ACUTE RENAL FAILURE

by

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ABSTRACT

A brief review of the literature on traumatic anuria (acute tubular necrosis, lower nephron nephrosis) has been presented, including a complete bibliography. Special attention was paid to the pathology and pathogenesis of the syndrome and it was concluded that Oliver's recent work (271) probably comes closest to presenting the true picture. He describes tubular necrotic lesions for which the chemical toxins (mercuric chloride, carbon tetrachloride) were responsible, and tubulorhectic lesions which were characteristic of the shock kidney. These lesions could appear at any level in the renal tubule and were characterized by destruction of the basement membrane. Pigment casts were apparent if intravascular pigment release was associated with the illness. The work of Phillips, Van Slyke and associates (291, 292, 355, 356), of Oliver (271) and of Block et al (41) lead one to conclude that renal ischemia is the chief pathogenetic mechanism, though it is obvious that specific extrinsic renal toxins play a major role in specific cases. The role of hemoglobin appears to be chiefly in the production of obstructive casts later in the course of the disease; these pigments are precipitated in the lower nephron where urine is concentrated and acidified, and dehydration and oliguria contribute to their formation.

Three hundred rats were studied in eighteen experiments concerning crush syndrome. It was concluded that the most

important single factor tending to aggravate the renal effects of crushing injury is the antecedent state of dehydration. Myoglobin is not an essential factor in the development of renal damage but tends to aggravate the existing uremia. Acute renal failure was seen to be a late effect of shock; animals developed acute tubular necrosis only if initial shock was severe, but not severe enough to produce death from circulatory failure. Development of this delicate balance of factors was aided by reduction of renal reserve by unilateral nephrectomy. A seldom described but distinct and consistent phenomenon was observed in the development of marked, immediate and persistent diuresis in response to the trauma of limb ligation. This polyuria was of a dilute urine and was taken as an indication of initial increased glomerular filtration followed by decreased reabsorption of water because of tubular damage. It was not an indication of a recovery phase as is recorded in the clinical syndrome.

Testosterone propionate, desoxycorticosterone acetate, cortisone acetate and Compound F did not appear to be promising as therapeutic agents, although in one experiment Compound F showed some promise. Neither did combined therapy with testosterone and cortisone reduce the mortality rate or decrease uremia.

Although there was no doubt that the syndrome of acute renal failure due to acute tubular necrosis could be produced in large numbers of these relatively inexpensive laboratory animals by dehydration and limb ligation, production could not altogether be standardized and the syndrome ran such a short course that

serial observations were difficult to obtain and separation of shock deaths was occasionally impossible. It is felt that future work might well make use of some other laboratory animal, perhaps the dog or cat, and that an initial stress of controlled hypotension or renal artery occlusion could be used. It is also our opinion that further investigation into the value of Compound F as a therapeutic agent in this syndrome is justified.

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INTRODUCTION

In the early years of the recent World War the heavy bombing of British cities resulted in a great number of injuries to the population from falling masonry. The subsequent course run by many of these injured people was such that a "new" clinical syndrome was described. Because the injuries sustained were consistently the result of prolonged exposure to the pressure of destroyed brick and concrete structures, this syndrome was first named the Crush Syndrome (69) and was typified by apparent early recovery from the crushing injury followed by a state of progressive, acute renal failure, with oliguria, anuria and uremia, frequently ending in death. In the ten years since that time much work has been carried on to investigate the possible pathogeneses of the condition, with some progress being made. In this thesis, an attempt will be made to synthesize the great multitude of papers published on the subject, to present principally the experimental aspect of its pathogenesis and to formulate a workable pathogenetic basis for treatment of the syndrome in the light of more recent concepts. It was soon realized that only a brief summary of this voluminous literature was practical but an attempt

has been made, nevertheless, to include as complete a bibliography as possible. In addition, experiments designed to reproduce consistently in rats a syndrome resembling that seen in human crush injuries are reported, as well as the results of using certain agents to lessen the effect of the presumably temporary cessation of renal function.

Since Bywaters (69) first described the crush syndrome, a similar clinical picture has been noted in a great many other conditions and has been described under various titles. "Traumatic Anuria" is perhaps a more general term, indicating that the anuria and its outcome is a result of various forms of trauma. The pathological picture has been taken into consideration together with a slightly different etiological agent in the description "Hemoglobinuric Nephrosis" (229) and in 1946, Lucke (213) summarized the various conditions known to give rise to this syndrome and described the lesion in the kidney as "Lower Nephron Nephrosis". Maegraith (226), emphasizing his opinion that the kidney damage is a result of oxygen lack, has insisted that the "Renal Anoxia Syndrome" is a better name, and more recently, other investigators (55) have tried to remain general in their description of the pathological picture, at the same time avoiding the use of the undesirable term "nephrosis", by referring to it as "Acute Tubular Necrosis". All these descriptions, varying in specificity and point of view, are descriptions of forms of acute renal failure which are closely allied and must be discussed in any consideration

of traumatic anuria itself.

If one must be restricted by the narrowness of definition then one could describe the clinical syndrome and its experimental counterpart as a state of acute renal failure as exhibited by oliguria or anuria, retention of nitrogenous wastes within the body (i.e., uremia) and histological evidence of renal tubular damage which follows trauma. It is obvious that this statement best defines "traumatic anuria", but to include all conditions likely to end in this picture one needs merely to add the various other etiologies such as intravascular hemolysis, extrinsic chemical toxins and so on.

HISTORY

As is the case with most "new" clinical entities, the syndrome of traumatic anuria and its pathological picture are not new at all. Renal deaths with hemoglobinuria after unmatched blood transfusions were apparent as long ago as 1667 when Denys (12) cross-transfused blood from sheep to man. Experimental work with and clinical trial of blood transfusion continued through the subsequent years, notably in the late nineteenth century, and in early editions of Osler's Principles and Practice of Medicine (277) reference is made to "acute parenchymatous nephritis" as a type of acute Bright's disease caused by various toxic agents such as turpentine, phenol and potassium chlorate, acting on the kidney. The same pathological picture could be seen as a late

effect of burns and in toxemias of pregnancy; in later editions, trauma and extensive surgery were added as causes of the subsequent renal damage. Adami (3) in 1909 added salicylic acid, phosphorus, bichloride of mercury and cholera as agents giving rise to the picture of "acute degenerative parenchymatous nephritis".

