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Use of cortisone in the treatment of experimental glomerulonephritis in rats

Robert S. Behrns
University of Nebraska Medical Center

THE USE OF CORTISONE IN THE TREATMENT OF EXPERIMENTAL GLOMERULONEPHRITIS IN RATS

Robert S. Behrns

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INTRODUCTION

At the onset of this piece of work it was our objective to find out if a glomerulonephritis that had been induced in rats by injections of rabbit anti rat kidney serum was modified enough by treatment with cortisone to significantly increase the life span of rats treated with cortisone over the life span of those not so treated. Undertaking such work as this by inexperienced workers who are at the same time trying to obtain an education in medicine presents several difficulties. Foremost among these are lack of time when the experiment requires a good deal of time and inexperience in working with laboratory animals. It has been our misfortune in our experiment to have had a great deal of pneumonia in our rats; in fact, nearly all of our rats that died after surviving the initial shock of receiving the anti rat kidney serum died of pneumonia, which will be described later. Very few of our rats have died with a typical nephrotic syndrome nor have many shown pale, scarred, and contracted kidneys, which one expects to see in death from renal failure from chronic glomerulonephritis.

Although our original goal has not been attained in a pure and simple form, we have been able to make

certain observations that allow us to arrive at some opinions concerning the subject under discussion. Before discussing the experiment, however, it might be well to review the physiologic effects of cortisone, the etiology of glomerulonephritis in man and its pathology, and explain our reason for believing that cortisone might modify the course of this pathology.

CORTISONE

Although an extensive review of cortisone is not feasible here, I would like to consider most of the effects of cortisone since several of those due to high dosages of this hormone were seen in our rats. These results of cortisone therapy, at least in several instances, occur only with high doses, such as 200 mg. in and adult human. They will be considered in this order: Effects on carbohydrate, fat, and protein metabolism; electrolyte balance; lymphatic and hemopoetic systems; on various tissues and systems; connective tissue, inflammation, and healing; allergy and sensitivity; and stress, and resistance.

1. Effect of Cortisone on Carbohydrate Metabolism:

Cortisone has a diabetogenic effect upon the carbohydrate metabolism. It increases blood sugar levels and often causes glycosuria, though this usually doesn't

occur in humans without latent or frank diabetes (Sprague, 1). There is some indication that there may be a decreased renal threshold for glucose (Sprague, 1). Gluconeogenesis is increased since amino acid and fatty acid levels in the blood are up, according to Bartter (2) this is apparently an essential part of Selye's alarm reaction to increase the metabolic pool. This all contributes to the availability of a large energy resevoir resulting in increased glycogen and fat storage (Bartter, 2).

2. Effect on Fat Metabolism:

As stated above ketone bodies in the blood and fat storage increase under cortisone therapy. Sprague (3) reports that the storage of fat to give the moon face and buffalo hump appearance typical of Cushing's syndrome may appear even on an inadequate diet. Ingle (4) reported an increase in serum lipids from 19% to 61% in 8 of 10 patients with an accompanying though slower rise in serum cholesterol. Booker (5) and Sprague (3) likewise reported increases in serum cholesterol due possibly to liberation of cholesterol with vitamin C storage in the adrenal (Booker, 5) or to a decreased metabolic rate so often seen in patients on cortisone therapy plus decreased requirements of cholesterol esters for

synthesis of cortisone or related compounds (Sprague, 3). Decreases in serum cholesterol with an accompanying decrease in esters has been demonstrated with ACTH therapy (Sprague, 3).

3. Effect on Nitrogen and Protein Metabolism:

Cortisone and related compounds have a great effect on nitrogen metabolism. Shortly after administration of cortisone is begun urinary excretion of nitrogen increases greatly to reveal a negative nitrogen balance. In a study by Thorn (6) using ACTH this increase in urinary excretion of nitrogen increased up to 58%. This increase appears to be due to both catabolic and anti-anabolic effects on the protein metabolism. In experimental animals this causes a loss of both normal and abnormal protein (tumor protein) and results in an overall retardation of growth or a weight loss (Ingle, 4, 7, 8). In addition to the negative nitrogen balance these effects also cause increased blood levels and urinary excretion of amino acids and increased urinary excretion of phosphorus, and potassium (Bartter, 2). Whitney and Bennett (9) have presented some evidence that diets high in potassium chloride decrease the catabolic effect of ACTH on nitrogen metabolism in rats. According to Sprague (3) the body adapts

somewhat to the induced negative nitrogen balance since the peak nitrogen excretion in urine is not sustained. Marked weight loss in laboratory animals receiving high doses of cortisone are often due to infection resulting from decreased resistance (Selye, 10). According to Ingle (4) cortisone allowed rat muscle to function longer because it prevented formation of urea. It also necessitates an increase in liver arginase, which is necessary for urea formation and is involved in detoxification of amino acids. Under cortisone stimulation the serum albumin usually increases while the globulin decreases more so resulting in an increased A:G ratio with a decrease in TSP. Urine uric acid and creatinine excretion are also increased, however Sprague (1) contends that after two to three weeks of therapy cortisone and ACTH lose their effect of increasing urinary excretion of uric acid. Ingbar (11) states that this change is due to increased tubular excretion and decreased tubular reabsorption of uric acid.

4. Effect on Electrolyte and Fluid Balance:

Selye (12, 13) contends that cortisone and similar compounds are primary gluco-corticoids, while desoxycortico sterone (DCA) and similar compounds are mineralo-corticoids. By this he means that cortisone has

its main action on carbohydrate metabolism (including the fat and protein metabolism which effect this), while DCA has its main effect on electrolyte balance, though each compound has properties of the other, but only to a slight degree. According to Sprague (3) cortisone has about one fiftieth the Na Cl and water retaining power of DCA. Antagonistic effects of DCA and cortisone on electrolyte metabolism as well as on artificially induced lesions in the cardiovascular and renal tissue have been described by Davis (14) and Selye (12, 13, 15). It would be well to recall the statement made earlier that changes mentioned here occur only with higher doses of cortisone. According to Sprague (1) prolonged administration (to 30 days) with 100 mg. cortisone daily in an adult usually causes no change in electrolyte balance or water metabolism unless the patient being treated is suffering from adrenal insufficiency. Doses of ACTH producing similar clinical changes as in rheumatoid arthritis, rheumatic fever, etc. produce greater changes in electrolyte and fluid balance than does cortisone since it stimulates production of DCA as well as cortisone. (Sprague 3, Selye 13).

Perhaps one of the first effects on the electrolyte metabolism is that of increased excretion of

potassium. This increase roughly parallels the increase in urine NPN (Ingle, 8) and as stated earlier probably results in part from increased protein catabolism and probably from an increased renal excretion (Ingbar, 11), this K is probably mainly intracellular (Sprague, 1; Bartter, 2). There is likewise an increased urinary excretion of P and Ca. Cortisone usually causes a transcient (2-4 day) retention of Na Cl and water often followed by an increased excretion of them (Sprague, 1; Ingle, 4). Without concommitant heart disease the Na retention is usually insignificant (Sprague, 3). With high doses of cortisone a hypokalemia and hypochloremia often develop producing an alkalosis. Sprague (1) decreased the K loss by concurrent administration of 25 mg. testosterone proprionate. Ingbar (11) thought K loss was decreased with a low Na diet which resulted in a decreased renal tubular exchange of K with Na. In patients given DCA on low Na diets receiving cortisone Ingbar (11) did not get increased K excretion.

5. Effect on hemopoetic and lymphoid systems:

With cortisone therapy there is usually some degree of lymphopenia, regression of lymphoid tissue, a decrease in eosinophils and often a slight polymorphonuclear leucocytosis. According to Sprague (3) this decrease

in lymphoid tissue results from increased destruction of lymphocytes. Eosinopenia results from the effect cortisone has of decreasing the allergic response. The response of the patient to cortisone is more accurately measured by the decrease in circulating eosinophils since much larger doses are required to cause a decrease in circulating lymphocytes. With cortisone therapy there is usually a decreased hematocrit and hemoglobin thought to be due to hemodilution (Sprague, 1).

6. Effect on Various Tissues and Systems:

The effects of cortisone on various tissues and systems probably result from both specific primary physiologic changes and changes resulting from altered metabolism of fat, protein, carbohydrate, electrolytes, and water. On the skin acne and hirsuitism are often produced from androgenic effects of cortisone, although baldness is often produced at the sites of injection in animals, probably from protein catabolism (Ingle, 4). Cutaneous striae, increased pigmentation may result and less often keratosis pilaris (sand paper like skin) (Sprague, 1).

Cortisone causes an increased secretion of uropepsin in the urine. It also stimulates the appetite. The increased formation of pepsin plus protein catabolism

a poor tissue response to inflammation, and poor healing caused by cortisone given a patient who needs just this boost to develop an ulcer has been responsible for an ulcer developing (Sprague,2; Selye, 13). Perforation with peritonitis has occurred with no symptoms other than absent peristalsis and decreased liver dullness (Beck, 16).

Effects of cortisone upon blood pressure and the heart are not marked in the normal person. However, most patients with renal disease develop or show a more marked hypertension while receiving the hormone (Sprague, 1, 3). This effect is decreased somewhat with a low salt diet. Cardiac failure is sometimes precipitated by cortisone therapy in patients with antecedent heart disease. This complication also is decreased with low salt diets (Sprague, 3).

Excitability of the brain is increased by administration of cortisone. Treatment with cortisone or ACTH is occasionally accompanied by convulsions, less often by coma. These people usually show an abnormal EEG. The most common psychiatric changes are a mild euphoria, restlessness, manic behavior, or depression. Psychotic reactions occur probably only in predisposed patients. The cause for all these is not

clear (Sprague, 1).

Almost every endocrine gland is effected by cortisone. It depresses the activity of the anterior pituitary. The action it has on other glandular tissue may be mediated in part through this depression, though most of these changes occur with ACTH therapy also (Sprague, 3). Usually thyroid function is decreased, though in Addison's disease it usually increases. As stated before the action of insulin is antagonized. Amenorrhea often occurs with this type of therapy as well as decreased libido in males. As stated before hirsuitism in females may occur due to androgenic effects of cortisone. Except for an increase in basophils in the hypophysis and the replacement of basophilic granules by lumpy masses of basophilic hyaline material and a decrease in the size of the adrenal cortex, principally in the fascicular and reticular zones, there are no marked changes in the endocrine glands in man. According to Ingle (4) there have been reports of increase in the weight of the thyroid and a decrease in the size of the genital organs of rats receiving ACTH or cortisone. Antagonistic effects of ACTH and cortisone with DCA and the somatotrophic hormone of the anterior pituitary will be discussed later.

