0.005. By the ordinary criteria applicable in human pathology, the disease was classed as severe in 7 of the 24 animals, an incidence of 29% within confidence limits of 9 - 57%. These results are in keeping with the successful accomplishment of the initial experimental objective.

Other results make it appear very likely that the incidence and severity of this experimental disease were increased when animals were exposed continuously to cold throughout the treatment period. Nephritis occurred in 100% of 16 animals treated in this way. Using a 1:100 Probability factor, it appears just possible, though unlikely, that this result might have occurred fortuitously. The incidence of severe lesions in these same animals (56%) again coincided with the upper confidence limit for an unlimited number of similar animals treated at room temperature(233). It thus appears probable that both incidence and severity of lesions were greater in animals treated in the cold.

It should be emphasized that in the estimations of incidence and severity of lesions, grading was done before any statistical calculations had been made, rendering it unlikely that <u>a priori</u> reasoning was involved. Furthermore, in the grading criteria employed, the difference between severe (+++ and ++++) and mild (+ and ++) lesions was so striking as to make incorrect classification in these categoties very unlikely. It was more difficult to make this decision in the case of mild (+) lesions, and for this reason, where the diagnosis was considered doubtful, nephritis was considered not to be present.

The results of Experiment V appear to indicate complete prevention of nephritis resulting from moderate restriction of the animals' food and water intake. All other procedures in this experiment were carried out in the same manner as in previous experiments in which nephritis had been produced. The amount of bovine globulin injected was the same in relation to the body weight of the animal, and the particular lot of globulin used was the same as that which had given rise to nephritis in other experiments (Experiment IV). The animals used appeared comparable in every way to those used in other experiments, and were obtained from the same dealer who supplied animals for Experiment IV. It, therefore, seems unlikely that a strain difference in susceptibility was involved, such as has been found in experimental nephritis in rats(173).

Explanation must be sought therefore for three main observations emerging from these experiments:-

1. The failure of all animals treated at room temperature to develop nephritis.

2. The increase of incidence and severity of nephritis in animals treated in the cold.

3. The complete prevention of nephritis by the simple expedient of moderate dietary restriction.

It is possible, of course, that all observed differences in incidence and severity are fortuitous, and unrelated to variations in treatment. The application of statistical methods of analysis to such

small numbers can be misleading. However, at probability odds of 1:100, it seems quite unlikely that the observed differences could have occurred anyway. There remains, however, the long-shot possibility that they might have.

The first of the above problems may well be the most important one, and it is concerning this that the smallest amount of relevant information is available. Despite numerous observations made during the course of the experiments, nothing was found which seemed to give a clue as to which animals would develop nephritis, and which would remain free of the disease. It must, therefore, be assumed that the important information needed on this point remains concealed among the unknown etiologic factors covered by the term 'constitutional differences'.

Considerably more information is available with respect to the differences in incidence and severity related to exposure to cold, and to dietary restriction. Assuming that these differences are in fact resultants of the added factors, two possibilities present themselves. The potentiation by cold, and the inhibition by diet may be the result of opposite effects upon one particular part of the mechanism by which nephritis is produced, or they may be the result of entirely different and unrelated functions. Since this mechanism is at present imperfectly understood, the sounder assumption would seem to be that the differences are opposite effects mediated by a common factor.

Hypersensitivity is the common factor shared by all experimental diffuse glomerulonephritis thus far reported (see: pp. 36 to 57). All have resulted from the intravenous injection of animals with rather large amounts of foreign protein. Furthermore, a number of quite different substances have given rise to nephritis. Among these are normal horse serum (148, 149, 151, 153, 154), normal duck serum (151), anti-kidney sera (160, to 180, 183), anti-placenta serum (185) and horse (152) and bovine (156, 157) serum gamma globulins. Although some of these materials contain more or less specific anti-kidney antibodies, most of them do not. In the present experiments, anti-kidney antibodies could not be demonstrated in the bovine serum gamma globulin used to produce nephritis. Moreover, the nephritis produced by the injection of anti-kidney serum follows virtually the same clinical course(170, 234) to that produced by bovine serum gamma globulin. In both, the urinary manifestations of nephritis do not appear until 7 - 10 days after the injection of protein. In both, the nephritis appears at about the same time as antibodies to the injected protein become demonstrable (171, Experiment III, above). It has been found that the inhibition of antibody production by X-radiation will also prevent the development of so-called nephrotoxic nephritis(171).