However, in the reshuffling of classifications of kidney pathologies based on the work of Volhard and Fahr about thirty-five years ago, this particular entity was largely dropped or divided so that it received less emphasis, at least in the English literature, until its rediscovery and description as "Crush Syndrome" by Bywaters and Beall (69) in 1941. One must nevertheless be careful not to malign the quite adequate powers of observation of the many clinicians and experimenters of those first forty years of the century, for cases which we would now classify as lower nephron nephrosis or traumatic anuria were noted and carefully described. Bell (27) considered under the description of clinical acute nephritis not only acute glomerulonephritis, but also tubular disease due to mercuric chloride and hemoglobin obstruction. In a large measure, these entities which we are to consider were included in the term "extra-renal (pre-renal) uremia" (133). This azotemia stands in contrast to that of primary renal disease in which morphological kidney damage is obvious. Under extra-renal azotemia, Bell includes such causes as diabetic coma, peritonitis, hypochloremia and external or internal haemorrhage, and specifies the absent or minimal kidney structural changes. Fishberg (133) anticipates to a large

extent our present classifications by listing prolonged vomiting, diarrhea (as in cholera), hepato-renal syndrome, diabetic acidosis, Addisonian crisis and shock(traumatic, post-operative, peritonitis, burns, coronary thrombosis, etc.) as frequently giving rise to pre-renal uremia. Also, focussing on a less prominent feature of the pathological lesion in the kidney, Kimmelstiel (192) described cases of "acute hematogenous interstitial nephritis" dying in uremia as a result of septic abortion, burns and severe infections. He recognized that his entity was part of a picture of delayed renal tubular pathology in infections and septicemia, conditions associated with hemolysis and the hepato-renal syndrome.

In any case, Bywaters and Beall (69) in 1941 noted that casualties brought in to hospital after being released from under fallen buildings soon developed signs of shock which went on to a picture of renal failure, uremia and death. They reported the first four such cases in the British Medical Journal of March 21, 1941. Bywaters soon discovered that the same entity had been described adequately in the German literature about the time of World War I, notably by Dr. Siego Minami (243) in 1923, though von Colmers (245) had also encountered it with the German relief expedition to the Messina earthquake of 1909. Hackradt (245) in 1917 described a case of burial for nine hours with resultant renal damage in which he emphasized tubular damage but Minami's description of the tubule damage with pigment casts

following "Verschuttung" (burial), resulting in death on about the seventh day (Figure 1), was more complete both clinically and pathologically. His cases tallied well with those of

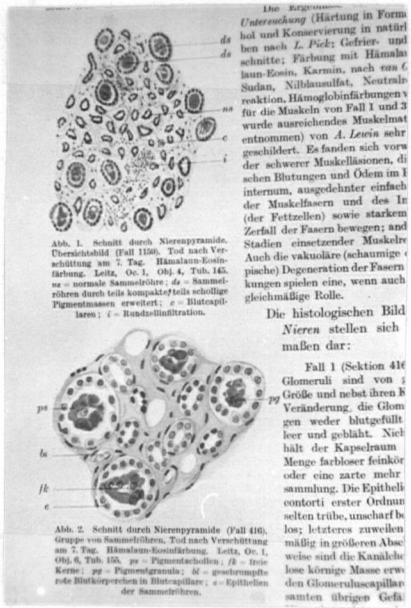

Abb. 1. Schnitt durch Nierenpyramide. Übersichtsbild (Fall 1150). Tod nach Verschüttung am 7. Tag. Hämalaun-Eosinfärbung. Leitz, Oc. 1, Obj. 4, Tub. 145. *ns* = normale Sammelröhre; *ds* = Sammelröhren durch teils kompakte teils schollige Pigmentmassen erweitert; *c* = Blutcapillaren; *i* = Rundzelleninfiltration.

Abb. 2. Schnitt durch Nierenpyramide (Fall 416). Gruppe von Sammelröhren. Tod nach Verschüttung am 7. Tag. Hämalaun-Eosinfärbung. Leitz, Oc. 1, Obj. 6, Tub. 155. *ps* = Pigmentschollen; *fk* = freie Kerne; *pg* = Pigmentgranula; *bl* = geschrumpfte rote Blutkörperchen in Blutcapillare; *e* = Epithelien der Sammelröhren.

Die Ergebnisse der *Untersuchung* (Härtung in Formalin und Konservierung in natürlichen nach *L. Pick*; Gefrier- und schnitte; Färbung mit Hämalaun-Eosin, Karmin, nach van G Sudan, Nilblausulfat, Neutralreaktion, Hämoglobinfärbungen für die Muskeln von Fall I und 3 wurde ausreichendes Muskelmat entnommen) von *A. Lewin* sehr geschildert. Es fanden sich vorw der schwerer Muskelläsionen, di schen Blutungen und Ödem im I internum, ausgedehnter einfach der Muskelfasern und des In (der Fettzellen) sowie starkem Zerfall der Fasern bewegen; and Stadien einsetzender Muskelre Auch die vakuoläre (schaumige pische) Degeneration der Fasern kungen spielen eine, wenn auch gleichmäßige Rolle.

Die histologischen Bild *Nieren* stellen sich maßen dar:

Fall 1 (Sektion 41 Glomeruli sind von Größe und nebst ihren K Veränderung, die Glom gen weder blutgefüllt leer und gebläht. Nich hält der Kapselraum Menge farbloser feinkör oder eine zarte mehr sammlung. Die Epitheli contorti erster Ordnu selten trübe, unscharf be los; letzteres zuweilen mäßig in größeren Absc weise sind die Kanälch lose körnige Masse erw den Glomeruluscapillar samten übrigen Gefä

Figure 1

Bywaters and he compared them with paralytic myohemoglobinuria of horses. Although his description of pigment casts in the tubules of the renal pyramids and Bywaters' rediscovery of the syndrome and identification of the pigment as myoglobin (myohemoglobin, muscle hemoglobin) were separated by some twenty years, work had been going on, clinically and experimentally before and during this period, in the field of intravascular hemolysis. Blackwater fever and incompatible transfusions in particular were

involved and an end result of renal failure with uremia and pigment cast formation in the kidney tubules had been noted. The obvious similarity of these syndromes was soon realized and much of the older investigative work was applied to the new crush syndrome, with investigation in both fields receiving great impetus from the renewed importance of the clinical entity. In 1941, then, investigation into the possibility of a common pathogenesis for these various illnesses attracted new interest and since that time much experimental work has been carried out, many treatments tried and volumes of papers written. Basing their opinions on the work of Baker and Dodds (15) in 1925, Bywaters and Beall (69) at first carried on the idea of obstruction of renal tubules by pigment casts into their theory of the pathogenesis, substituting myohemoglobin for hemoglobin. Because this concept did not satisfy all the observed facts, the idea of renal anoxia was upheld by Maegraith (226) in his work on blackwater fever and he postulated some sort of short circuit of blood through the renal parenchyme (223). When Trueta and associates (353) in 1947 described just this phenomenon (which has come to be known as the Trueta or Oxford Shunt) it was felt that the answer, the common factor, had been found. Trueta's classical work, however, was soon followed by reports which cast doubt on the importance -- and perhaps even the fact -- of this bypass and at present the idea of pathogenesis appears to be in a state of flux, in which several modes of development appear to be acceptable, rather than one.