7. Effect on Connective Tissue, Inflammation, and Healing:

The action of cortisone on the mesenchymal tissue and the reaction of this tissue to stress of one sort or another constitute most of the reasons for its use clinically. It is common knowledge that cortisone decreases tissue response to inflammation and delays healing. Just how cortisone causes such effects upon the connective tissue is unknown, though the effect this hormone has on protein metabolism probably plays some part (Ragan, 17). The fact that cortisone has no effect in reducing pre-existing granulation tissue (Spain, 18), however, tends to make on suspect that any such effect would be caused from antianabolic effects of cortisone and not by its catabolic effects on protein metabolism. The ability of cortisone to cause regression of lymphoid tissue seems to be specific for such tissue and is not usually shared by all mesenchymal tissue. It should also be noted that restoration of a positive N balance with testosterone proprionate does not alter the response of patients with rheumatic fever to cortisone or ACTH (Sprague, 3). At the site of stress in connective tissue in patients receiving cortisone one sees a poor fibroblastic response, fibroblasts

present appear undersized (Baker, 19), there is also a poor response by macrophages, decreased growth of capillary endothelium from what is normally seen, and a decrease in ground substance. Cortisone also exhibits noticeable anti hyaluronidase or an anti spreading activity. This action is antagonized by the somatotrophic hormone (Seyle, 10). Spain (18) noted that this poor response of the C. T. was similar in several ways to that seen in vitamin C deficiencies. (On the other hand Schaffenburg (20) pointed out the presence of similar symptoms in scurvy and adrenal insufficiency. These include: 1. Clinical symptoms (asthenia, adynamia, weight loss), 2. Disturbance in water (edema), salt (K retention), and carbohydrate and protein metabolism (decreased liver glycogen and increased blood urea nitrogen). Both Schaffenburg (20) and Booker (21) noted that ACTH and cortisone tended to decrease the effects of scurvy while DCA antagonized them.) Baker (19) believed that this action of cortisone was probably on peripheral cells and even on the interstitial matrix of the C. T. He believed the primary site was the fibroblasts. The local action was demonstrated by areas of baldness, impaired growth of granulation tissue, and atrophy of dermal C. T. at sites of administration;

these were not predicated bya nitrogen loss from the body. This decrease in fibroblastic activity was also noted by Creditor (22). In attempting to promote wound healing in patients receiving ACTH he injected hyaluronidase and penicillin into the wounds with the result that much fibrin was deposited between the wound edges, although invasion by fibroblasts and true healing did not occur. The failure of the C. T. to respond to injury, inflammation, etc. under influence of ACTH or cortisone probably accounts for the usefulness of these hormones in most of the diseases in which they produce beneficial results, though a full explanation for this is not available.

8. Effect on Allergy and Sensitivity:

Cortisone is often capable of causing marked relief in allergic diseases though in many instances it does not prevent allergic reactions from occurring. Rose (23) observed that cortisone decreased urine histamine while it increased urinary excretion of histidine. Somewhat contrary to this Grot (24) noted an increase in urinary excretion in both histidine and histamine after injection of histidine under cortisone treatment, that of histidine showing the greatest increase. This was due to decreased tubular reabsorption of these

compounds. Both Carryer (25) and Spain (26) found that cortisone had no effect upon preventing histamine formation during antibody-antigen union in vitro. Selye (27) experimentally significantly reduced the anaphylactoid reactions of single intraperitoneal injections of egg white in rats with cortisone; however, Gray (28) and Dworetzky (29) presented experimental evidence to show that it did not prevent the anaphlactic shock that followed succeeding doses of horse serum and egg white, and Friedlander (30) showed that it did not prevent bronchospasm in guinea pigs or whealing reactions in human skin caused by histamine. However, Stoerck (31) observed a decrease in the tuberculin reaction in guinea pigs and Marcus (32) a decrease in the Schwartzman phenomenon in rabbits proportional to the amount of ACTH or cortisone given. Stoerch (31) believed this was due to the degradation of antibody protein by cortisone while Marcus believed the decrease in the Schwartzman phenomenon was due to a decrease of the inflammatory response. Although there is a transcient increase in antibody titer following cortisone administration due to the increased destruction of lymphocytes, followed by a decrease in circulating antibodies (Ingle, 4) which continues through treatment

with ACTH or cortisone, it has been shown that at least certain antibody formation is not altered. Mirick (33) showed that neither of these hormones altered the formation of antibodies to pneumococcal polysaccharides although there was a decrease in serum globulin and skin sensitivity to the antigen. If Mirick's (33) observations hold true for all antibodies, it would appear that the decrease in skin reactions is due to a decreased response of this tissue to the inflammatory reaction as Marcus (32) believed. Decreased vascular damage from vascular reactions due to horse serum were noted in rabbits being treated with cortisone compared with those not receiving treatment by Ebert (34), however, exacerbations in the treated animals occurred when cortisone therapy was stopped. Ebert (34) feels that the suppression of effects due to the horse serum is due to non specific effects of cortisone and not to suppression of antibody formation or antibody and antigen union. Sprague (3) similarly believed that the beneficial effects of cortisone in allergic reactions are due to prevention of the C. T. response to stimulation. He does not believe cortisone has any antihistaminics properties. However, Selye (12), noted the similarity of results of ACTH and cortisone and the antihistaminics

in many conditions and suggested that it might be well to try the antihistamics in some of the diseases that have been treated effectively with ACTH and cortisone where antihistamics have not been used previously. It might be well to remember at this point that ascorbic acid has also been used in allergic diseases in the past (Bicknell, 35) though with less success than either cortisone or the antihistaminics.

9. Effect on Stress and Resistance:

Although the complete roles of the endocrine glands in the alarm reaction have not been worked out, observations concerning them, particularly the anterior pituitary and the adrenal glands, are very interesting and probably pertinent to this thesis. Selye (13) has pointed out that it is a non-specific reaction and occurs in a normal human or animal from any type of stress whether it be a burn, exposure to cold, infection, irradiation, emotional stress, etc. Bartter (2) pointed out that the effects of cortisone on the metabolism lead to a pool of glycogen at the expense of body protoplasm to create a supply of easily mobilizable energy; however other observations of Selye (10, 12) lead him to believe that a well adjusted reaction to stress involves a balance between ACTH, cortisone, DCA,

and the somatrophic hormone (STH). On the one hand Selye (10) showed that rats treated with high doses of ACTH or cortisone usually lost weight and died of infections due to normally non-pathogenic bacteria, usually infections of the lung and pleura. The lesions produced showed very little or no granulation tissue forming in an attempt to wall off the bacterial colonies. When STH was administered in conjunction with ACTH or cortisone no rats died. It was also noted that STH enhanced the spreading of hemoglobin in soft tissue while ACTH and cortisone decreased it.

High doses of DCA or STH, on the other hand, produced a weight gain with hypertrophy and hyperplasia of renal, hepatic, and splenic tissues (Selye, 10). In rats with one kidney removed and 1 % Na Cl solution continued high doses of STH produced nephrosclerosis, periarteritis nodosa, hypertension, and myocarditis. These results were decreased with similtaneous treatment with cortisone, but aggravated by both ACTH and DCA. It is also interesting to note that Selye (12) has prevented arthritis in rats produced by formaldehyde injected into the joint by eliciting the stress reaction previously with either forced exercise or exposure to cold, or with extrinsic cortisone, while continued stress,

such as prolonged exposure to cold has caused lesions similar to those produced by DCA or STH. These results make one wonder if the failure to maintain adaptation doesn't lie with a possible exhaustion of the gluco-corticoid side of a balance between these various hormones. The necessity of the two sets working in harmony has been pointed out in one instance by Selye (10), at least hypothetically. He points out that during invasion by bacteria cortisone decreases the spread locally by its anti-spreading effect while STH decreases the spread by promoting formation of granulation tissue. Though much remains to be uncovered concerning the balance between these hormones in the reaction of the body to stress, this hypothesis probably correctly shows that such a balance does exist and is very necessary.

USE OF CORTISONE IN GLOMERULONEPHRITIS IN MAN

At the onset of this discussion it might be well to review briefly the etiology and pathology of glomerulonephritis in humans and also explain our reasons for using cortisone in the treatment of the nephritis we produced artificially.

It is generally agreeed on that glomerulonephritis results from an allergy or hypersensitivity of the

kidney tissue involved to a bacterial product, possibly to an antigen formed by union of bacterial products with kidney tissue (Fashena and Harrison, 36). Glomerulonephritis has been produced experimentally by inoculating homolagous kidney extracts combined with killed streptocci (Fashena & Harrison, 36). The infection preceding, usually about one to three weeks, is most always caused by beta hemolytic streptococci, usually an upper respiratory infection, though staphylococcal skin infections and others, usually of the coccal group, may cause it. The development of high antibody titers during this interval has suggested an allergic cause of the disease also. It is interesting to note that during World Wa rs I and II the disease occurred with considerable frequency among men exposed to prolonged cold and exhaustion, (Fashena and Harrison, 36) though these factors are considered important by Fashena and Harrison only to the extent that they predisposed to the infection.

The disease usually begins in the first two decades, though an onset up to the age of 40 is not too uncommon. Mild cases very likely often go undiagnosed. It is characterized by proteinemia, casts and red blood cells in the urine, and sometimes by edema, impaired renal

function, and hypertension. In death from acute glomerulonephritis the kidney is usually swollen, the outer surface smooth, and often pale with small reddish dots from minute hemorrhages in the cortex. The cut surface usually shows a pale, bulging cortex and congested, reddened medulla. Microscopically the glomeruli are enlarged, bloodless, and quite cellular as a result of endothelial proliferation. Intracapillary fibers, eventually resembling collagen fibers, may appear. Often there is proliferation of the epithelial cells in the glomerular tufts, though this occurs less regularly and to a lesser extent than that of the glomerular endothelium. Adhesions between tuft and capsule may occur, especially if there is much proliferation of the cells of the epithelium of the capsular lining. Tubules show varying degrees of degeneration, usually from fatty changes and sometimes hyaline droplet accumulation. Protein and red cells are often present in the lumen of the tubules. Desquamation of tubular cells usually occurs. The interstitial tissue is usually edematous and infiltrated with leucocytes.

The acute form may progress into a subacute form which results in termination in uremia in a few months. The main features here are albuminuria, edema, and

hypertension resulting from renal insufficiency. At autopsy the kidneys are enlarged, pale and soft. The surface may not be smooth and the capsule may adhere in places. The cut surface reveals a pale, thickened cortex. Microscopically epithelial proliferation is prominent and crescents, formed by proliferation of capsular epithelium, are numerous. Adhesions obliterating the capsular space due to this growth are common. Hyalinized glomeruli are not common in this stage. Tubular degeneration and atrophy are common, tubular casts are often present. Interstitial material is slightly increased or not at all. Blood vessels likewise show slight or no changes.

Chronic glomerulonephritis may result from a slow progression from the acute phase; however, more commonly it results from repeated streptococcal infection with added renal damage after each infection. Often there is no history of acute phases which were mild and passed unnoticed. Patients with chronic glomerulonephritis often appear well until almost no renal function remains. Death is usually similar to that seen with subacute glomerulonephritis. Post Mortem examination of these kidneys reveal a small, scarred, pale kidney with a quite adherent

capsule. The cortex is narrow and scarred. Microscopically many glomeruli are represented by rounded hyaline masses, tubules are for the most part atrophic or have disappeared. There is a great increase in the connective tissue. Small and medium sized arteries show varying degrees of intimal and medial proliferation and thickening. In the more rapidly progressing cases the arteries are said to show more cellular intimal thickening and arteriolar necrosis, while the slower cases show mainly intimal fibrosis, splitting and reduplication of elastic lamellae, and arteriolar hyalinization. (This section on Pathology in Man from Anderson, 37).

RESULTS OF TREATMENT OF GLOMERULONEPHRITIS AND NEPHROSIS WITH CORTISONE IN MAN:

During the past three or four years cortisone and ACTH have been used to a fair degree in renal disease, though for the most part this has been for promoting diuresis in the edema of the nephrotic syndrome, mostly in children with nephrosis. It would probably be well to review a few of these articles not because of the occasional occurrence of glomerulonephritis and nephrosis together, but because beneficial changes are possibly not specific for just one of the diseases,

but may occur with both.