Kay(171) explained these features of nephrotoxic nephritis by postulating fixation of the injected anti-kidney antibody. Later, when antibodies to the injected serum had formed, they produced nephritis (according to Kay) by reacting with the injected antigen, which presumably had remained fixed in the kidney throughout this period. Tentative support

was given to this concept by the work of Sarre and Wirtz(179), who found that nephrotoxic nephritis could be completely prevented if the renal artery were clamped for a short period during the injection of serum. It was presumed that this procedure prevented the local fixation of anti-kidney antibody. In more recent observations, Roda et al. (234) supported the Kay hypothesis by demonstrating an increased protein content of renal tissue following the injection of anti-kidney serum.

An entirely different hypothesis is put forward here, which appears to offer partial explanation of the production of nephritis in animals injected with either nephrotoxic serum, or with other serum proteins. In the present experiments efforts to demonstrate anti-kidney antibodies in the injected globulin, or in the sera of treated animals, were unsuccessful. It is considered unlikely,therefore, that the mechanism proposed by Kay could have been operative.

It is suggested that the development of nephritis in animals treated by any of these methods may be related to the amount of globulin in the injected serum, rather than to its content of antibodies. Anti-kidney serum (being immune serum) is almost certain to have a higher globulin content than normal serum. It has been found that injection of animals with foreign serum globulins(152, 156, 157) may result in nephritis, and the disease has also appeared following the injection of large amounts of normal duck serum(151) (i.e. large amounts of duck globulin). In experiments in which horse serum injection has resulted in nephritis, large amounts of serum have been used, and the globulin content of horse serum is higher (3.5 to 4.5%) than that of most other sera.

It can be further suggested that the production of experimental nephritis may be related ultimately to the quantitative antibody response of the organism, rather than to the particular antigen used to elicit the response. The globulin content of various inocula producing nephritis may be important in furnishing anti-body-building material of high biological potency, given at the same time as the antibody response is stimulated. The quantitative antibody response has been but little investigated in relation to nephritis, although Bukantz et al.(236) have recently found that the production of allergic arteritis in animals appears related to the amount of antibody produced.

It has been seen that antibody production may be increased in rabbits by repeated chilling(228), or by continuous exposure to dry cold(229). The work of Schoenheimer et al. (204) indicates that the globulin (and therefore, presumably the antibody) fractions of the serum participate in general metabolic exchanges at about the same rate as do other serum and tissue proteins. Furthermore, Cannon et al.(205) have shown that rabbits maintained on a low protein diet have an impaired capacity for antibody formation. Thus, animals exposed to cold, while on unlimited dietary intake, might have a generally increased metabolic turnover, associated with a corresponding increase in antibody activity. By the same argument, dietary restriction involving reduction in protein supply, might be expected to result in impaired antibody production.

Some support is given this view by the present experimental results. An increased metabolic combustion of ingested food is indicated in the animals of Experiment V, which were maintained on restricted food intake. Weight gain in animals kept in the cold room during the experimental period was only 20% of that of otherwise comparable animals kept at room temperature. On the other hand, the animals of Experiments III and IV, kept in the cold room while on ad libitum food intake, gained approximately the same amount as animals kept at room temperature. It is likely, therefore, that in order to maintain this rate of weight gain, a general increase in metabolic activity must have occurred, and that associated with this there may have been a related increase in antibody formation.

Although quantitative antibody studies have not been done, there is some indirect evidence on this point in the present results. In the serum protein studies in Experiment IV it was found that untreated animals exposed to cold showed an increase of total serum globulin. This was most marked three and ten days after the animals were placed in the cold room, and had returned to normal after 17 days. The main globulin increase was in the fraction considered by Wolfson et al.(232) to correspond with the alpha globulin component. A much more marked globulin increase was found in all animals injected with bovine serum Fraction II (gamma globulin). In this instance, the increase was mainly in the fraction precipitated by 1.39 M. ammonium sulfate, which Wolfson et al. consider equivalent to serum gamma globulin. This increase was at its highest point in sera drawn at

the end of the experimental period (i.e. 6 days after the second injection), but was also found in sera taken 7 and 10 days after the initial injection. Since it occurred on occasions when antibodies to the injected protein were known to be present, it seems unlikely that the increase represented injected globulin. The rise in G-globulin* was rather more marked in animals treated at room temperature than in those treated in the cold.