It should be mentioned here, too, that a third line of investigation has been carried on in the ten year period from 1941 to 1951, based on early observations of the toxic action of such chemical agents as mercury, uranium and phosphate on the renal tubules. Nephrotoxins, acting directly on the renal tubules, have been said to be released from ischemic muscle, and such workers as Eggleton (125), Bywaters (67, 75) and Bielchowsky and Green (30) have named breakdown products of muscle protein, myoglobin derivatives and released intracellular components as being responsible for the renal damage. Two of the pathogenetic theories are drawn together in work on shock which produces hypotension and thus renal anoxia. Corcoran and Page (85), among many others, have identified a vaso-depressor substance released from tissues in trauma which causes a prolonged lowering of blood pressure, which in terms of Maegraith's concept (226) of renal anoxia, would damage the kidney in such a way as to produce the acute renal failure seen clinically.

It can be seen, then, that the syndrome described by Bywaters (69) in 1941 was not new, but had been encountered in similar circumstances earlier in the same century and described adequately by Minami (243) in 1923. In addition, the same end result had been recognized and investigated in conditions of release of hemoglobin into the bloodstream, notably in incompatible blood transfusion and Blackwater fever. The pathological picture was described, at the turn of the century, as acute tubular nephritis, but with the identification of etiologies

responsible in recent years, specific descriptions such as crush kidney, hemoglobinuric nephrosis and lower nephron nephrosis were suggested. As might be expected, with the realization that the kidney damage was the common end of multiple etiological factors, the pendulum has returned, so that at the present time the term suggested by Bull, Joekes and Lowe (55), acute tubular necrosis, seems more satisfactory. Three main theories of pathogenesis, to be discussed later, remain but these are perhaps being viewed in their proper perspective as each contributing in varying degrees to the end result of acute renal failure.

AETIOLOGY

In the years since the crush syndrome was rediscovered the concept has broadened to include many more etiologies producing the same end result. As mentioned previously, the similarity between this syndrome and the renal deaths encountered in Blackwater fever and incompatible transfusion was soon realized. These etiologies are so numerous and appear so diverse that it seems advisable to name the syndrome on the basis of a common pathological picture. For this reason, the term acute tubular necrosis seems satisfactory. In Table A, an attempt has been made to group the causes of acute tubular necrosis under eight headings. Indicative of the confusion as to the pathogenesis of the condition is the rather large column under "Miscellaneous", and it will be noted that several etiologies appear under more than one heading, a fact which indicates that more

TABLE A

INTRAVASCULAR HEMOLYSIS

1 Transurethral prostatectomy ... 219, 207, 94, 367.

2 Blackwater fever ... 371,137,223.

3 Incompatible transfusion ... 12, 229,96,45,365,16,106,109, 128,129,10,343,341,121,151,14,105,246,310,107,135,127.

4 Quinine ... 349, 278.

5 Burns ... 236, 53, 328, 153, 272, 122.

6 Malaria ... 307

7 March hemoglobinuria ... 293, 310.

8 Paroxysmal cold hemoglobinuria ... 345, 310, 333, 109.

9 Paroxysmal nocturnal hemoglobinuria ... 333.

10 Paralytic myohemoglobinuria ... 72, 199.

11 Toxins: Favism ... 333, 310, 137.
Snake venoms
Mushroom poisoning ... 213.

12 Myanesin ... 176.

TRAUMA and SHOCK

1 Hemorrhagic shock ... 84, 322.

2 Traumatic shock ... 87,88,175,229,100,355,280,249,250,251, 252; 102,283,317,164; 26,59,60,61,93,208,305; 116,162, 163,347,78,329,297,348,291,111,319.

3 Burn shock ... See "Burns".

4 Crush injury ... 63,64,65,66,67,69,70,71,72,74,75,25,173, 212,227,228,239,245,247,351.

5 Peritonitis ... 244,177,193,221,315,364,187.

TABLE A
continued

INFECTION

1 Typhus ... 150

2 Cholera ... 352

3 Malaria ... 307

4 Weil's disease ... 362

5 Welch infection ... 178

6 Septic abortion ... 51

ELECTROLYTE IMBALANCE

1 Pyloric obstruction ... 54, 82, 133,187,28,333,214,221.

2 Alkalosis ... 214,193,221,339,9,224.

3 Acidosis ... 160, 161.

4 Hyponatremia ... 315

5 Hypochloremia ... 177

6 Hypokalemia ... 136

CHEMICAL TOXINS

1 Mercuric chloride ... 77, 123, 303, 174, 357, 23.

2 Carbon tetrachloride ... 368,284,90,331,333,271,23.

3 Diethylene glycol ... 271,23.

EXPERIMENTAL TOXINS

1 Uranium ... 304,43,174,264,363.

2 Oxalates and Urates ... 118, 119, 120

3 Phosphates ... 222, 218, 30.

TABLE A
continued

4 Potassium chlorate ... 271

5 Sodium tetrathionate ... 332

7 Potassium dichromate ... 174

MASSIVE DESTRUCTION OF TISSUE

1 Burns ... See "Intravascular hemolysis" and "Shock".

2 Prolonged labor ... 373, 372

3 Toxemia of pregnancy ... 83, 360

4 Concealed, retroplacental hemorrhage ... 372, 285, 112.

5 Welch infection ... 178

6 Abortion ... 178, 278, 268, 269.

MISCELLANEOUS

1 Pulmonary infarction ... 198

2 Electroshock ... 152

3 Gastrointestinal hemorrhage ... 340, 188, 356, 34.

4 High altitude anoxemia ... 252, 76, 197

5 Hepatorenal syndrome ... 48, 172, 275, 338.

6 Heat stroke ... 252, 213, 146.

7 Intravenous soap ... 359

8 Sulphonamides ... 2, 104, 290, 142, 132.

9 Allergy ... 211, 142, 132.

10 Myelomatosis ... 254

11 Lymphosarcoma ... 289

12 Volkmann's ischemic contracture ... 165.

than one pathogenetic factor is involved. Also, in attempting to find a common factor in these many causes, it is often impossible to decide just what factor contributes to the renal failure, so that such headings as "Massive destruction of tissue", "Electrolyte imbalance" and "Infection", though unsatisfactory are, in the present state of our knowledge, unfortunately necessary.

It is impossible to review in detail the many interesting intricacies of individual entities leading to the end picture of acute tubular necroses. However, it was felt that the many references to these etiologies encountered in the literature might well be included in the Table for future reference.

INCIDENCE

A brief review of the incidence of the syndrome as presented in the literature is advisable in order to place it in its true perspective as a clinical entity. Bywaters (64) stated the incidence of ischemic muscle necrosis (crush syndrome) as one to five per cent in air-raid casualties. Presumably these were cases requiring hospital care. Douglas (114), in a very complete work, considered a random group of casualties, 764 in all, admitted to hospital following an air raid. Of these 764, 77 (10.1 %) were buried for two or more hours, and six of these 77 (7.8%) developed crush syndrome. That is, six (0.79%) of the total of 764 casualties were cases of crush syndrome and one of the six (16.6%) died. The works of Lauson et al (208), of

Cournand et al (93) and of Burnett et al (59, 60) present very complete renal function studies in cases of trauma with or without shock, but these records are mainly in the acute phase of trauma. Darmady (99) also found that blood urea nitrogens done in 79 battle casualties were elevated in 35% of cases. In 10,000 casualties reviewed by him there were 44 deaths, twelve of them due to renal failure; all of these suffered shock from blood loss. Snyder et al (335) considered 1411 battle casualty deaths and found 68 deaths from lower nephron nephrosis, with 31 other deaths in which lower nephron nephrosis played a part. Of these 99 fatal cases, 56 had blood pressures below 100 mm. of mercury and only five had "no evidence of shock". Moyer (256) examined renal function following major surgical procedures but again considered only the immediate post-operative period in random cases in which impairment of renal function was not clinically apparent. Gaberman et al (146) searched widely for cases of the "renal anoxia syndrome" and found few - 22 cases in two years of admissions to two large Chicago hospitals.