Encouraging results in the treatment of the nephrotic syndrome with ACTH have been reported (Metcoff, 38; Rapoport, 39; Hecker, 40; Barnett, 41; Farnsworth, 42; Bjorneboe, 43), while less encouraging results have been reported with cortisone (Rapoport, 39 and Leutcher, 44). Metcoff (38) used ACTH in such cases to see if ACTH would favorably alter the permeability and reaction of the glomerular basement membrane and if this alteration would lead to improved tubular cell function with a secondary renal hemodynamic adjustment. Observations by Bjorneboe (43) lend support to this idea. Bjorneboe and his associates observed that when human serum albumin was administered in these cases without previous treatment with ACTH an increase in proteinuria followed, but when ACTH was previously given proteinuria did not increase. These authors felt that the difference was due to the fact that the tubules and glomeruli had become less permeable with regard to protein.

Thorn (45) and his associates observed six patients in the nephrotic syndrome of chronic glomerulonephritis, four of whom were treated with ACTH while two were treated with cortisone. The former diuresed well, but

the latter poorly. He believed the diuresis was due to a "renal tubular fatigue." He supported this theory by the observations that after a few days of therapy with ACTH the kidneys show a marked reduction in sensitivity to DCA and posterior pituitary extract plus the fact that patients with Cushing's syndrome have an increased capacity for eliminating a test sodium load following administration of DCA. Thorn thought this decreased sensitivity might result from a qualitative change in the ratio of DCA and cortisone-like compounds by the adrenal cortex under continued ACTH therapy. He believed that increased amounts of extracellular fluid and electrolytes, a temporary adrenal insufficiency following cessation of ACTH or cortisone therapy, and decreased in over all tissue edema (including kidney) also favored diuresis and decreased proteinuria. Bjorneboe (43) supports Thorn's theory of "renal tubular fatigue" as well as Metcoff's belief discussed earlier, but did not believe decreased edema favored decreased proteinuria. Bjorneboe thought diuresis and decreased proteinuria to be independent processes since the diuretic effect could occur after a fall in proteinuria had begun. Metcoff thought a mild adrenal insufficiency following cessation of

therapy had nothing to do with diuresis since in his series diuresis usually began while therapy was going on. Nearly all observers agree that increase in extracellular fluid volume and increase in plasma electrolytes aid the diuresis that occurs. (It should be noted that the decreased responsiveness of the kidney to DCA agrees with Selye's (13) theory that cortisone and ACTH decrease the effect of DCA, possibly by decreasing the reactivity of cells to DCA. However, why ACTH, which also stimulates production of desoxycorticosterone is more effective than cortisone, remains to be seen. In Thorn's work cortisone was used in less satisfactory patients than ACTH.)

In the larger series (Metcoff, 38; Rapoport, 39) approximately 75% of the patients diuresed after treatment. Recurrence varied, though in Metcoff's series 50% remained without edema in three months and in 16 patients 6 were without recurrence in 6 months and 6 more without recurrence in 12 months. In five cases where cortisone was used Rapoport (39) got no diuresis, however Leutcher (44) got complete disappearance of edema in 6 of 11 cases using cortisone. Favorable findings indicative of improved renal function were decreased proteinuria, increased TSP with increase in

the A:G ratio, decreased serum cholesterol, restoration of water and electrolyte balance, and an increased glomerular filtration rate. In one case of Farnsworth's, and 2 of Leutcher's 11 patients proteinuria cleared completely. It should be remembered, however, that about 25% of these cases clear spontaneously and also that remissions may occur spontaneously. All of these authors agreed that for beneficial effects to occur the kidney parenchyma must be potentially physiologically reversible and not morphologically irreversible. Though most of them were hopeful that these hormones might alter the course of nephrosis, none said anything other than time would tell. Rapoport believed the effect would be temporary as in rheumatic fever or lupus erythematosis.

In a fairly complete review of the literature only four articles pertaining to treatment of acute or subacute glomerulonephritis with ACTH or cortisone were found (Thorn, 45; Farnsworth, 46; Burnett, 47; and Jacobsen, 48). Of these four only Farnsworth thought the course of the disease was forably altered with ACTH. It is noteworthy that her cases were treated for longer periods than the others (one to two months) and that her patients were 5,9, and 12 years old. In most instances

renal function improved while patients were receiving the hormones. In Thorn's four cases 3 benefited slightly from ACTH by decreases in hematuria, azotemia, and proteinuria. In 3 cases there was a temporary increase in hamaturia when therapy was stopped. One of Thorn's patients with psoriatic glomerulonephritis, showed marked improvement of psoriasis and arthritis but very little if any improvement in the renal disease. Of Farnsworth's 3 cases, 2 subacute and 1 acute, hematuria, hypertension, and azotemia were greatly decreased in two cases, including one subacute glomerulonephritis that had been present for six weeks, and one acute case. In one case of subacute GN present for 5 months the patient improved while on treatment with ACTH, though hematuria recurred a few weeks after therapy was stopped.

Jacobson observed 2 cases of acute and one of subacute GN. In one case of acute GN treated a total of 4 weeks (3 weeks and 1 week with 2 weeks intervening) a routine urinalysis 3 weeks after treatment was stopped showed only a slight trace of albumin, though an Addis count revealed presence of red cells and casts. This was a 58 year old man who developed the GN following streptococcal pneumonia. The other 2 patients, who

had much greater albuminuria and elevated NPN initially, showed no beneficial effects other than improved renal function during treatment. Burnett's case treated with 200 mg. cortisone for 12 days showed slight aggravation of hematuria, proteinuria and Na retention shortly after treatment with cortisone was started, though they all began to decrease before cortisone was stopped. Hematuria, by 11 days after treatment was stopped, was still above pre-treatment levels. Burnett did not beleive any beneficial effects from cortisone were due to favorable changes in the usual pathologic process of this disease. All of these authors realized that the majority of cases of acute GN improve spontaneously and that a very large series of cases showing beneficial results from hormonal therapy would be required before any definite conclusions could be drawn.

PRODUCTION OF GLOMERULONEPHRITIS IN RATS

The glomerulonephritis that we artificially induced in this experiment was done using anti-rat kidney rabbit serum according to the method of Smadel (49, 50). There are other methods of producing this lesion in laboratory animals, and there are objections by some to the use of Smadel's method. These subjects are discussed in detail in the thesis of my co-worker in this experiment,

M. M. Barelman. Smadel (49) thinks that the nephrotoxin causes only the acute renal damage and that subsequent pathological changes, except simple connective tissue replacement of structures destroyed by the nephrotoxin and some of the residual thickening of the glomerular membranes, arise as a result of a slightly diseased organ attempting to function under conditions that are unfavorable.

PRODUCTION OF NEPHROTOXIN

The first step in preparing the nephrotoxin (rabbit anti-rat kidney serum) was to prepare a suspension of sterile rat kidney. This was done by removing the rat kidneys under sterile conditions, then removing the capsule, perinephric fat, and as much of the kidney pelvis as possible. The kidneys were then cut into 3 or 4 pieces and washed with distilled water about six times, then 3 or 4 times more in sterile saline. This was done to remove as much of the blood present as possible and also to improve the sterile technique. The kidney was then mixed in a Waring Blender from 5-10 minutes to give approximately a 10-15% suspension (by volume). Sterility was tested by placing 2-3 drops of the suspension in Brewer's medium and observing for cloudiness in 24 and 48 hours. Two white rabbits were injected with gradually

increasing doses of the kidney suspension over a five week period. At the end of this period 5 cc. of blood was withdrawn from a ventricle of one animal and the serum separated. This serum was then treated with small amounts of rat blood until no reaction occurred between the rabbit serum and rat red cells or plasma. One milliliter of the end product was then injected into the dorsal penile vein of an adult male white rat, which was then placed in a urine collection cage over night. The urine of this animal showed a 4+ albuminuria (using sulfosalycilic acid technique). With this result, intracardiac punctures were done on the two rabbits one week after the last injection of rat kidney suspension and as much blood withdrawn as possible. The blood was treated as the sample withdrawn a few days before, the serum (actually sera) was separated after approximately 20 hours. Then it was treated with rat blood until no gross or microscopic reaction occurred between the rabbit serum and rat red cells or plasma. This end product, the nephrotoxic serum, was then pooled and 1:10,000 merthiolate in alcohol added, approximately 1 drop to 10 cc. of the serum. Just before the preservative was added, however, a few drops of the nephrotoxic serum was placed in Brewer's medium

and observed for clouding, None was observed in over a week. This main pool was then separated into smaller amounts to decrease the possibility of contamination by repeated needle punctures when injecting the experimental aeries. Sterility of these smaller amounts was also tested using Brewer's medium. These were stored in a refrigerator at 4° C.

STANDARDIZATION OF NEPHROTOXIN BY BIOASSAY

The purpose of this bioassay was to determine the least amount of nephrotoxin that would produce a good 4 + albuminuria and the dose of nephrotoxin producing death from ap arent renal failure. Since we planned to use rats of the Long-Evans strain in this work, five male L ng-Evans rats weighing around 200 gm. each were selected. These were injected with 0.4cc., 0.6 cc., 0.8 cc., 1.0 cc., and 1.2 cc. per 200 grams respectively, since previous nephrotoxins produced using the method used here usually had an optimal dosage somewhere in this range. The rat injected with 0.4 cc. per 200 gram showed a 1 + albuminuria the next d y; those receiving 0.6, 0.8, and 1.0 cc. per 200 gram all showed a 4 + albuminuria, while the rat receiving 1.2 cc./200 gm. died the day after receiving the injection. With these results a standard

dose of 0.8 cc. of the nephrotoxin per 200 gm. rat was decided on.

SELECTION OF RAT STRAIN, DIVISION OF SERIES, AND INJECTION WITH NEPHROTOXIN

In Smadel's experiments three strains of rats were used, Whelan, Long-Evans and Wister rats. Of these three strains the Whelan rats seem to be the most susceptible to nephritis. Rats of this strain, however, are difficult to obtain and quite expensive. Whelan rats progress directly from the acute to the chronic form of glomerulonephritis. The Evans rats appear to be the next most susceptible to the nephritis; however, many of these rats appear to recover from the acute nephritis within 2 to 5 months (according to Smadel, 49). before they progress into a chronic stage. This occurs regardless of whether the rats receive a high or low protein diet. Wister rats follow a pattern similar to that of the Evans rats though the percentage of those recovering or being only slightly affected is greater than in the Long-Evans strain. Long-Evans were used in this experiment because of their intermediate position in susceptibility and their availability.

At the onset of our experiment it was planned to treat about 25 rats injected with the nephrotoxin

with cortisone; to have 25 untreated control rats injected with nephrotoxin; 5 untreated, uninjected controls; 5 rats not injected with nephrotoxin treated with cortisone; and 10 rats treated with nephrotoxin that had been neutralized with rat kidney suspension (though this had been done successfully by Smadel (50). However, since the combination of a lung infection and anaphylactic shock produced by the rabbit serum killed several of our rats shortly after they were injected, we were able to have only 22 rats injected with nephrotoxin that received cortisone; 18 untreated rats that were given the nephrotoxin; 5 controls receiving cortisone, and 5 rats that received neither the nephrotoxin nor cortisone.