Nevertheless, there appears to have been some correlation between the G-globulin levels and the severity of nephritis. These values have been plotted for two pairs of animals of the same sex and weight. One pair (Text-fig. 55)+ showed nephritis of about equal severity (+++) at autopsy. In the second pair, one animal had severe nephritis (+++), while the other had much milder (+) lesions. (Text-fig. 56)+ In these, it is seen that the G-globulin level in the animal with mild lesions is much lower on all occasions than that found in either of the animals with severe lesions.

These findings, though not sufficiently impressive to be conclusive, are in keeping with the suggestion that quantitative antibody response is involved in the production of nephritis.

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* Designated thus to distinguish it from true gamma globulin, the estimation of which must be done by electrophoresis. Wolfson et al.(232) consider the two equivalent, but this has not been demonstrated conclusively in the present work.

+ Page 149.

Evidence of antibody inhibition due to dietary restriction was found in these experiments. All animals on unrestricted diet in which antibody tests were performed ten days after the initial globulin injection had positive tests at this time (Table XI), page 130). On the other hand, only six of twelve animals on restricted diet showed detectable antibodies at this time, and only one of these was an animal treated in the cold. Even the positive reactions recorded in this group were less pronounced than those found in other groups. It is perhaps worthy of emphasis that this result occurred even though the amount of dietary restriction was relatively slight. Weight gain in the room temperature animals was about half that of controls, whilst the weights of cold room animals remained almost stationary. Insofar as the two experiments are comparable, these results are in agreement with those of Cannon et al.(205).

There is no evidence from these experiments concerning possible participation of Selye's General-Adaptation-Syndrome. The thymus and spleen weights of our animals nearly all fell within normal range, suggesting that if the adaptation syndrome was involved, the stage of resistance had been reached. It has recently been reported that administration of adrenal cortical extract to mice is associated with an increase of total serum protein, without significant differences between the various fractions(237). It has also been found that the administration of cortisone is without apparent influence on experimental nephrotoxic nephritis in rats(238). These results, as well as our own, leave the relation of the adaptation syndrome to experimental nephritis undetermined.

The evidence presented above is no more than suggestive, but is in keeping with the assumption that the development of experimental nephritis is related in some way to quantitative antibody response. The question of the mechanism by which this relationship may actually bring about lesions must be left open for the time being. It is suggested that more detailed investigation of this aspect of the problem may yield more clear-cut results, from which, perhaps, more definite conclusions may be drawn.

The results of Experiment V, in which nephritis appears to have been prevented by the simple expedient of restriction of diet, may contain a warning for future investigations. In the experimental evaluation of any treatment intended to prevent or inhibit nephritis, it seems essential to establish that any effect produced is not due simply to interference with the animals' appetites, since it appears that this alone may produce a result which will be erroneously ascribed to some other feature of the treatment.

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It was stated in the introductory portion of this communication that the ultimate objective of the experiments carried out was the advancement of the knowledge of acute diffuse glomerulonephritis in man. Even tentative conclusions in this regard may only be drawn if it can be shown that the experimental disease is similar in all important respects to human nephritis. Even a brief comparison of the clinical and morphological features of the two conditions shows that this is indeed so.

The urinary findings of proteinuria, cylindruria and haematuria are similar qualitatively and quantitatively in both instances. In the experimental disease impairment of renal function with accumulation of blood urea nitrogen occurred in about the same small proportion (6%) of animals as is found in humans with nephritis (3 - 5%). In some of the animals some degree of cardiac enlargement was found, suggesting early hypertension, such as may also occur in human nephritis. Serum protein analyses showed a fall in albumin and elevation of globulin similar to that found in humans, and, if our interpretation of the results of chemical fractionation is correct, a comparable rise in gamma globulin(63).

The rapid onset of the disease, and absence of marked oedema, suggest that this experimental nephritis is probably in the same general class as human Type I nephritis(6). (See Table II, page 13).