Although these incidence figures are not high and the frequency of the syndrome in hospital practice will not be great, it is apparent that in times of violence cases of traumatic shock will increase this incidence to perhaps 1% of cases treated. An understanding of its pathogenesis and an adequate regime of treatment therefore become of some importance.

PATHOLOGY

With the great number of etiologies recorded, it is apparent that many minor variations of essential kidney pathology would be expected. There must be, however, a basic common factor in the pathologies in order that the syndrome be described as an entity in itself. This essential factor is, of course, by definition renal tubular degeneration to necrosis.

Although Adami's (3) description of the pathology of "acute degenerative parenchymatous nephritis" in 1907 cannot essentially be improved upon today, this pathologic entity received less and less emphasis in the early 1900's, principally because of the reclassification of kidney pathologies based on the work of Volhard and Fahr. Nevertheless, such men as Bell (27) continued to describe it, partly as "acute haemorrhagic glomerulonephritis" with its tubular obstruction by blood and hemoglobin casts, and also as "acute interstitial nephritis" and "pure tubular degeneration" as in mercury poisoning. Again, Kimmelstiel (192) described a number of cases in which he emphasized the focal interstitial edema and infiltration by naming the renal pathology "acute hematogenous interstitial nephritis".

In 1942, Bywaters (71) followed his report of the new crush syndrome with a full description of its pathology. The most outstanding gross feature of the kidneys was the almost constant appearance of cortical pallor contrasting with a congested,

reddish-purple medulla. Microscopically, the glomeruli were noted to be essentially normal, except for the frequent appearance of intracapsular granular eosinophilic debris and occasionally cubical metaplasia of the capsular epithelium. Again, the essential lesion was in the renal tubules; Bywaters describes a catarrh of the proximal tubule and descending loop of Henle, while in the ascending limb and distal tubule, degeneration and necrosis of tubules and herniation and rupture of casts through tubular walls were seen. Outstanding were pigment casts formed of myoglobin derivatives, in the distal convolution and collecting tubule. Bywaters' first report (69) emphasized the severe degeneration of proximal tubules, but in his more extensive consideration (71) he localized the severe changes to the distal convolution. It will be seen later that, in Oliver's (271) opinion, the renal lesions in crush syndrome were perhaps most accurately noted by Dunn, Gillespie and Niven (117).

Bywaters, of course, noted the similarity of this pathology to that described in transfusion reactions and Blackwater fever as hemoglobinuric nephrosis, as did Dunn et al (117) to the lesions described by Dunn and Polson (120) with uric acid nephritis, and McFarlane (218) with phosphate nephritis. Mallory (229) carefully listed the characteristics of the hemoglobinuric kidney. Grossly, the kidney is enlarged with a pale cortex and purplish pyramids, but may be normal. Microscopically, the glomeruli are again largely normal. The first change noted is

fatty vacuolization of the ascending loop of Henle, becoming severe degeneration by three days, with herniation and rupture. Little change is seen in the proximal segments. Prominent are the casts and these are of two types: pigment casts, staining red-orange in Hematoxylin-Eosin sections, are seen in the lower nephron, while hyalin casts are encountered higher up. A controversial feature described is dilatation of the proximal tubules, which appears to be more prominent in formalin fixed material. It will be seen later in the discussion of pathogenesis that the problems of frequency of casts and presence or absence of tubule dilatation are key points in the argument. In addition, a focal and diffuse inflammatory infiltration of the interstitial tissue is seen with a granulomatous reaction around herniated casts.

A report which has dominated the literature on this syndrome since its publication in 1946 is that of Lucke (213), who recognized the similarity in clinical picture and renal pathology in these various clinical entities. He described the common pathological picture and because he believed it was essentially a lesion of the distal convoluted tubule, he named it lower nephron nephrosis. This name has persisted even though opinions as to the location of the lesion have changed. Lucke's description (213) of the pathology as lower nephron nephrosis remains in common usage.

By means of an extremely meticulous technique, Oliver (270) has been able to study the nephron as a unit in continuity. By his microdissection technique, complete individual nephrons are dissected out and stained. Ten years of careful investigation (271) of human material -- 54 kidneys of crush injuries, burns, transfusion reactions, Blackwater fever, obstetrical deaths, surgical shock, paroxysmal cold hemoglobinuria, sulfonamides, mercuric chloride, diethylene glycol, carbon tetrachloride, potassium chlorate and mushroom poisoning -- and of animals subjected to induced shock or toxins, led Oliver and his coworkers (271) to conclude that there are two essential tubular lesions in the kidney of acute tubular necrosis. These lesions are: (1) Nephrotoxic tubular necrosis -- here the epithelium disintegrates between intact basement membranes, the lesions are seen only in the proximal convolution and are evenly distributed in all nephrons of a damaged kidney. Such agents as mercuric chloride, potassium chlorate, diethylene glycol and carbon tetrachloride produce the typical nephrotoxic lesion, but it must be remembered that the second essential tubular lesion may also be observed in these cases, presumably because the toxins also induce shock. (2) Tubulorhexis, in which there is a localized destruction of the entire tubular wall. The basement membrane disintegrates and there may be intralumenal material such as pigment casts in a fortuitous distribution. There may be an interstitial granulation tissue reaction associated, and regeneration, though

it may begin, is impossible without the support of a basement membrane. As to the localization of this tubulorhectic lesion, Oliver states that it has been found anywhere from the proximal convolution at the glomerulus to the lower nephron at its junction with the collecting tubule. Maximum development is usually in the terminal portion of the proximal tubule which is in the outer stripe of the outer zone of the medulla. The distribution of the lesion in any one kidney is irregular -- irregular among nephrons and within a nephron.

This view that there are two characteristic lesions, tubulorhexis and nephrotoxic tubular necrosis, in the entity acute tubular necrosis would seem to be most acceptable because it satisfies all the known facts -- the actual cytological disruption and the observed distribution of both types of lesion -- and because it is as well based on a pathogenetic concept which is becoming more widely accepted. These ideas are hardly new. Almost all the observations had been made previously. But Oliver's work organizes and classifies these observed facts and places them on a sound basis by persistent, meticulous and patient techniques.