Of the 40 rats injected with nephrotoxin 26 were males and 14 females. All of the others were females. Most of the injections were made after the rats had been anesthetized with ether. However, after pneumonia began cropping up among the rats, intraperitoneal nembutal alone was tried, and injections were done without any anesthesia, but in the long run ether proved to be as safe as anything. At best it was far from satisfactory. The males were injected in the dorsal penile vein at the base of the penis. The females were injected

in one of the femoral veins. This was done by making a skin incision lateral to the vein and injecting the nephrotoxin. The needle was then withdrawn and the vein compressed with a sponge until homostasis was obtained if necessary. After this the skin incision was closed with silk, usually 2 or 3 sutures being required. We do not believe that injection of females offers any greater hazard than that of males if good sterile technique is followed. The skin incisions in the females treated with cortisone appeared to heal with no complications.

The diet of the rats consisted of Purina Fox Chow and water throughout the experiment.

METHOD OF TREATMENT WITH CORTISONE

In all of our work injectable cortisone acetate was used which contained 25 mg. of cortisone acetate per cc. This was kindly donated to us by the Merck Chemical Co., Rahway, N. J. This form of cortisone is for intramuscular injection in humans; we used it subcutaneously in our rats. Cortisone was begun 1 or 2 days after the rat received nephrotoxin. One injection was given daily.

With our first (of 3) group of rats treated with cortisone the dose at the onset was 12.5 mg. per 200 gm. of body weight daily. During the last week (5th week) of treatment this was tapered to about 9 mg./200 gm. With this dosage only 2 of 6 rats survived. Four rats

died from an extensive pneumonia. Our five female controls also died within 3 or 4 days after administration of cortisone was stopped. All of the control rats had extensive pneumonia also.

Of the next group injected 6 of 9 survived, though 2 of these died several months after cortisone was stopped. The cause of death in these last cases was not determined since these rats were put in with other dead rats which were marked similar to our due to an oversight by the animal room custodian. The initial dose among these rats w s 7.5 mg./200 gm. a day. This dose was decreased gradually to 5.0 mg. in 3 weeks. Thereafter the does was decreased to about 0.5 mg. the day it was stopped. In this group cortisone was given from 30 to 40 days.

In the last group injected the initial dose was 6.3 mg./200 gm. daily. Cortisone was given from 39 to 40 days. The initial dose had to be decreased after two weeks in most animals to approximately 4 mg./200 gm. daily. This was decreased about 0.6 mg. the following two weeks, then about 0.6 mg. bi-weekly until treatment was stopped. Of seven animals treated with this dosage all 7 survived the treatment period. Two animals died from pneumonia about 3 weeks after cortisone was stopped. Observations concerning these rats will be considered subsequently.

OBSERVATIONS AND FINDINGS IN THE RATS

Clinical Observations:

These will be divided into four main categories: (1) injection with nephrotoxin, (2) treatment with cortisone, (3) the usual clinical picture before death in rats with pneumonia, and (4) miscellaneous observations.

Before going further it might be well here to identify the rat groups by letters. The following designations will be used:

1. NT - Rats receiving nephrotoxin but no cortisone.
2. NT-T - Rats receiving nephrotoxin and cortisone. Small letters a, b, and c. will be added to to indicate the different dosage groups:
    a. Those starting on 12.5 mg./200 gm. daily.
    b. Those starting on 7.5 mg.
    c. Those starting on 6.3 mg.
3. CC - Cortisone controls--which were treated initially with 12.5 mg./200 gm. daily.
4. UC - Untreated controls.

1. Reaction to Neprhotoxin:

It was mentioned earlier that nearly all of the rats that were injected showed some degree of shock

(anaphylactic) which did not seem to be altered by the type of anesthesia used, of if no anesthesia was ued at all. It was observed that occasionally a rat died as the result of excessive pulmonary secretion from the anesthesia, especially ether, but also nembutal, where the rat received no nephrotoxin whatsoever. It was definitely noted that rats with "sniffles" were much more susceptible to this shock than those in good health. At one time we thought that the increased blood volume might be causing the lung edema and increased secretion at least in part; however, the same amount of saline injected intravenously did not produce this shock in three rats even when ether was used. We concluded that the shock was due mainly to the rabbit serum, but was made much worse if the rat had a lung infection, and that deaths that occurred from anesthesia without nephrotoxin being given probably occurred only in rats with a marked lung infection.

The usual picture of this acute anaphylactic shock started within a few minutes after the nephrotoxin was injected. The shock picture began with moist wheezes in the animal, and th rat would become weak and prostrate. As the condition grew worse the rat would usually amble around with an ataxic gait. Frothing

at the nose and mouth occurred. Finally the rat would take a deep gasp every 4-5 seconds and death usually followed shortly. Tracheotomy near death was usually accompanied with the pouring forth of copious amounts of frothy fluid which contained no blood. Suction was of no avail. This usually occurred in 10-20 minutes. There were a number of rats that appeared in satisfactory condition 2-3 hours after being injected, though somewhat weak or listless, that were found dead the following day with a dried up froth around their mouth and nose. Occasionally this froth would be blood tinged. The animals that survived appeared to recover from this initial shock completely, though there is little doubt that this condition would favor development of a pneumonia, especially if the shock were very severe.

2. Treatment with cortisone:

Actually there are only a few observations concerning the animals treated with cortisone that we didn't find in the untreated animals as well. The two prominent ones were falling out of the hair around injection sites and the formation of hard nodules 2-3 mm. in diameter at the injection sites. In two of the males (NT-Ta-3 and 4) there seemed to be a decrease in the coloration of the black hair along with

the development of baldness. It was noted within a few weeks after cortisone was started that the hair came out easily when the rats were held for injection. These areas were noticeable months after injections were stopped but did fill in before the animals were sacrificed (about 11 months after injections were stopped).

Hard shot-like nodules developed at the sites of injection within 3 to 4 weeks after injections were begun. These contained a hard yellowish homogenous material similar to the caseous nodules of lungs in rats dying of pneumonia. The significance of these nodules will be discussed later. They appeared to clear up within a short time after cortisone therapy was discontinued.

Most of the rats receiving cortisone lost weight while being treated; though this was not always true. This loss was usually made up within 2-3 weeks after cortisone therapy was stopped. Besides this it was observed that rats not receiving cortisone underwent a marked weight loss if they developed pneumonia.

Increased excitability was not markedin rats receiving cortisone if present at all. After the rats became accustomed to being handled and receiving injections, usually within a week, treatment was quite

easy, We could not tell whether or not our furry friends became euphoric while receiving cortisone.

3. Pneumonia

To us pneumonia was a constant menace. Not only did it kill several of our animals from days to months after they received the nephrotoxin, but, as mentioned earlier, it made the shock from receiving the nephrotoxin fatal where it very probably would not have been so otherwise. We lost over 35 animals from this disease, including 5 cortisone controls and 5 untreated controls, not including those dying from massive lung secretions directly after receiving the nephrotoxin.

Shortly after the onset of the disease the animal would become inactive and sit still with back arched and fur ruffled. As the disease progressed the marked weight loss became evident. The arch in the back became more pronounded. Often increased lacrimation was evident from eye watering. These rats would creep around like shrunken old folks. Bloody stools, often a bloody diarrhea, were frequent.

After death there were nearly always hemorrhages under the toenails and evidence of bleeding from the respiratory tract (blood on external nose and mouth) and the G. I. Tract. At one time we thought the rats

might have scurvy from some unknown cause; Since cortisone has been shown to decrease symptoms of scurvy (Booker, 21; Schaffenburg, 20) and since ascorbic acid serum levels increase somewhat par allel to urea levels with impaired renal function (Sendray, 51) we tended to discard this idea. The fact that the disease swept through one cage so rapidly, along with the autopsy findings also lead us to beleive that this could not be scurvy. We must say, however, that as long as the rats got lettuce once a week there were no deaths like this. The only time the rats did get lettuce was during the summer months. We have assumed that the reader knows that rats are not normally subject to vitamin C deficiency disease.

According to Ratcliffe (52) rats are quite subject to pneumonia. In 487 Wister rats about 75% were involved at one year of age. The disease was rare before 3-4 months; then the rate gradually increased to reach the high at one year. About 75% had some degree of lung abscesses and bronchiectasis at autopsy. In his description of the disease, Ratcliffe made no mention of signs of hemorrhage present before or after death.

4. Miscellaneous:

Only one rat, NT-8, developed ascites. This was

This was not evident until autopsy. It is possible that out obser vations were not close enough to detect edema that might have been present shortly after injection with nephrotoxin.

One rat appeared to develop a labyrinthitis. He was observed with his head turned 90° on the horizontal axis of his body and was ataxic. In Ratcliffe's series the incidence of otitis media with labyrinthitis was 4%. In ours this one case would make the incidence less than 2%. This rat, which received cortisone, was not included in the series because of a possible mix up with a non-treated rat.

Four cortisone treated rats that had received nephrotoxin were treated with penicillin for three days for pneumonia receiving 10-20,000 u. daily of a 3:1 mixture of procaine and potassium penicillin. Our 5 untreated controls were also treated with penicillin once but died within 24 hours. The treated animals were NT-T 13, 14, 15 and 20. The controls were not treated sooner because they were n t handled every day and their condition progressed too far before they were observed and treated. The cortisone treated animals were treated with penicillin only while receiving cortisone. The pneumonia, if diagnosed early enough, seemed to respond satisfactorily to the penicillin.

LABORATORY FINDINGS

Unfortunately the only laboratory results we have are roughly measured urine protein concentrations and animal body weights. When correlated with microscopic findings in kidney these may be of some importance. Since Smadel (49) found that albuminuria often decreased during the first five months, only animals surviving at least six months will be considered here.

To determine proteinuria we used Exton's method (sulfosalicylic acid and sodium sulfate). Our grading was as follows:

tr: Minimal clouding

1+: Noticeable clouding but not opacity.

2+: Milkiness of the urine with or without slight flocculation.

3+: Heavy flocculation with a large precipitate on standing.

4+: Curdiness of the precipitate.

According to Addis (53) rats normally excrete protein in their urine. Adult male rats may normally excrete up to 1.0 mg. per hour; females excrete less. This proteinuria is greatly decreased by adrenalectomy according to Addis. It is restored fully by cortisone, to a large extent by DCA, and to a slightly less extent

by adrenal cortical extract (in male rats). In non-adrenalectomized rats these hormones aggravated the proteinuria according to Addis. (This does not agree with Bjorneboe's (43) work, although his was done on humans with nephrosis).

In our series the 5 female controls receiving only cortisone showed from 0 to 1 + proteinurias the one ti e they were tested. Yeck (54) observed only an occasional trace of albumin in urine of normal controls in Long-Evans rats. He did not state whether or not these were male or female rats, though it is more likely that they were females. (He was working on a project at the University of Nebraska College of Medicine for a senior thesis and prob ly used all his male animals in his nephrotoxin series).