The morphological findings in the kidneys of our animals appear also to correspond with those of human Type I nephritis. There was considerable renal enlargement (see Frontispiece), and the kidney was pale and

yellowish-white in many cases. On microscopic examination all the principal features of human Type I nephritis were found in our animals(6,19). In both, the glomerular damage is diffuse, with nearly equal involvement of each tuft in all glomeruli. There was glomerular ischemia, endothelial and epithelial swelling and proliferation. The degree of this similarity is seen in a comparison of Fig. 9 (human Type I nephritis) with Figs. 2 - 8, in which the experimental lesions are illustrated. Epithelial proliferation was usually marked. It had often led to adhesion of the tufts, glomerulo-capsular adhesions, and outspoken crescent formation. In none of the kidneys in which nephritis was found could the condition be considered the almost entirely intracapillary type of lesion which Ellis(6) and Davson and Platt(19) found almost constantly associated with the clinical features of Type II nephritis.

It is considered, therefore, that any contribution to the evaluation of the pathogenesis of this experimental disease may well have important bearing upon human glomerulonephritis.

In Experiment II of the present work, animals were killed on the day when at least some of them might have been expected to be developing early changes of acute nephritis. The morphological findings in the kidneys of these animals are in general agreement with those of previous experimentors(160,183,184). There appeared to be a diffuse glomerular capillary engorgement, together with a local increase of polymorphonuclear leucocytes. The glomerular leucocyte content was increased about ten times, in comparison

with normal kidneys. It is considered unlikely that this increase could be accounted for on the basis of general leucocytosis, or of variation in thickness of the sections examined. Similar findings have been reported by Dunn(92) in early human cases of acute nephritis. Support is thus given to Dunn's contention that the fundamental tissue response in early acute nephritis is probably an inflammatory one, similar to inflammatory responses in other tissues.

Blood coagulation studies were carried out in these experiments on the assumption that altered blood coagulability might play a role in the pathogenesis of nephritis. This approach was suggested by the frequent finding of glomerular capillary thrombosis in both human(15) and experimental(160) acute nephritis. The report of a favourable influence upon experimental nephritis brought about by heparin therapy(195) lent further stimulus to this study. An apparent increase in blood coagulability was found in globulin-treated animals of Experiment I, during the first week after the initial globulin injection. However, further studies of the same type in animals of Experiment III failed to confirm this finding. At this point, it was concluded that the capillary tube technique which had been used in these determinations was probably unreliable, being too easily effected by external changes in temperature after the test was set up. Since more careful coagulation studies would have necessitated increasing the amount of blood already being drawn from the animals, this line of investigation was not pursued beyond this point. The recent publication by Forman et al.(235) does lend support to the earlier observation of increased

blood coagulability during the first week after a single globulin injection. These authors found that in rabbits injected with normal horse serum, there was a marked increase in blood platelets during the first week after a single injection, but that a corresponding rise did not occur after a second injection.

The possible role of blood coagulability changes in the pathogenesis of acute glomerulonephritis has not been clearly established. The evidence at hand does suggest that future investigation of this aspect of the problem might be pursued with profit.

It is tempting to suggest, on the basis of our experimental results, that dietary restriction may be expected to have a favourable effect upon human nephritis. If, however, the assumption is correct that quantitative antibody response is involved in the pathogenesis of the disease, such therapy appears to involve serious risks. Even though nephritis might be inhibited by such therapy, cessation of dietary measures before eradication of a presumed source of allergic insult might be expected to have disastrous results. The Amsterdam outbreak of acute nephritis(35) actually coincided with a period in which the population as a whole was in a state of dietary rehabilitation, an observation which offers some support for this suggestion.

A clue to the pathogenesis of human Type I nephritis may be suggested from the results of these experiments, and from those of others. The clinical and morphological manifestations of the human disease and of

experimental nephritis show striking similarities. The immunological insult inflicted upon the animals in our investigations was of a sudden and rather extreme character. This appears also to be true of the nephritis induced by massive horse serum injection (153,154), and by large doses of potent anti-kidney serum (160-163). In Type I human nephritis a history of known acute infection shortly preceding the onset of renal symptoms is usually obtained(6). Such history is usually lacking in cases of Type II nephritis. It seems reasonable to suggest, therefore, that a rather sudden, massive introduction of antigen, followed by a similarly large scale antibody production may be the fundamental processes underlying this type of nephritis. The fact that such insult may not be repeated could account for the high percentage of recoveries noted in this type of disease(6, 19).