Though most reports have dealt with glomerular changes as being absent or consisting of, at most, ischemia, intracapsular eosinophilic debris and swelling of the capsular epithelium, it is true that some observers have searched for more significant changes in the renal corpuscles of these kidneys. Goormaghtigh (155, 157, 158), in particular, has examined the glomerular

structures and found minute changes which he feels are important functionally. French (143) believes that tubular changes are not sufficient to explain the oliguria of lower nephron nephrosis, and uses Fahr's term "glomerulo-nephrosis" to emphasize his view that the glomerular changes of decreased blood, thickened capillary wall, thickened capsular epithelium and granular precipitate in the capsular space are functionally important.

These glomerular changes may well be present, but the large majority of investigators are agreed that most of the important faults in function of the kidney of crush or trauma can be explained by the tubular lesions. In any case, the dominant tubular changes provide a suitable contrast to the many glomerular pathologies in classifications of renal diseases.

PATHOGENESIS

That an understanding of the pathogenesis is the key to successful treatment of a disease entity is an obvious truth which is no less a fact in the case of acute tubular necrosis. For this reason, much of the experimental work done and the articles written on this subject are concerned with the pathogenetic mechanism. Presentation of this material necessitates retracing steps to consider early opinions on the matter, then following them to their logical end in the most acceptable theories of today. In doing this it is advantageous to group ideas into three categories: (1) simple mechanical obstruction of tubules;

(2) toxic action of agents on renal tubules; and (3) renal ischemia. It will be seen that these three theories of pathogenesis overlap a good deal and are in no way mutually exclusive.

Obstruction:

The earliest theory put forth, chiefly because first observations were made on the pigment nephropathies, was perhaps the simplest and most obvious, that of simple, mechanical obstruction of renal tubules by the prominently seen pigment casts. Yorke and Nauss (371) and Foy, Altmann et al (137) trace the origin of this theory to the German literature of as long ago as 1883 and Yorke and Nauss themselves (370, 371), after observing rabbits injected with homologous hemoglobin solutions, believed that the renal tubules secreted the hemoglobin into the lumena where it was precipitated, forming casts which plugged the tubules chiefly in the thin loop of Henle. They observed dilatation of tubules, presumably as a result of the plugging, and believed that this in turn impinged on adjacent patent tubules thereby increasing the obstruction. They recognized the fact that a lowered blood volume (shock) in Blackwater fever aids production of anuria and precipitation of hemoglobin casts.

A paper which appears to be the basis of many present-day opinions was published in 1925 by Baker and Dodds (15). They examined kidneys from two fatal cases of transfusion reaction and were struck by the widely dilated tubules and capsules, together with casts, seen in one. Experimentally, they concluded

that the hemoglobin released intravascularly was excreted by the kidney as oxyhemoglobin, but in the presence of acid urine (pH less than 6) this was converted to methemoglobin, which was precipitated by a concentration of inorganic sodium salts of at least 1%. The precipitate was thought to be hematin and this process was aided by the normal concentration of tubular fluid as it proceeded down the renal tubules. The hematin casts then obstructed the tubules and accounted for the observed dilatation. Baker and Dodds' conclusions were logical and attractive, but nevertheless were based on experiments carried out on only a small number of rabbits. The work does not merit the devoted attention it has been given over the last 25 years.

Baker and Dodds' work was at first supported by De Gowin et al (108) in 1937, but in the following year, De Gowin (106) observed that in five deaths in renal failure following transfusion reactions, there was no microscopic anatomic basis for renal insufficiency -- few casts and little degeneration and dilatation of tubules. De Navasquez (109) followed this work with the opinion that pigment cast obstruction was not the cause of oliguria because too few casts were seen and dilatation of tubules was rarely seen. He believed that hemoglobinuria did no harm with pH at 5.5 to 6.3, and that if the glomerular flow was sufficient, urine flow would wash out any casts formed. This opinion, it will be seen later, is more or less returned to by Jean Oliver in his classical report of December, 1951 (271).

In the past 10 years, the controversy of whether or not pigment casts can account for anuria by simple obstruction has continued and the role of aciduria in the precipitation of hemoglobin casts has also been thoroughly discussed (96, 10, 137, 32, 62, 117, 202-206). One of the chief objections to the obstruction theory has been suggested by Bywaters and Dible (71): that not enough casts are seen to account for obstruction and urinalyses indicate that there is abnormal tubule function. They point out that, if obstruction alone accounted for oliguria, that urine which was excreted would be from normal tubules and would be of normal makeup; this, of course, is not the case.

Ayer and Gould (10) concluded that necrosis of the distal convolution was the only progressive change seen in the renal pathology and that casts do not produce the structural and functional changes. They point out that kidneys of jaundiced infants may show frequent casts without any evidence of renal dysfunction in life and quote Huber as stating that slight dilatation of the tubules is a normal variation.

A number of investigators (137,32, 377, 202-206) record their belief that precipitation of pigment is a sequel to, not the cause of renal failure, implying that renal damage and dysfunction is present before pigment casts appear and therefore the casts, if they do obstruct the tubules, merely add to renal damage already present. They add that oliguria appears within hours of initial injury and that the earliest structural change seen in the kidney is lipid vacuolization in the ascending loop of Henle (229).

Maegraith and Findley (223) in a preamble to their conclusion that a redistribution of renal blood flow is responsible for anuria, list four objections to the simple pigment cast obstruction theory: (1) Casts are not extensive enough in distribution to discount the high renal reserve; (2) dilatation of tubules and capsular spaces is not always present; (3) reaction of urine played no role in production of anuria (in an analysis of 35 cases of Blackwater fever); and (4) the anuric state is reversible.

In contrast to these opinions, there are several arguments for the obstruction theory. Corcoran and Page (85) conclude that renal damage in pigment nephropathy is due to three factors -- obstruction by casts, ingestion of pigment by cells and a cytotoxic action of hematin on distal tubules. They refer to Oliver's work (270) of the same year, in which he points out that a renal lobule which in histological section appears only partially obstructed by pigment casts may be in fact completely occluded since the casts form at different levels, as seen by microdissection. Experimentally, Flink (135) on the basis of studies in dogs injected with hemoglobin and examined by needle biopsies of an explanted kidney, concluded that the most severe renal insufficiency developed in those animals with most casts, and the amount of tubular epithelial injury correlated with the number of casts. He believed that hemoglobin casts and tubular epithelial damage were equal factors in the production of anuria and insufficiency.

Harrison and co-workers (168) also believe that renal impairment is partly explained by obstruction to flow of urine in tubules and Maluf (232) comes out strongly in favor of the obstruction theory: "The mechanism of renal failure from the intravascular introduction of a moderate quantity of lysed red cells is primarily due to tubular obstruction from casts of hemochromogen combined with a low rate of glomerular filtration."

The answer to these strongly held opinions is probably the compromise stated so convincingly by Oliver in his monograph of 1951 (271). He points out that, as a pathologist, he cannot ignore the fact that, in micro-dissected kidneys from fatal cases of pigment nephropathy, renal tubules are plugged with heterogeneous casts, often massive in extent and obviously contributing to the anuria by obstructing the tubules which normally conduct fluid. He points out that pigment casts are found in all cases where myoglobin or hemoglobin is liberated into the blood, but that there is no correlation between pigment casts and tubular damage. He concludes that simple mechanical obstruction of renal tubules by pigment casts is certainly a factor contributing to the oliguria and anuria seen in the pigment nephropathies.