Only seven rats in our series, NT-4, 12 and NT-T 4, 5, 13, 14, and 17, showed a definite decrease in proteinuria during the first 5 months following injection as described by Smadel (39). Two rats NT-12 and NT-T-14 showed a mild, transcient exacerbation of proteinuria, while only one rate, NT-T-5, showed a marked and persistant recurrence. Three rats not living six months also showed this trend rather definitely, NT-5, NT-6 and NT-Tc-21. Six other rats in those living over six

months were not followed closely enough to say whether or not this occurred. Although the number of rats followed is not enough to be valid statistically, we can see that a severe nephritis was produced in 14 of 20 rats. Of these 14 only 4 were in untreated animals. One of these four showed a gradually decreasing proteinuria over a period of 175 days. Of 10 rats that had a rather marked nephritis from the nephrotoxin that received cortisone, 6 rats showed improvement as evidenced by a rather persistent decrease in proteinuria from a 3 or 4 + to a 1 + to a trace. In our opinion this indicates that cortisone was of value in reducing the kidney damage produced by the nephrotoxic serum. We know of no good explanation for the erratic proteinurias of the non-treated females. It is doubtful if NT-Tb-15 ever developed the experimental lesion.

From the weight and proteinuria tables, (Table 3 and Table 4) it can be seen that a prompt and rather severe weight loss occurred with cortisone treatment. A rather severe weight loss is also observed in animals just before death from pneumonia as seen in the untreated control rats (all females). Because of the intercurrent infections, no definite conclusions can be

drawn from the effect the nephritis played on the weight. Comparing rats kept in the same cage, NT-1 gained less weight than NT-2 with a similar degree of proteinuria, NT-17 and 18 gained about the same weight despite the fact that 18 had a more severe proteinuria. NT-T-4 and 5, 7 and 8, and 21 and 22 showed a greater over all weight gain in animals with the least amount of proteinuria. It does appear that the degree of proteinuria did influence the weight gain, even though our method of measuring urine proteins was not accurate enough to make these comparisons with any degree of exactness. With three variables involved, treatment with cortisone, presence of a nephritis, and presence of pneumonia and probably residual abscesses and bronchiectasis, no definite single cause could be ascribed to all of the weight loss when it occurred.

It should be noted that the long time average weight gain in the rats receiving cortisone is only slightly less than that of those not receiving cortisone. We would also like to point out the fact that NT-10 was an abnormally large rat (compare with NT-1 and 2) and by itself should not be used for comparison.

TABLE 1: Proteinuria in Rats Surviving 6 Mos. or Longer

	Oct. 1951	Nov.	Dec.	Jan. 1952	Feb.	Mar.	April	May	June	July	Aug.	Sept	Oct.	Nov.	Dec.	Jan. 1953	Days living if not sacrificed**	
NT-1	4+	4+				4+		4+		4+	4+	4+		4+		Exp.	411	
NT-2	4+	4+				4+		4+		4+	4+	4+		4+		Exp.	413	
NT-4	4+					2+		Exp.									175	✓
NT-10			4+			4+		4+		4+	4+	3+				4+		
NT-12 ♀					2+ 3+	3+	1+	2+		2+	1+	1+				1+		✓
NT-17 ♀						2+	3+	3+		2+	2+	3+			Tr.	1+		
NT-18 ♀						2+	3+	3+		4+	4+	4+			2+	1+		
NT-Ta-4		3+		2+		Tr.		Tr.		Tr.	1+	Tr.		Tr.		1+		✓ *
NT-Ta-5		4+		2+		Tr.		3+		3+	3+	3+		3+		3+		✓
NT-Tb-7			4+				3+	3+		1+	1+	1+		1+		1+		*
NT-Tb-8			4+				4+	3+		4+	4+	4+		4+		4+		
NT-Tb-10			4+				2+	1+		Exp. 1+							198	*
NT-Tb-13					4+		2+	Tr.		Tr.	Tr.	Tr.			Exp		✓ 312	*
NT-Tb-14					3+		2+	2+		Tr	1+	2+				Tr.		✓ *
NT-Tb-15					1+		Tr.	Tr.		1+	Tr.	Tr.				Tr.		
NT-Tc-16 ♀					4+		4+	4+		4+	4+	4+				4+		
NT-Tc-17 ♀					4+		1+	Tr.		Tr.	Tr.	1+				Tr.		✓ *
NT-Tc-18 ♀					2+		3+	3+		2+	2+	2+				Tr.		
NT-Tc-19 ♀					4+		4+	3+		4+	4+	4+			4+	3+		
NT-Tc-20 ♀					2+		3+	Tr.		2+	2+	3+			3+	3+		

✓ Rats showing decreased proteinuria in first 5 mos.

* Rats having received cortisone showing sustained decrease in proteinuria.

** Days surviving between injection of nephrotoxin and death (not birth and death). The first proteinuria indicates month rats were injected. Blank spaces mean that urine was not tested that month for protein.

TABLE 2: Proteinuria in rats not surviving 6 mos.

	Days Alive *	1st Month	2nd	3rd	4th	5th	Cause of death
NT-3	38	4+	4+				Cysto-pyelonephritis
NT-5	144	4+	2+			Tr.	Pneumonia
NT-6	148	4+			Tr.		"
NT-7	148	3+			3+		"
NT-8	41	4+					" , possibly peritonitis also
NT-9	16	2+					"
NT-11	36	4+					"
NT-13 ♀	64	4+		4+			"
NT-14 ♀	64	3+		3+			"
NT-15 ♀	67	1+		Tr.			"
NT-16 ♀	67	3+		4+			"
NT-Ta-1	12	4+					"
NT-Ta-2	32	4+					" , pyelonephritis
NT-Ta-3	36	4+					"
NT-Ta-6	15	4+					"
NT-Tb-9	43	2+					"
NT-Tb-11	38	3+	3+				"
NT-Tb-12	38	2+	2+				"
NT-Tc-21 ♀	62	4+	4+	4+			"
NT-Tc-22 ♀	62	2+	2+	Tr.			"

* Days alive between injection of nephrotoxin and death (not birth and death).

TABLE 3: Weight in Grams and Proteinuria in NT Rats

(Rats receiving nephrotoxin only)

Month	NT-1	NT-2	NT-3	NT-4	NT-5	NT-6	NT-7	NT-8	NT-9
1st	212 4+	204 4+	152 4+	213 4+	235 4+	107 4+	106 3+	184 4+	129 2+
2nd			4+	229	262 2+	265	204	242	Exp.
3rd	234 4+	274 4+	177*	299	364			249*	
4th	294	329				327 Tr.	265 3+		
5th				352	393	365	291		
6th	315	385		346 2+	403	Exp.			
	328 4+	367 4+			333*		Exp.		
7th				350*					
8th	326 4+	366 4+							
9th									
10th	326 4+	368 4+							
11th	336 4+	383 4+							
12th	330 4+	395 4+							
13th	4+	4+							
14th									
	Exp.	Exp.							

*Weight after found dead

TABLE 3 (Continued)

Month	NT-10		NT-11		NT-12 ♀		NT-13 ♀		NT-14 ♀		NT-15 ♀		NT-16 ♀		NT-17 ♀		NT-18 ♀	
1st	224	4+	213	4+	155	2+ 3+	194	4+	214	3+	171	1+	200	3+	220	2+	195	2+
2nd	270		264 224*		160	3+	222		238		223		196		224	3+	200	3+
3rd					193	1+	207 Exp.	4+	230 Exp	3+	217 Exp.	Tr.	157 Exp.	4+	240	3+	218	3+
4th	365 405	4+			220	2+												
5th															238	2+	217	4+
6th	465	4+			220	2+									252	2+	229	4+
7th					230	1+									249	3+	225	4+
8th	496	4+			236	1+												
9th	518	4+																
10th	456	3+														Tr.		2+
11th															261	1+	237	4
12th					230	1+												
13th	505																	
14th	475	4+																

TABLE 4: Weight in Grams and Proteinuria in NT-T Rats

(Rats receiving nephrotoxin and cortisone).

Month	NT-Tb-4		NT-Tb-5		NT-Tb-7		NT-Tb-8		NT-Tb-10		NT-Tb-13		NT-Tb-14		NT-Tb-15		NT-Tc-16?	
1st	128	3+	234	4+	260	4+	216	4+	145	4+	216	4+	232	3+	224		169	4+
	113		180		195		160		128		202		240		217		168	
2nd	105		165								195		223		217		156	
	109		168		189		166		132		229		174		190		148	
													192		235			
3rd	152	2+	142	2+								2+		2+		Tr	177	4+
4th					264		250		225		264	Tr	261	2+	281	Tr	193	4+
					300		285		265									
5th	222		250			3+		4+		2+								
	265	Tr	285	Tr														
6th					320	3+	295	3+	306	1+	299	Tr	299	Tr	304	1+	205	4+
7th	255	Tr	323	3+							310	Tr	301	1+	318	Tr	213	4+
8th					387	1+	358	4+	Exp	1+	286	Tr	283	2+	294	Tr	209	4+
9th	285	Tr	344	3+	398	1+	370	4+										
10th	288	Tr	350	3+	390	1+	356	4+										
11th	290	Tr	351	3+							Exp.							
12th						1+		4+					286	Tr	230*	Tr	213	4+
13th		Tr		3+														
14th	301	1+	366	3+	405	1+	346	4+										

Receiving Cortisone.

* Had bowel obstruction at ileo cecal junction when sacrificed.

TABLE 4 (Cont.)

NT-Ta-1	NT-Ta-2	NT-Ta-3	NT-Ta-6	NT-Ta-9	Month	NT-Tb-11	NT-Tb-12	NT-Tc-21♀	NT-Tc-22♀
75 4+ Exp	229 4+	234 4+	137	253 2+	1st	168 3+	144 2+	202 4+	192 2+
	176	181	118 4+	200		132	123	200	202
	145 Exp	136*	79*	189 Exp.	2nd	132	106 2+	185	191
						107 3+ Exp.	89*	160 4+	165 1+
					3rd			204 4+ Exp.	210 Tr. Exp

TABLE 5: Cortisone Controls

CC-1	CC-2	CC-3	CC-4	CC-5	Month	NT-Tc-17♀	NT-Tc-18♀	NT-Tc-19♀	NT-Tc-20♀
163	105	102	95	98	1st 1st	207 4+	185 2+	225 4+	188 2+
131 Tr	87 1+	95 Tr	90 0	93 Tr		195	173	225	188
114	78	100	85	84	2nd 2nd	186	159	209	166
107 Exp	78 Exp	81 Exp.	43* ✓	80 Exp		183	159	173	133
					3rd	202 2+	3+	201 4+	197 3+
					4th	229 4+	205 3+	247 4+	207 Tr
					5th				
					6th	245 Tr	221 2+	260 4+	229 2+
					7th	257 Tr	230 2+	265 4+	224 2+
					8th	256 1+	225 2+	266 4+	226 3+
					9th				
					10th				
					11th			4+	3+
					12th	250 Tr	225 Tr	275 3+	233 3+

TABLE 6: Untreated Controls

** UC-1	UC-2	UC-3	UC-4	UC-5
193	218	178	217	189
144*	174*	142 Exp.	174 Exp.	147*

┌── Receiving Cortisone.

✓ Cannibalized.

* Weighed after found dead.

** All UC rats weighed 11 days apart.

GROSS AND MICROSCOPIC FINDINGS

Gross Findings:

These will be considered first in animals found dead and then in the animals sacrificed. In animals dying a short time after receiving nephrotoxin the only noticeable finding was presence in the lung of a large amount of edema fluid. The lung was sometimes thought to be somewhat congested grossly.