On the other hand, experiments conducted by McLean et al.(154), in which continued small injections of horse serum led, after a prolonged period, to a diffuse glomerulonephritis showing considerable similarity to human Type II nephritis. Although many of the details of this work are not known to the writer, and it has not yet appeared in published form, it seems reasonable to suggest that the pathogenesis of Type II nephritis may involve continued, rather low-grade, allergic assult, rather than the acute variety associated with Type I nephritis.

The question of why some people develop nephritis, while others under apparently similar circumstances do not, may have some relation to the capacity for immune response, the factors governing which are incompletely understood

VIII. SUMMARY.

The literature concerning the etiology and morphology of acute diffuse glomerulonephritis in man and experimental animals has been presented, and theoretical consideration of the pathogenesis of this disease has been given.

Experimental work has been presented in which it is shown that acute diffuse glomerulonephritis, similar in its clinical and morphological behaviour to the human Type I nephritis can be produced in unilaterally nephrectomized rabbits by giving them two massive intravenous injections of purified bovine serum gamma globulin (Fraction II) at ten to twelve day intervals. 75% of animals killed were found to have diffuse glomerulonephritis and the disease was considered severe in 29%.

Other experiments suggest that the incidence, and the severity of this experimental nephritis may be increased if animals are kept in a cold environment throughout the experimental period, and given food and water ad libitum.

Results of a third series of experiments indicate that clinical and morphological manifestations of nephritis may be prevented by moderate general restriction of diet.

Preliminary observations of the early morphological changes of experimental nephritis were made, and it is suggested that these consist mainly of glomerular capillary dilatation and congestion associated with

leucocyte accumulation.

The thesis that the development of acute diffuse glomerulonephritis is related to the quantitative antibody response of the orgamism is discussed. It is also suggested that the pathogenesis of human Type I nephritis may be related to massive allergic insult, whereas Type II nephritis may be the result of long continued irritation of similar type.

IX. CLAIM TO ORIGINALITY.

The method employed to produce experimental acute diffuse glomerulonephritis has not been reported previously. For this reason, the main body of clinical and morphological observations on this type of experimental nephritis are entirely original. Studies of the effects of exposure to cold upon experimental glomerulonephritis have not been reported by others. The effect of general, moderate dietary restriction upon experimental nephritis is also an original observation. The hypothesis that acute nephritis may be related to quantitative antibody response does not appear to have been presented before, nor has the relation of experimental nephritides to human Types I and II nephritis been examined in detail by others.

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ALPHABETICAL INDEX OF FIRST AUTHORS.

APPENDIX.

Technical Procedures not fully described in Text.

NEPHRECTOMY.

Anaesthetic: Soluble Nembutal (Wyeth) was used as basal anaesthetic for all operations. A dosage scale of 1.0 c.c. of the prepared solution per 2.3 kg. was found satisfactory. Basal anaesthesia could be smoothly achieved when this was injected slowly intravenously. With rapid injection, respiratory arrest and loss of the animal were not uncommon. Open ether was then administered by means of a metal cone containing a cotton sponge lightly soaked with ether. Excessive struggling or respiratory arrest could be avoided by slow induction; it seemed particularly important not to completely cover the animal's nose when the cone was first put in place. In a few animals 1% procaine (without adrenalin) was infiltrated into the subcutaneous tissues about the area of incision, and next to the peritoneum when it was exposed. Either of these anaesthetics was quite satisfactory, the latter method having the advantage that it could be carried out by the operator, but having the disadvantage of being very slow. Local anaesthesia was abandoned after a few trials.

Sterilization: Lap sheets, skin towels, rubber gloves and gauze sponges were sterilized by autoclaving. Instruments and suture material were boiled, needles and scalpel blades were sterilized in 70% alcohol. Separate pairs of gloves were used in the earlier operations, but it was found later that washing the gloved hands in Dettol between operations allowed faster progress without change of gloves, and gave rise to no increase in infection rate.