Myoglobin:

Though most of the above work has been based on clinical observations on cases of intravascular hemolysis and experimental injection of hemoglobin solutions, it is obvious that the

theory applies equally well to those conditions in which myoglobin is the released pigment. Muscle pigment entered the discussion with the reporting of the crush syndrome by Bywaters and Beall (69), who noted the loss of this pigment from pale, edematous, crushed skeletal muscle and were able (70) to identify it in the urine of these injured patients.

Millikan (242) reviewed the properties of muscle hemoglobin thoroughly and reported that it was first isolated and crystallized by Theorell in 1932. Myoglobin, with a molecular weight of 17,500 (as compared to hemoglobin's 68,000) has a renal threshold one-fifth that of hemoglobin, is very soluble and is easily oxidized to the 'met' form. According to Morgan (253) it is extremely soluble in phosphate buffers at pH 6.6. It occurs in red muscle and has a characteristic spectrum. It has the typical hemoglobin oxygen-carrying capacity but probably acts chiefly in storing oxygen rather than transporting it. Its iso-electric point has been reported as 6.78 (7). Newman and Whipple (265) stated that this pigment was not taken up by renal tubule cells, a fact with which Yuile and Clarke (375) agree. These workers found that the pigment was rapidly cleared from the blood -- 25 times more rapidly than hemoglobin -- and its threshold value was 20 mg. per cent.

When Bywaters and Beall (69) first reported the crush syndrome they suspected that the circulating muscle pigment might be responsible for the kidney damage. They soon identified the

myoglobin spectrographically in the urine of air-raid casualties (70), though they could not identify it in the plasma because of its rapid clearance. Bywaters and Dible (72) reviewed seven reported cases of acute paralytic myohemoglobinuria in man, and added an eighth case in which the kidney pathology was the same as that seen in the crush syndrome. Kreutzer, Strait and Kerr (199) reported a ninth case in which the pigment was again identified spectrographically in the urine.

It was natural that experimental work involving the injection of myoglobin solutions would follow these observations. Bywaters and Stead (75) prepared such solutions of human myoglobin by Theorell's method (242) and injected amounts calculated to approximate that released in a typical crush injury (150-200 mg. per Kg.). Using rabbits, they found that myoglobin alone produced no kidney damage in eight animals; myoglobin injections following leg compression produced oliguria, uremia and renal dysfunction with casts in six of six animals; and the pigment injected into animals with ammonium chloride acidified urine resulted in four deaths in 27 animals, with a rise in urea nitrogen of 100 to 860 mg. % in 15 rabbits.

Corcoran and Page (87, 88) were also able to produce kidney damage in rats, comparable to that seen in human crush syndrome, by injecting myoglobin following limb ligation for five hours. Their dose was 75 to 180 mg. per Kg. and in experiments on dogs (85) with urine acidified by diet and sodium acid phosphate they produced partially recoverable renal injury which they

attributed to obstruction by casts and a cytotoxic action of hematin, a split-product of myoglobin. They also injected hematin itself and observed efferent, then afferent, arteriolar constriction and toxic cellular changes with resultant depression of renal function. Kidney damage as a result of hematin injection had been reported previously by Anderson et al (6). These workers believed that the resultant renal failure was produced by a vascular effect rather than a nephrotoxic or obstructive one.

On the basis of these few reports, no definite conclusions can be drawn as to the role of myoglobin in the production of renal damage. It seems probable, however, that the muscle pigment will contribute in much the same way as hemoglobin itself does, be it obstructive or toxic. As Oliver has stated (271), it is difficult to say that pigment cast obstruction does not contribute to renal dysfunction when microdissected specimens show the tubes to be plugged. This effect may well be a later phenomenon, but appears to be a definite one. Whether or not the pigments have a cytotoxic effect is best considered under the discussion of the nephrotoxic theory of renal dysfunction.

Mechanism of Anuria:

The problem of pathogenesis in acute tubular necrosis can be viewed to advantage as a question of the mechanism of anuria. Four possible mechanisms have been suggested.

First, mechanical obstruction by casts, as discussed above, is an obvious cause of anuria. Oliver (271) points out that when a plumber sees a plugged pipe he concludes that fluid will not flow. So it is with the renal tubules obstructed with pigment casts. This plugging contributes to the anuria only later, however, and may well be only a minor factor.

Second, increased plasma osmotic pressure due to hemolysis has been considered by Foy et al (137) to be a possible cause of oliguria in Blackwater fever. With 50% of the blood hemolyzed, plasma protein would be increased by 8%, thus increasing the plasma osmotic pressure to counteract the hydrostatic pressure, reduce filtration and result in oliguria. They concluded, however, that this change made no contribution to oliguria because both albumin and globulin levels in plasma dropped in proportion to the hemoglobin rise so that the total osmotic pressure remained unchanged.

Third, a decreased hydrostatic pressure could also conceivably result in reduced urine output. This is an obvious cause of anuria in the initial stage of shock, where renal blood flow may be interrupted completely. With a prolonged low blood pressure, below 60 to 100 systolic (291), renal blood flow continues to be nil, so that obviously no urine can form. This mechanism, then, involves the renal ischemia theory of pathogenesis and will be discussed under that heading.

A fourth mechanism has been discussed as early as 1925 by Dunn and Jones (119) in their experiments with oxalate nephritis. This is the "back diffusion theory". They believed that the urea retention and oliguria seen in "experimental tubular nephritis" could be explained on the theory that the damaged tubule cells are unable to prevent indiscriminate reabsorption of glomerular filtrate with urea from the tubules into the connective tissue and vessels of the kidney. They carried this idea into their work on uric acid nephritis (120) and recalled it in a consideration of two cases of crush syndrome in 1941 (117). In 1929, A. N. Richards (303) had reported that in frogs made anuric with mercuric chloride, glomeruli were more active and remarked, "The only explanation which I can reach is that under these abnormal conditions the osmotic pressure of the blood proteins is unobstructed by the normal qualities of the tubular epithelium and is able to draw all or nearly all of the glomerular filtrate back into the blood stream." The idea appealed also to Nicholson et al (266) in their work with sodium tartrate nephrosis, as it did as well to Hayman et al (171) in uranium nephrosis and to Bywaters and Dible (71). In more recent functional studies, Redish et al (299) determined clearances and tubular maximums in a case of sulphonamide anuria and found, in the third week, that these values were negative. They became positive at six weeks. They concluded that the initial decreased clearances (mannitol) were not indicative of the true glomerular

filtration rate and that some back diffusion had occurred. Similarly, a negative Tm_{PAH} indicated back diffusion of PAH (para-amino hippuric acid) through the tubules. This same conclusion was reached by Govaerts (159) in comparing urea and creatinine clearances in mercury and bismuth poisoning cases and uranium and oxalate poisoning in dogs.