In the cortisone treated animals that were found dead, all appeared to die from pneumonia. There was always extensive consolidation of one or more lobes of lung. At several autopsies one lung was almost a complete firm yellowish cheesy mass with part of the other lung being involved also. Often the lungs were spotted with several small abscesses. This was usually accompanied with several areas of infarction. It is probably that these represented an earlier stage than where complete consolidation of a lobe occurred. In one animal one lung was very atelectatic, being about 1/4 the size of a normal lung. A few adhesions between lung and pleura were often present though never extensive.

All of the cortisone treated rats dying within a week after cortisone was stopped had several hard

yellowish plaques around the injection sights in the groin. These plaques were about 2mm. in diameter. Those dying later (NT-T 21 and 22 and others) did not show the plaques at autopsy.

Only one rat NT-Tb-9 showed gross abscesses on the liver surface. It is possible, however, that other rats had abscesses within a viscus and were over looked by us.

All the animals, regardless of how extensive the lesions of the lung and viscera, showed evidence of hemorrhage from nose, mouth, anus, and under toenails.

Kidneys grossly were usually essentially normal. Often there was little or no perinephric fat remaining, especially in animals that had become quite aachectic before death.

A similar picture to this was found in 11 of 14 untreated nephrotoxic rats; however, in only four of these were multiple abscesses or consolidated lobes noted. In the others the lung showed varying degrees of congestion and edema but on the surface or cut surface where sections were taken no abscesses were visible. All of these eleven showed evidences of hemorrhage as described before. One rat had a small amount of light brown sero sanguinous fluid in his abdomen (NT-8),

though no inflammation of bowel or peritoneum was seen. One rat of this gro , NT-3, died of a cysto-pyelonephritis with hydroureters and hydronephrosis. The bladder, ureters, and kidneys of this animals contained a large amount of purulent sanguinous fluid. The cause of death in NT-1 and 2 was not evident.

All of the controls appeared to die from pneumonia. This was more marked in the cortisone controls than in the untreated controls.

In animals that were sacrificed the only significant gross findings were those in the lung and pleura, except in one animal, NT-T 15, which had a bowel obstruction apparently at the ileocecal junction. The lung findings included light grayish areas, slightly depressed from the surface, from 1-2 mm. in diameter, adhesions to pleura and pericardium (possibly myocardium), and apparently active abscesses that were well surrounded by fibrous tissue. The adhesions and abscesses were seen only in animals that had received cortisone.

Microscopic findings:

Kidney Findings:

Findings in both cortisone treated and untreated controls in the kidneys were essentially negative.

In the animals receiving nephrotoxin alone that died within about the first month after receiving the nephrotoxin the kidneys usually showed adhesions between the glomular tuft and Bowman's capsule, increased segmentation of the tuft, fibrosis of the tuft, narrowing of the capillary lumen, and in one instance crescents were present (NT-9). Tubules were affected to a much less extent, tubular findings were limited to cystic dilitation with hyaline like casts or precipitated protein being contained in the lumen. These changes were thought typical of subacute and chronic glomerulonephritis. In one rat, NT-11, that had had a 4 + proteinuria 34 days previously, there were no significant changes in the kidney. These sections were from rats NT-3, 8, 9, and 11.

In a similar group of nephrotoxin injected rats that had received cortisone (NT-T-2, 3, 9, and 12) the reaction in the glomeruli was less severe. Adhesions between capsule and tuft occurred but were rare. Only NT-T-2, which had a pyelonephritis, contained casts in the tubules; these were of a protein-like material. Both NT-T-2 and 3 showed cloudy swelling and granular degeneration in the tubular cells. Sections of kidney of NT-T-12 showed rather advanced antolytic changes

and were difficult to interpret, though no significant reaction of glomerulitis was suspected from the kidney structure that could be interpreted.

In these two groups of rats those receiving cortisone showed fewer glomerular chang s typical of glomerularnephritis in general than did the untreated animals. All of these rats were males.

All of the rats that died at about 2 months were females. Slides of these rats include NT-13, 14, 15, and NT-T-21 and 22. In all of these sections the amount of renal damage microscopically and proteinurias corresponded quite well. Findings in the untreated animals included adhesions between Bowman's capsule and the tuft, increased segmentation of the glomerular tuft, and fibrosis with some shrinkage of the tuft. Surrounding tubular degeneration and an occasional markedly dilated tubule were present in NT-13. Findings were slight in NT-15.

Sections of both NT-T-21 and 22 showed rather advanced antolytic changes. However, it could be seen that the reaction in # 21 was quite severe while that in # 22 was minimal. This reaction consisted mainly of adhesions between capsule and tuft. The lesions in NT-T-22, though rather marked, did not appear as

advanced as those seen in NT-13. Tubular changes were not as noticeable in the cortisone treated animal.

In the next group of animals, which lived 5 to 6 months we had only untreated animals. Unfortunately NT-Tb-10 had been dead too long when we found him, and NT-Tb-13 was misplaced after death by the animal room attendent so we have no slides of these rats. These animals lived about 6 1/2 and 10 months respectively. Slides are those of NT-4, 5, and 7. Changes in these kidneys included thickening of the basement membrane of Bowman's capsule, adhesions between the tuft and capsule, and an increased fibrosis of the tuft. Kidney sections of NT-4 showed little change in the glomerular tuft, but the entire kidney tissue was somewhat swollen and moderate size collections of polygonal and mononuclear cells were present surrounding the glomeruli. The reaction in NT-7 did not appear quite as severe as might have been expected from a 3 + proteinuria, though the picture in the kidney sections of the other two animals correlated well with the proteinuria. None of these kidney sections showed severe lesions.

The histologic findings in the treated and in the

untreated rats surviving over one year will be considered in this section. The total number of rats surviving one year or over was 17, of which 15 were sacrificed at the end of the experiment and two (NT-1 and 2) died before the end of the experimental period. Of the total 17 rats 6 were untreated nephrotoxin injected controls and 11 were cortisone treated nephrotoxin injected rats. In the untreated nephrotoxic group 2 rats (NT-1 and 2) died before the end of the experiment leaving a total of 4 animals that were sacrificed. The significance of the death in these two rats will be considered subsequently.

The histologic findings in the untreated nephrotoxic group will be considered first. Of the two untreated rats (NT-1 and 2) which were not sacrificed, sections of NT-2 showed an advanced diffuse chronic glomerulonephritis with changes ranging from complete obliteration of the glomerular tufts by dense fibrosis to tufts showing adhesions between the base of the glomerular tuft and a thickened Bowman's capsule. Tuft hyalinization was present but not marked. The tubules in the region of the fibrotic glomeruli showed cystic dilatation and were filled with hyaline material and casts. In areas where less marked alteration of the

glomeruli were present the tubules showed better preservation. There is also increased interstitial fibrosis. In view of the normal appearance of the lungs, renal insufficiency could have been the cause of death in these animals, but since blood chemistry studies were not done, we can little more than speculate as to the cause of death in NT-1 and 2. The only other rat in this group which showed a severe and constant proteinuria was NT-10. In this rat the kidneys showed a marked progressive chronic glomerulonephritis. with fibrosis of tufts and Bowman's capsule. Some of the fibrotic glomeruli were surrounded by mononuclear cells. Many tubules were dilated and contained casts. The disease in this animal was chronic and progressive and correlated well with the clinical proteinuria which this animal exhibited when sacrificed. The other three rats in this group (NT-12, 17 and 18) seemed to recover from their disease clinically. Histologically the renal changes varied from normal (NT-12) to a picture of a mild old inactive glomerulitis in the other two animals (NT-17 and 18). The histologic picture correlated well with the clinical picture. Proteinuria in NT-12 cleared up clinically in a very short time while NT-17 and 18 had a slight relapse of

proteinuria before the urine protein decreased toward normal.

The cortisone treated groups also exhibited three different clinical courses. Clinically rats NT-Ta-4, NT-Tb-7, 14, 15, 13, NT-Tc-17 and 18 recovered as evidenced by their proteinuria levels. Histologically the renal findings ranged from a moderate, inactive glomerulitis with some segmentation of tufts and thickening of Bowman's capsule, and casts present in the collecting tubules (rats NT-Tb-7, 14) to minimal to negative renal findings (NT-Ta-4, NT-Tb-15, NT-Tc-17 and 18). Rat NT-Tb-13 followed the same clinical course with early recovery from proteinuria. Since no histologic specimen was obtained on this animal, we can only assume the nature of the kidney lesion. In most of the above cases the proteinuria correlated favorably with the histologic findings. Rat NT-Tb-8 was an exception. This rat exhibited severe continuous proteinuria with histologic renal lesions similar but perhaps slightly greater than NT-Tb-7. The lesion in NT-Tb-7 consisted of a mild old glomerulitis, only slight evidence of fibrosis of tufts and numbers of old adhesions between tufts and Bowman's capsule.

The histologic renal lesion in rat NT-Tc-20

showed a minimal reaction of an old glomerulitis, though this rat showed a persistent 3+ proteinuria for 5 months before it was sacrificed.

Other cortisone rats that showed a persistent severe proteinuria also showed advanced and severe changes of glomerulonephritis histologically. These include rats NT-T-5, 16, and 19. In NT-T-5 a few glomeruli were shrunken and fibrotic. Others showed thickening of Bowman's capsule and fusing of the tuft to Bowman's capsule. The tubules were moderately dilated and contained sizable numbers of casts. The lesions were advanced and progressive. Rats NT-Tc-16 and 19 also exhibited continuous severe proteinuria. Their renal lesions consisted of moderate chronic glomerulitis with advanced fibrosis of many tufts, adhesion of tufts to Bowman's capsule and segmentation of tufts. Many glomeruli appeared to be univolved in the process. The tubules in the area of the damaged glomeruli were dilated and filled with hyaline casts. Histologically the process did not seem active as in NT-Ta-5.

OTHER TISSUES:

No reactions that could be attributed to the nephrotoxic rabbit serum were seen. The only significant changes seen in sections of tissues other than kidney sections in animals that died within five or six

months after receiving nephrotoxin were those due to inflammation. In the non-treated animals this was confined mostly to the lung. Lung lesions varied from mild to severe and included pictures typical of lung abscesses, lobar pneumonia, and bronchopneumonia. The main differences between these animals and the cortisone animals were that nearly all of the cortisone animals showed extensive involvement in every case; the inflammatory response to the bacteria was greatly reduced from that seen in untreated animals, and often distant bacterial colonies of varying sizes were seen in heart, liver, spleen and also kidneys.

In untreated rats that were sacrificed at the end of the experiment the only abnormal finding was in the lungs of NT-12. This rat had large collections of lymphocytes around the bronchioles. Sections of heart, liver, and spleen were all negative. No lung sections were obtained from NT-1 and 2.

Histologic findings in remaining sections of cortisone-treated animals were negative in NT-T-4, 5, 17, 18, 19 and 20. Lung sections of NT-T-14 showed an old inflammatory reaction which was inactive at the time of sacrifice. The liver of this animal also showed degenerative changes. Liver sections of NT-T-15

revealed a few inactive granulomata. Lung sections in this rat showed a considerable amount of interstitial infiltrate of lymphocytes and plasma cells. In NT-T-16 sections from the right lower lobe of the lung revealed an old abscess which appeared to be well-walled off with fibrous tissue at the time the animal was sacrificed.