Procedure: Midline abdominal incision was used in earlier operations, but this was later abandoned in favour of a posterior approach. The animal was tied down with the left flank upright, the skin was shaved and sterilized with iodine and alcohol. A longitudinal incision beginning just cephalad to the lower ribs and at the margin of the posterior longitudinal spinal muscles, was carried perpendicularly downward for a distance of 3 to 5 cm. Skin towels were clipped in place and the apponeurosis joining the oblique abdominal muscles with the longitudinal spinal group were incised. The centre of the kidney was then located by palpation, and the obliques over this region were split. Perirenal fascia was partially freed with blunt forceps, and the kidney delivered into the wound. The renal pedicle, consisting of renal artery and vein and ureter were tied with a single piece of silk. The incised apponeurosis was then closed with continuous 000 chromic gut suture on a curved fused needle. The skin was then closed with interrupted silk mattress sutures, or with Michel clips. Whichever method was employed, it was found essential to achieve accurate and complete skin closure. Failing this, infection of the wound usually supervened, with ultimate loss of the animal. No dressing was placed over the wound. Healing was usually complete and suture were removed after five to seven days.

PREPARATION OF 10% PURIFIED BOVINE SERUM GAMMA GLOBULIN SOLUTION IN NORMAL SALINE.

Purified crystalline bovine serum gamma globulin was received from Armour and Company (Chicago, Illinois) as a light, pale yellow dry powder. The amount of globulin required was weighed out in a beaker. About two thirds the amount of sterile normal saline required to give a 10% solution of globulin was then placed in another beaker. The globulin was added slowly, with continuous stirring, to the saline. When solution was complete, the whole was transferred to a volumetric flask, and both original beakers were washed into the flask until made up to the mark.

As soon as the globulin solution was thus made up, it was sterilized by passage through a large Seitz filter. Head and tail portions of the filtrate were cultured, and in no case were bacteria grown in the filtered solution.

BLEEDING AND SEPARATION OF SERUM.

While being bled animals were held firmly in a box of special design, constructed so that the animal's head protruded through a hole at one end of the box, more or less like that of a prisoner in stocks. The skin of the external surface of the ear was shaved with a razor blade, exposing the central ear artery and its terminal branches. The under surface of the ear was then swabbed with xylol, and a sufficient period allowed to elapse for florid vasodilation to occur throughout the ear. The xylol was then thoroughly rubbed off with 95% alcohol. A small slash passing obliquely across the distal end of the central artery was then made with fragment of sharp razor blade. The gushing arterial blood was collected into a test tube, or other appropriate receptacle.

After bleeding was finished, a small spring-type pinch-cock was placed on the animal's ear, above the bleeding point, and residual blood on the surface of the ear wiped away. After five minutes or so, the vessel was usually occluded by clot, the clamp was removed and the animal returned (gingerly) to his cage.

Blood thus collected was allowed to clot, and then to stand for one half to one hour. After this period, the upper end of the clot was freed from the wall of the tube with a piece of fine steel wire, or capillary glass tube. Where serological tests were contemplated, care was taken to use a fresh piece of wire, or capillary tube, to free the clot from each serum sample, in order to avoid possible cross-reactions. The

blood was then centrifuged at about 2,000 r.p.m. for 15 minutes, after which the supernatant clear serum was drawn off with a Pasteur pipette. Serum samples were then stored in frozen state at -20°C. until required.

BLOOD STUDIES.

Sedimentation Velocity: A sample of oxalated blood was used. Blood for this purpose was drawn into a 10 c.c. test tube onto the walls of which one drop of 5% potassium oxalate solution had been dried for every c.c. of blood to be collected. It was necessary to shake the tube for thirty seconds to one minute after bleeding, in order to ensure prevention of clotting. Sedimentation tests were always set up within two hours of the time the blood was obtained, in order to eliminate ,as far as possible, artefacts produced by rouleaux formation. The test tube was thoroughly agitated, and the proper amount of blood transferred with a pipette into a Wintrobe tube. This tube was set up in a rack, on which a spirit level indicated when the tubes were completely perpendicular. After exactly one hour, the height of the column of clear plasma above the red cells was read and recorded as the sedimentation velocity.