It would seem reasonable to conclude that anuria is first the result of reduced or absent renal blood flow in the initial stages of shock. If glomerular flow is restored but tubules have been damaged, anuria and oliguria may be continued by back diffusion of tubular fluid through the dead membrane of necrotic tubules into the more osmotically active plasma. When intravascular hemolysis and pigment cast formation is involved, tubular obstruction is undoubtedly a factor.

Nephrotoxin:

The nephrotoxic theory of renal damage in the syndrome has, like the obstruction theory, been held for many years. Implied in this theory is that a substance toxic to the kidney is released into the circulation in trauma, shock, crush, intravascular hemolysis or destruction of tissue, resulting in the clinical picture of acute tubular necrosis. Two possible modes of action of the toxin are considered: first, a direct cytotoxic action whereby tubular cells are poisoned and put out of action; and second, a vasospastic action in which case the renal ischemia theory of pathogenesis is embraced to some extent.

In specific instances, the validity of the nephrotoxic theory cannot be doubted. For many years, specific inorganic compounds such as mercury bichloride (123, 174), uranium (174) and carbon tetrachloride (331, 368) have been known to damage renal tubular cells directly even to the point where various levels of action, with reference to the nephrons, have been noted (271).

The more controversial aspect of the nephrotoxic theory is encountered in the belief that various intracellular components are released from damaged or shocked tissues, which, in effect, have the same cytotoxic action as the chemical agents referred to above. Reviews of recent years (213, 88) have always included this explanation but of late it has received less attention. The observation of MacKay and Oliver (222) in 1935 that rats fed an excess of inorganic phosphate developed lesions in the terminal portions of the proximal convolution were confirmed by McFarlane (218) in 1941, though the affected portion according to this observer was lower in the ascending limb of Henle.

These observations were a prelude to the work of Green (162, 163, 164) and of Bollman and Flock (44) and others, who attempted to isolate a shock-producing factor from striated muscle. Green (162) isolated an adenylic acid derivative from crushed muscle, which Bielchowsky and Green (30) identified as adenosine triphosphate. This compound, when injected into

animals, could produce hemoconcentration, fall in temperature and anuria with casts and elevated NPN, results which compared favorably with results of experimental limb ischemia. Stoner and Green (342) followed this work with analyses of blood phosphate and adenosine levels following limb ischemia and shock and found these to be increased anywhere from 25 to 129%. They concluded that with a diminished blood supply to a large muscle mass, the blood returning from the area has increased adenosine-like action, and believed that adenosine triphosphate may play a role in the renal failure death in rabbits. Bollman and Flock (44), however, in careful experiments based on Green's work, were unable to demonstrate a toxic product released during exercise after limb ischemia, saw no renal impairment and concluded that adenosine triphosphate was not released from ischemic muscle; the adenosine triphosphate was hydrolyzed and only non-toxic products were liberated. Green continued in his opinion, however, reiterating in 1945 (163) that adenosine triphosphate is toxic to the kidney and in crush syndrome its effect is magnified by that of myoglobin. But later (164), in discussing a case of traumatic uremia, he concludes that renal anoxia (vasospasm) and toxic metabolites (myoglobin) are responsible for the renal damage.

A second approach considered the toxicity of thoracic duct lymph in crush injury, trauma and tourniquet shock. Blalock (38) concluded that lymph from dogs suffering five hours

of crush to one limb contained some factor lowering blood pressure and producing casts in urine. Katzenstein et al (190) also found that blood pressure was lowered following injection of thoracic duct lymph taken from animals subjected to tourniquet shock. The effect was, however, variable.

The work of Eggleton (124, 125, 126) on crush injury in the cat is intriguing and has not been altogether discounted. She first reported in 1942 that crush syndrome could be reproduced by a sudden release of compression whereas gradual release prevented development of impaired renal function. An intact liver appeared to be essential for the detoxification of an agent gradually released from the ischemic muscle. She was able to show (125) that an extract of ischemic muscle depressed the creatinine clearance while an extract of fresh muscle did not; that extracts of muscle dead for from four to ten hours proved to be toxic; and that the extract appeared to be a breakdown product of a large protein molecule formed anaerobically.

Also in 1944, Mirsky and Freis (244) injected trypsin intraperitoneally into rats and rabbits and produced renal damage. They concluded that their findings supported the theory that extensive tissue damage released proteolytic enzymes which in turn released "catabolic factors" responsible for renal and hepatic injury.

Most searches for these biochemical factors have been

concerned with the etiology of shock and many agents have been described since Chambers, Zweifach et al (78) demonstrated a vaso-excitor material (VEM) in early shock and a vasodilator material (VDM) in later shock. This work has been carried on to present times (329), but both Reinhard et al (302) and Frank and his co-workers (139) have discounted the importance of this VEM-VDM mechanism in shock. They do, however, substantiate the importance of an intact, functioning liver in the prevention of irreversible shock. Page (297, 302, 348) has also been active in this field, having described a serum vasoconstrictor, serotonin, which he believed was the ultimate effector mechanism in renal ischemia. He believed the primary stimulus to renal vasoconstriction was neurogenic.

Frank et al (138, 139) found also that in dogs suffering haemorrhagic shock and treated by peritoneal irrigation or artificial kidney, the blood chemistry picture improved but survival was not prolonged. They concluded from their work that plasma electrolyte disturbance, azotemia and hypoglycemia were not responsible for irreversibility of shock, and that there is no circulating depressor substance in irreversible shock.

To attempt to untangle the voluminous literature on the subject of humoral agents in shock is beyond the scope of this thesis, but one other lead is of interest. Moyer and Handley (260) injected dogs with norepinephrine and epinephrine and found that both drugs produce a diminution of the number of active nephrons as indicated by functional tests. It is evident that

the newer investigations in the field of nephrotoxins have been directed to the discovery of an agent which is responsible first for the abnormal vascular responses of the body in shock and also for the apparent lasting renal ischemia seen as a late complication of shock. The search for a specific cellular nephrotoxic agent has therefore been overshadowed by this new concept, which is obviously an outgrowth of a strengthened faith in what will be described below as the renal ischemia theory of pathogenesis.