DISCUSSION:

Before discussing the present piece of work let us consider what others have observed in similar experiments. The only other experiments using rats in which a nephrotoxic nephritis was produced and treated with cortisone were done by Hackel et al (55), Yeck (54) and by Hrnicek and Hoffman (56). Hackel used 10 mg./100 gm. of cortisone and sacrificed his animals 4 days after they were injected. He found no change between treated and untreated animals. We didn't consider this work significant since the rats were treated for such a short time. Yeck used ACTH over a four week period, about 3-4 mg./100 gm. daily and sacrificed his animals at various intervals over the four week period. He thought the basement membrane of the glomerular endothelium was thinner in treated animals than in the untreated ones. Hrnicek and Hoffman used this same dose of ACTH over a six week period. They noted no

significant change between the kidneys of treated and untreated animals over a 6 1/2 month period, though they did note that the capsule of the kidney in treated animals was easily removed whereas it was more dense and adherent to the kidney in untreated animals.

Rich, Berthrong et al (57, 58, 59) and McLean et al (60) treated rabbits with ACTH and cortisone in which glomerulonephritis had been induced with injections of sterile horse serum. In all of these experiments a necrotizing arteritis and valvulitis were also produced. Rich and Berthrong found that ACTH greatly decreased the nephritis and maintained the glomeruli in their normal state in most animals, but that cortisone early suppressed the glomerular lesions and later caused "aneurysms" of capillary tufts often with rupture and hemorrhage or with hyalizations resulting in formation of large globoid hyaline masses which were in some instances indistinguishable from the glomerular lesions of Kimmelstiel Wilson's disease in humans. These authors thought the difference between effects of ACTH and cortisone may have been due to too high a dosage of cortisone. McLean observed similar changes using cortisone. Teilum et al (61) treated rats with cortisone in which a nephritis was

induced with IV injection of killed Pfeiffer bacillus 3 times weekly for 7 to 16 months. These authors treated five rabbits with cortisone. They also noted deposition of this material in the glomeruli but thought the change in the kidney more closely resembled amyloidosis.

In our experiment using the technique of Smadel a glomerulonephritis was produced in Long-Evans rats that closely resembled the kidney lesion seen in human glomerulonephritis. This statement is born out by microscopic examination of control rats which showed changes in the kidney including adhesions between the glomerular tuft and Bowman's capsule, complete obliteration of the tuft by dense fibrosis, increased numbers of fibrocytes in the interstitial tissues, and cystic dilitation of tubules which contained proteinacious casts. All control rats did not develop such a severe lesion, however.

It is interesting to note that several workers have made the observation that proteinurias have not correlated well with the extent of renal pathology in a nephritis in rats produced with nephrotoxic serum. In our series of 17 rats that lived over 6 months we found that in 15 the kidney involvement one would have expected to find from the degree of proteinuria and

that which was seen in microscopic examination of kidneys correlated very well. In other words there was 90% correlation. In 7 rats living 2-5 months the correlation was 100%. In rats dying under one month the correlation was less. This was probably due to the fact that the rat died several weeks after the urine was first tested for protein and improvement occurred during the period between the time urine was tested for protein and the death of the animal. In cases where a discrepancy existed between proteinurias and microscopic findings the microscopic findings were always less severe than would have been expected from the degree of proteinuria.

It should be stated before going further that the limited number of rats that survived plus the additional variable for the pneumonia, which was responsible for the limited number of rats, do not permit our figures to be of significance statistically. This situation possibly tends to make us more speculative than we would have been had we had a significant number of rats and no pneumonia; and we would therefore have had more definite results.

Although the original goal of our experiment--to observe the effect of treatment with cortisone on the

TABLE 7:

		Untreated	Treated
1.	Number of animals at onset	18	22
2.	Number dying (not sacrificed)	14	11
3.	Number sacrificed	4	11
4.	Average survival time in days between injection with nephrotoxin and death (in animals not sacrificed).	131	77
5.	Number of animals dying within one month after injection with nephrotoxin.	4	7
	a. % of all animals dying (not including animals sacrificed)	27%	63%
	b. % if NT-1 & 2 are excluded (since these 2 animals lived longer than some rats that lived until sacrificed)	33%	63%
6.	Number of animals surviving after treatment period with cortisone.	14	15
	a. % surviving (until sacrificed)	27%	73%
	b. % surviving if NT-1 & 2 are included in surviving group.	43%	73%
7.	Animals surviving 6 mo. or longer.	7	13
8.	Animals living 6 mo. or longer with 3+, 4+ proteinuria at onset.	4	10
9.	Animals showing persistant decrease in proteinuria to 1+ or trace.	1	6

life span of rats with induced glomerulonephritis--was not accomplished, it is interesting to note a few observations concerning this point. Looking at Table 7, item 5 it is easy to see the marked effect cortisone has of decreasing the inflammatory response in that 7 of 11 cortisone rats that died from an inflammatory process died during treatment or shortly after treatment with cortisone was stopped. Only 4 of 14 untreated animals died during this period(4 of 12 if NT-1 and 2 are excluded for reasons given).

Considering item 6 on Table 7 we see that after treatment with cortisone was stopped 11 of 15 cortisone rats survived while only 4 of 14 untreated rats survived a similar period (6 if NT-1 and 2 are included as surviving rats). It must be remembered, however, that 4 of the surviving cortisone treated rats received penicillin and showed clinical improvement as a result. Assuming these rats were saved by the penicillin and that 4 untreated rats would have been saved had they received it, the per cent surviving until sacrificed would have been about the same. On the other hand if we recall the effect cortisone had on decreasing resistance it would seem that use of penicillin in these cortisone treated rats really served to reduce

an undesireable side effect of the cortisone. If one takes this attitude the figures in item 6 again assume some significance. (Remember that these rats received penicillin while also receiving cortisone.)

Looking at items 7, 8, and 9 on Table 7 one can see that only one of four untreated rats with an initially high degree of proteinuria showed spontaneous improvement while six of ten rats receiving cortisone showed improvement. Although this untreated rat showed a decreasing proteinuria and only mild changes in the glomeruli, it lived only 175 days--the shortest period in this group of twenty rats. We cannot be sure that this rat would have stayed in remission. It should be stated in passing that the two rats in the seventeen with microscopic findings less than was anticipated from proteinurias were cortisone treated rats, NT-T-8 and 20. NT-T-20 was not considered to have developed a severe lesion initially.

Histologic examinations were done on three untreated aminals and 8 treated animals of the 14 considered under item 8 on Table 7. Of the total of

20 rats living over 6 months kidney sections were examined in 17 rats.

Reference to the cor relation between histologic findings and urine protein findings have been made above. Kidney changes in severe cases of persistant proteinuria include thickening of Bowman's capsule, adhesions between Bowman's capsule and tuft, increased segmentation of the tuft and often complete obliteration of the tuft by fibrosis, cystic dilation of the tubules, casts in the tubules, and increased firbrosis of the interstitium. These changes usually appeared progressive. In milder cases where the proteinuria had persisted around 1 + to a trace for some time, kidney findings consisted of minimal changes which usually appeared inactive. These changes generally included no more than a few adhesions between the tufts and Bowman's capsule, slight increased segmentation of the tuft, and occasional tubules that showed collections of hyaline casts.

Although the number of rats concerned here is not enough to make statistically valid conclusions, we feel that careful scrutiny of the effect of cortisone upon the life span of these rats, changes in the excretion of protein in the urine, and the histologic

picture in the kidney indicate that this drug was definitely of value in these rats in reducing the connective tissue response in the kidney that normally follows intravenous injection of nephrotoxic rabbit serum.

CONCLUSIONS:

1. Using the technique of Smadel and his associates a glomerulonephritis can be produced in Long-Evans rats that is quite similar to that seen in humans.

2. Cortisone used in a high dosage over a long period of time causes a marked decrease in the inflammatory response permitting extensive bacterial invasion with very little inflammatory reaction on the part of the host.

3. Although the number of rats in our series was not sufficient to be of statistical significance since so many animals died from pneumonia, we are of the opinion that cortisone did decrease the severity of the glomerulonephritis produced as evidenced by the fact that more cortisone treated rats showed persistent decreases in proteinuria and only mild kidney changes seen microscopically.

4. Further experimentation is needed to prove our

opinion. In future work it would be wise to use an anti iotic in conjunction with cortis ne; and if pneumonia is found among animals not receiving cortisone, an antibiotic should be used in these animals also.

SUMMARY:

1. A brief review is made of certain metabolic and physiologic properties of cortisone.

2. A resume of the literature is made with regard to the use of cortisone and ACTH in the tre tment of nephrosis and acute glomerulonephritis i humans and in laboratory animals.

3. Production of experimental glomerulonephritis in Long-Evans rats using the method of Smadel is discussed along with treatment of a ortion of these rats with cortisone, and a c arison of treated and untreated rats with regard to clinical observations, laboratory findings, survival time, and gross and microscopic findings of autopsy aterial.

4. Conclusions are dr wn.

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2. The Merck Chemical Company for furnishing without charge the cortisone used in this experiment.

3. To Mr. Dave Rhea, Mrs. Barbara McCabe, Mrs. Mary Brady and other members of the department of Pathology for the help they gave us in preparing our material.

4. To Dr. H. W. McFadden for interpreting the sections of our autopsy material and for the advice and guidance he offered us as well as the encouragement he gave us on the numerous occasions when we were disheartened by the setbacks we encountered in our experiment.

KIDNEY SECTIONS:

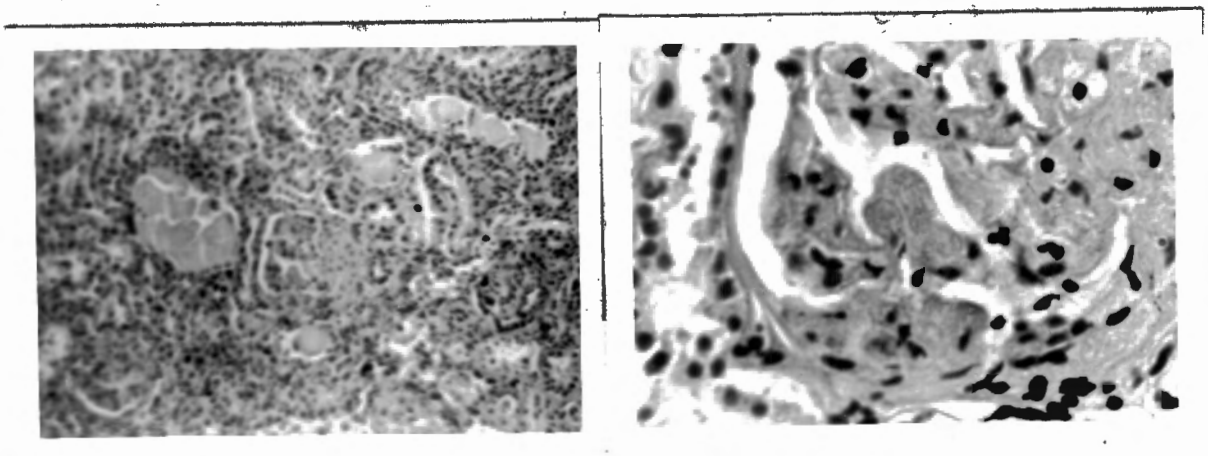

Fig. 1 NT-2 Untreated rat with persistant 4 - albuminuria, showing interstitial fibrosis, cystic dilitation of tubules fil ed with casts, and glomeruli with adhesions, fibrosis, and increased segmentation typical of advanced diffuse chronic glomerulonephritis. High power shows glomerular alterations.