Erythrocrit and Leucocrit: The Wintrobe tube in which the sedimentation velocity had been determined was centrifuged at 1,200 r.p.m. for exactly 30 minutes. After this time, the height of the column of packed red blood cells was measured and recorded as the Erythrocrit. By the same method, the thickness of the layer of white blood cells on top of packed red cells was measured, and recorded as the Leucocrit.

FRACTIONAL SERUM PROTEIN ANALYSIS.

(Modified, from Wolfson *et al*) (232)

REAGENTS:

1. 23.0% Sodium Sulfate Solution, made up in distilled water at 37°C. Kept at 37°C. Fresh solution prepared every week.

2. 28% Sodium Sulfite Solution. Made up in distilled water at 28°C. Kept at room temperature.

3. Ammonium Sulfate Solution. 19.3% solution in distilled water, made up and stored at room temperature.

4. Biuret Reagent, Weichselbaum. 90 gm. Rochelle salt dissolved in about 400 c.c. accurately titrated 0.2 N. NaOH. Following solution, 10 gm. $CuSO_4.5H_2O$ added and dissolved. 10 gm. KI then added, and solution made up to two litres with 0.2 N. NaOH. Stored in rubber stoppered flask at room temperature.

5. Folin's Phenol Reagent. 100 gm. sodium tungstate and 25 gm. sodium molybdate ($Na_2MoO_4.2H_2O$) dissolved in 700 c.c. water. 50 c.c. 85% phosphoric acid and 100 c.c. concentrated hydrochloric acid added.

Boiled with reflux condenser (stopper protected with tinfoil wrapping) for 10 hours. 150 gm. lithium sulfate, 50 c.c. distilled water and few drops liquid bromine then added. Boiled without condenser for 15 minutes to drive off excess bromine. Cooled, diluted to 1 litre and filtered. Finished reagent was pale yellow in colour. Was stored in glass-stoppered bottles in refrigerator.

Procedure:

1. Total Serum Protein:
   (a) 0.4 ml. serum pipetted into 10 ml. volumetric flask, then filled to mark with distilled water. After thorough mixing,
   (b) 5.0 ml. transferred to colorimeter tube. 5.0 ml. Biuret reagent added, and tube shaken well.
   (c) Blank prepared as above with 5.0 ml. distilled water.
   (d) Solution allowed to stand 40-50 minutes, then read on colorimeter at wavelength of 540 millimicra (Evelyn photoelectric colorimeter was used).
2. Serum albumin plus A-globulin:
   (a) 4.6 ml. 23.0% sodium sulfate solution placed in test tube. 0.4 ml. serum added by pipette, and mixed.

(b) After 20 minutes, solution filtered through double Whatman No. 40 filter paper. 2 or 3 filtrations sometimes required to clear filtrate of precipitated globulin.

(c) 2.5 ml. filtrate transferred to colorimeter tube, mixed with 2.5 ml. distilled water.

(d) 5.0 ml. biuret reagent added, and colorimeter reading made as in Step 1, using same blank.

3. Serum Albumin:

(a) 9.6 ml. 28% sodium sulfite solution placed in test tube, and 0.4 ml. serum added as before, and mixed.

(b) After 20 minutes, solution filtered as in Step 2.

(c) Analysis carried out using 5.0 ml. filtrate.

(d) Blank prepared with 5.0 ml. 28% sodium sulfite solution, and 5.0 ml. biuret reagent.

4. Serum Total Globulin:

Obtained by subtracting value for serum albumin (Step 3) from value for total serum protein (Step 1).

5. Serum A-globulin:

Obtained by subtracting value for serum albumin (Step 3) from value for albumin plus A-globulin (Step 2).

6. Serum B- plus G-globulin:

Obtained by subtracting value for serum A-globulin (Step 5) from serum total globulin (Step 4).

7. Serum G-globulin:

(a) 9.6 ml. ammonium sulfate solution placed in test tube, and carefully overlaid with 0.4 ml. serum. Mixed by slow shaking to point of apparent maximum turbidity.

(b) Then placed in incubator at 37°C. for 20-24 hours to complete precipitation.

(c) Again agitated on removal from incubator, and filtered through double Whatman No. 40 filter paper. Filtrate discarded.

(d) Precipitate washed with 3 successive 4 ml. lots of ammonium sulfate solution.

(e) Funnel with filter paper and precipitate placed in top of 50.0 ml. volumetric flask. Hole poked in bottom of filter paper with clean, pointed glass rod, and precipitate dissolved into flask with about 25 ml. 0.05 N. NaOH, being careful to unfold paper and get all of precipitate. Flask made to mark with distilled water.

(f) 2.0 ml. solution from flask transferred to colorimeter tube. 12 ml. distilled water added, shaken, 1.0 ml. 5 N. NaOH, then 1.5 ml. Folin's phenol reagent.

(g) Made up to 25 ml., shaken, and read on colorimeter, using filter with wavelength of 660 millimicra.

(h) Total protein value by Folin's reagent obtained by carrying out analysis on 1.0 ml. of 0.4 ml. serum diluted to 50 ml. in volumetric flask.

(i) Total protein value by Folin's reagent obtained by carrying out analysis on 1.0 ml. (otherwise as in 'f' and 'g' above) of solution of 0.4 ml. serum in 50 ml. distilled water.

(j) G-globulin value: $\frac{\text{Step 7g value}}{\text{Step 7h value}}$ X Total serum protein (Step 1).

8. Serum B-globulin:

Obtained by subtracting serum G-globulin (Step 7) from serum B- plus G-globulin (Step 6).

CALIBRATION CURVES:

Separate curves were prepared for estimations with Biuret, and with Folin's phenol reagent.

For Biuret, the curve was calibrated using a series accurately prepared dilutions of a solution of purified bovine serum gamma globulin, the protein concentration of which had been determined by nitrogen estimation. Analysis of each of these samples was then carried out by the same procedure as that employed for estimation of total serum protein.

For Folin's phenol reagent, the curve was calibrated from a solution containing exactly 100 mg. of Tyrosine C.P. per litre. Graded quantities of this solution were then transferred to duplicate colorimeter tubes, and the analysis completed exactly as in Step 7 f and g above.

Each time a fresh solution of either of the color reagents used in protein analysis was made up, a new calibration curve was prepared using duplicate tubes. In all serum protein estimations in which Folin's reagent was employed, duplicate tubes were also used, and where the final colorimeter readings differed by more than 1.5 calibration points, the test was repeated. Early in the use of this technique, duplicate determinations were also made in all tests employing biuret. It was found, however, that there was extremely little variation between the tubes, and this procedure was abandoned. In all determinations a blank tube was prepared, and where variation in the blank value occurred from one day to the next, fresh solutions were prepared.

BLOOD UREA NITROGEN ESTIMATION.

From - Hoffman, W.S.:Photelometric Clinical Chemistry, New York, 1941, pp.90-97.

REAGENTS:

1. Urease Solution: Tablet of Arlco urease ground up in beaker with 10 c.c. of 0.05 M. Na_2HPO_4. The latter solution made by dissolving exactly 8.9 gm. $Na_2HPO_4.2H_2O$ in distilled water to make 1,000 c.c. in volumetric flask. Urease solution was used within 2 - 3 hours.

2. Zinc sulfate, 7.5% aqueous solution.

2. Sodium Hydroxide, 0.375 N.

4. Nessler's Reagent (Koch-McMeekin). Obtained ready for use from Anachemia, Montreal.

Procedure:

0.5 c.c. whole, oxalated blood pipetted into dry test tube.

0.5 c.c. urease solution added.

Tube rotated rapidly to ensure mixing, stoppered.

Placed in water bath at 45°C. for 15 minutes.

7.0 c.c. distilled water added, while shaking tube.

When laking of blood complete, 1.0 c.c. 7.5% zinc sulfate added, tube shaken, and 1.0 c.c. 0.375 N. NaOH added.

Tube stoppered immediately and vigorously shaken.

Stopper removed, and tube placed in beaker of boiling water, containing just enough water to reach level of tube contents. Boiled for 2 minutes, then cooled to room temperature.

A new curve was calibrated each time a new lot of Nessler's solution was put into use.

Where the initial determination showed a blood urea nitrogen value of more than 60 mg.%, the original sample of blood was diluted with an equal amount of distilled water, and, after mixing, repeat analysis carried out on this material. Final value, multiplied by dilution factor, was taken as true urea nitrogen level.