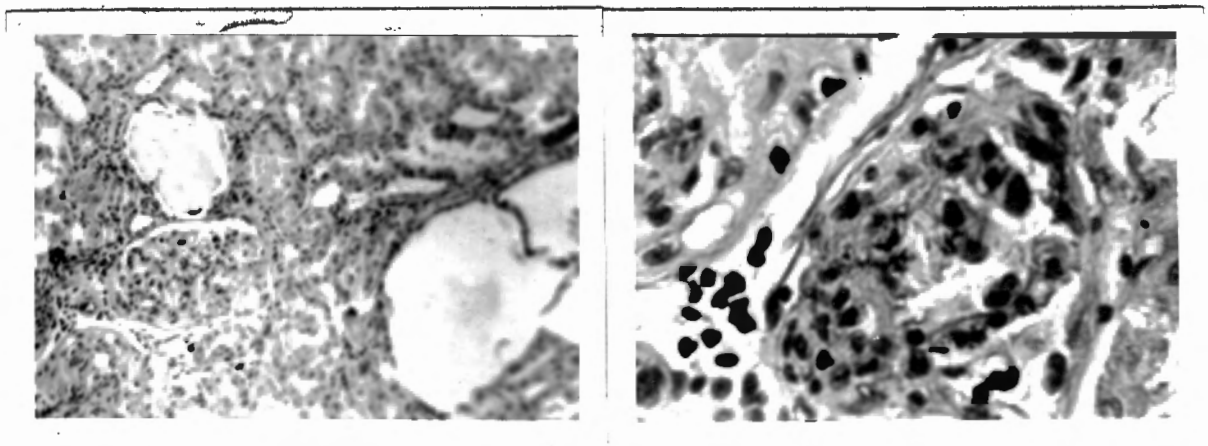

Fig. 2 NT-10 Untreated rat with persistant 4 - albuminuria with findings similar to rat NT-2 (Fig. 1) but appearing more progressive.

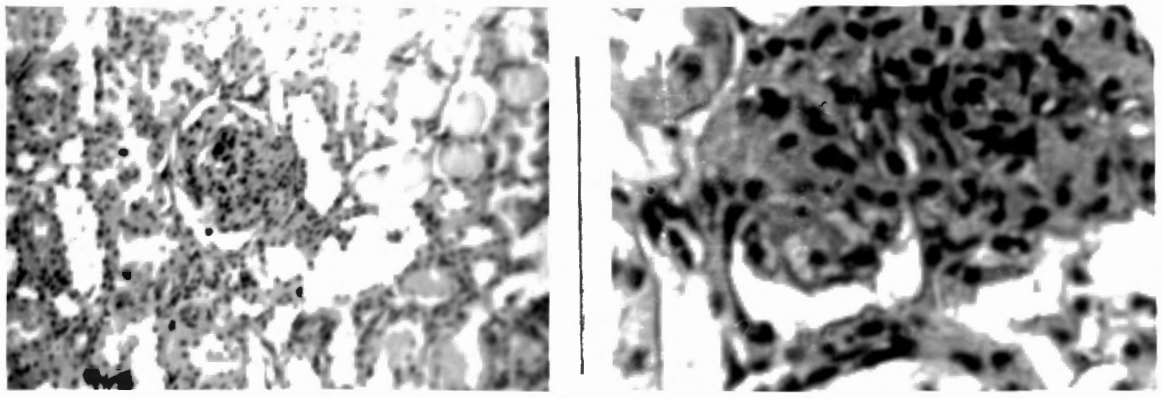

Fig. 3. NT-Ta-5 Treated rat with persistent 3 - proteinuria with findings similar to those in NT-10 (Fig. 2) though somewhat less severe.

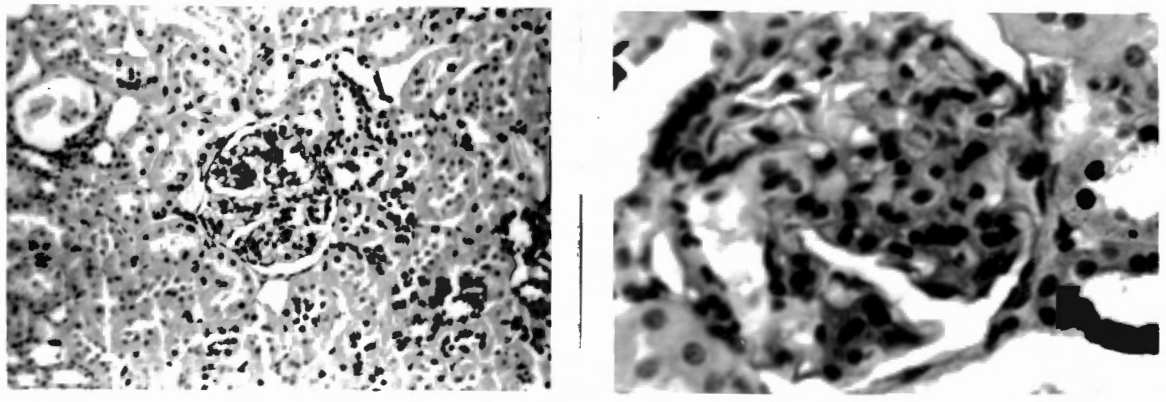

Fig. 4. NT-Tb-7 treated rat with persistent decrease in proteinuria from 4 - to 1 - showing moderate numbers of old adhesions between tuft and Bowman's capsule. This is a relatively old inactive glomerulonephritis.

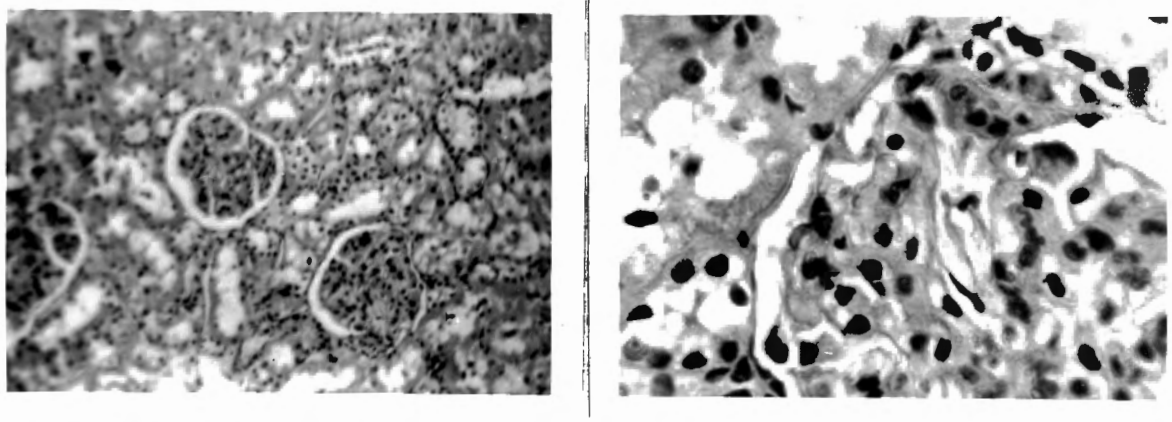

Fig. 5. NT-Tb-8 Treated rat with persistent 4 + proteinuria showing slightly greater reaction than rat NT-Tb-7 with occasional collection of casts in tubules and a mild old glomerulitis.

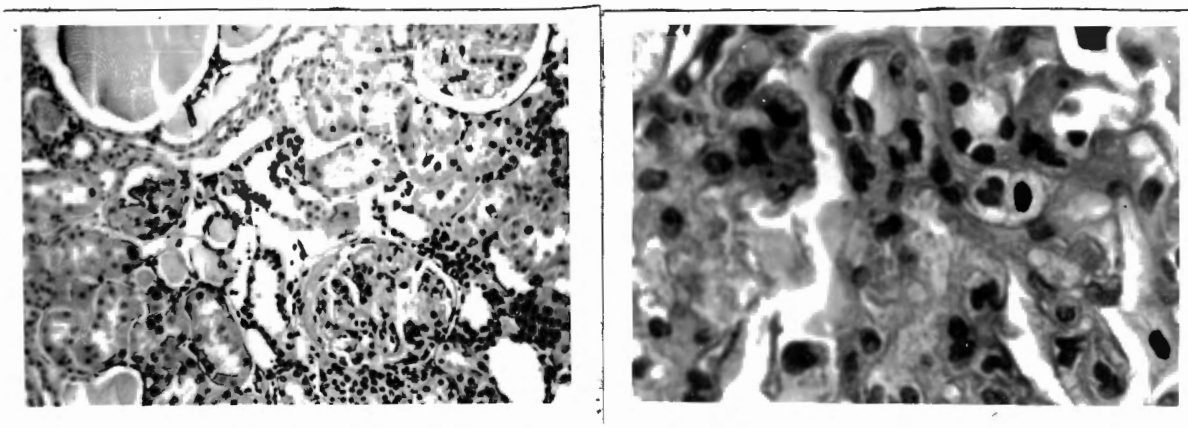

Fig. 6. NT-Tc-16 Treated rat with persistent 4 + proteinuria. These films show moderately advanced changes of chronic glomerulonephritis. In low power film upper tuft shows advanced changes while lower shows less involvement.

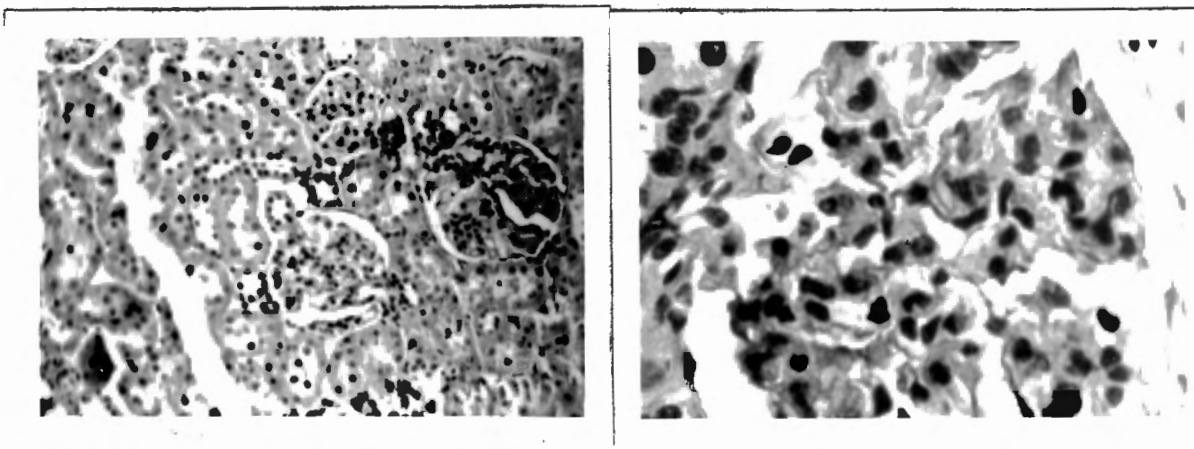

Fig. 7. NT-Tc-17 Treated rat with proteinuria decreasing from 4 + to trace showing mild changes evident of a minimal reaction of glomerulitis that appears to be inactive at present.

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