The role of hemoglobin (and by implication, myoglobin) in the production of renal failure has always involved the question of a direct cytotoxic action of the pigment, as well as a vasospastic action. Early opinion (21, 22) was that red blood cell stroma was responsible for the symptoms observed in hemoglobinemia. Conflicting opinions were soon recorded, however, and Sellards and Minot (323) injected small amounts of laked red cells and found no symptoms and no renal damage. Bayliss (24) considered the problem "Is hemolyzed blood toxic?" and concluded that results of rabbit experiments were not valid because of sensitivities in the animal and that incompatible transfusion damage was not due to hemolysis as such but was "rather an aspect of the action of foreign serum protein analogous to that responsible for anaphylactic shock". Reid (301) was the first to suggest a vasospastic action of hemoglobin when he noted a marked but transient decrease in kidney volume

following injection of distilled water or laked red cells. Mason and Mann (237) continued this work and found that associated with decreased kidney volume there were fewer blood filled glomeruli and more narrowed arterioles. It was about this time (45) that the idea of hemoglobin as a toxin began to be accepted but as Mason and Mann pointed out, most reports did not guarantee the purity of the injected pigment. Another variable, that of dosage, was emphasized in the work of O'Shaughnessy et al (276) who found that a 5% solution of hemoglobin in Ringer's was tolerated well as a blood substitute in doses up to 50 gm. of hemoglobin. In the years following the re-description of the crush syndrome in 1941, experimental work was profuse and contradictory, chiefly because little attention was paid to the amount of circulating hemoglobin involved (5). Other products of hemolysis (histamine, potassium) were implicated (147), as were related pigments (31, 32) and conflicting opinions as to the toxicity or vasospastic action of hemoglobin were frequent. One is forced to the conclusion that whatever the action of hemoglobin and related pigments is, it is at least not very dramatic and at most only contributory. That it does play a role is indicated in the work of Badenoch and Darmady (12) who concluded on the basis of rabbit experiments that hemoglobin per se is not toxic, but with renal ischemia produced by renal artery constriction, added hemoglobin plays a significant part in the severity and mortality of the illness. Yuile et al (377) believed that a specific renal vasoconstrictive action of hemoglobin was not an

important factor in the development of renal insufficiency, but more recent work by Miller and MacDonald (241) disagrees. These investigators injected homologous hemoglobin solutions into 25 normal males and on the basis of PAH and inulin clearances postulated again a vasoconstrictor effect of hemoglobin. The cytotoxic action of hemoglobin has also received recent support in the work of Rosoff and Walter (309) who suggest that "heme" competes with cytochromes in the oxidative processes of the tubular cells, resulting in damage, degeneration and necrosis of these cells.

In summarizing present opinion on the nephrotoxic theory, it is apparent that the original idea, the release of specific cytotoxic agents, has been somewhat neglected and replaced by the search for agents which are responsible for the early phenomena of shock. In this way, discussions of the nephrotoxic and ischemic theories fuse to some extent. The role of hemoglobin and related pigments also enters the field in that these agents have been reported to be both cytotoxic and vasospastic. These aspects of the theory remain controversial and only the very definite action of such chemical toxins as mercury, uranium and carbon tetrachloride, can be stated with certainty.

Renal Ischemia:

This third theory of the pathogenesis of acute tubular necrosis puts forth the idea that the renal disease is primarily due to a diminished or absent renal blood flow. As a result of

this ischemia the renal tubular cells undergo degeneration and necrosis, kidney function is disrupted and the clinical picture can progress from one of initial shock to acute renal failure with its olguria, uremia and death. Proponents of this theory believe that shock is the initial event in all cases, whether it be from trauma, crush, haemorrhage, dehydration, burns or anaphylactic reactions. With the initial lowering of blood pressure renal blood flow may cease altogether (probably at blood pressures of 60 to 100 mm. systolic (291)), so that glomerular filtration ceases, accounting for the immediate oliguria to anuria. The tubule cells are also deprived of their blood supply and, sensitive because of their comparatively high rate of metabolism, suffer varying degrees of anoxic damage. When the hypotensive or anoxic period is prolonged, this damage is severe and even though renal blood flow may be restored, degeneration continues to necrosis and the kidney recovers neither structurally nor functionally. Death in acute renal failure ensues. The disputable point in this theory is, what is responsible for the prolongation of renal ischemia beyond the period of initial low blood pressure? There appear in the literature cases of acute tubular necrosis, especially in the field of gall bladder surgery (hepatorenal syndrome), in which either no or only a very short period of hypotension was recorded and yet renal tubular degeneration occurred. It will be seen that nervous, humoral and hormonal agents have been described as producing renal arteriolar constriction to account for the prolonged ischemia, together with

the complicated Arterio-venous shunt suggested by Trueta (353).

Fishberg (133) in 1937 believed that a decreased renal blood flow, the result of decreased blood volume and cardiac output was the primary pathogenetic factor in the development of what he then called pre-renal azotemia. Tomb (351) also believed that the renal characteristics of the crush syndrome were due to anoxia and in 1945, Maegraith et al (226) came out strongly for the renal anoxia theory. These workers point out that pigments are not always present and therefore are not essential; that the nephrotoxic theory demands a wide variety of toxins; that circulatory collapse alone is common to all cases. They leave open the question of what causes the circulatory collapse, but foresee the work of Trueta by suggesting the possibility of a redistribution of renal blood flow or glomerular bypass (225).

In his classical review of lower nephron nephrosis in 1946, Lucke (213) devotes some time to the disturbed renal blood flow theory, pointing out that as Haldane said, "Anoxia not only stops the machinery but wrecks the machine." He appears, however, to feel that the heme pigments and nephrotoxins are of first importance. It was about this time that Trueta (353) published his work on renal vascular shunts and the shift of emphasis to the renal ischemia theory gained impetus. There were some objections to the idea, however, notably by Bywaters (68), who believed that the characteristic lesion of renal ischemia is cortical

necrosis and that renal ischemia short of this necrosis produces patchy degeneration of the proximal convolution. He adds that in crush and incompatible transfusion, the lesions are in the distal tubule.

In spite of these objections, much of the recent work on acute tubular necrosis has been concerned with its shock aspect. Page (280) believed that the entire cardiovascular musculature was altered in shock and pointed out that experimentally it is always necessary to produce shock in rats before injecting myoglobin solutions, if lower nephron nephrosis is to develop. Marshall and Hoffman (233) in the same year analyzed six cases of lower nephron nephrosis on the basis of mannitol and PAH clearances and concluded that the renal lesion was diminished renal blood flow and loss of function of the lower nephron. They defined lower nephron nephrosis as "a syndrome of oliguria with progressive renal insufficiency following a shock-like state produced by a variety of acute insults to the body, and in many cases associated with the deposition in the renal tubules of various derivatives of hemoglobin and myoglobin." This definition would appear to be a most satisfactory one in the light of present knowledge. Because of the swing towards ischemia as the chief pathogenetic factor, the description "Renal Anoxia Syndrome" suggested by Maegraith (226) has been emphasized by Gaberman, Atlas, et al (146) who review the problem, report 22 cases and suggest an etiological classification.

Clinical and pathologic evidence supports this ischemic theory well. The lesion, bilateral cortical necrosis has for some years been described as an ischemic lesion (115) and cases of concealed placental haemorrhage (112), surgical shock (286), burns (53) and so on have been described in which the renal lesion was anything from slight tubular degeneration to bilateral cortical necrosis. Functional and pathologic studies indicating a reduced renal plasma flow have been reported in alimentary haemorrhage (31) and particularly in traumatic shock (102, 99, 93, 208, 303, 322, 355, 356) and Herbut (175) lists twelve cases of "severe degeneration to complete necrosis" of renal tubules, all of different etiology, in which he emphasizes the common factor of shock. An interesting case of cardiac arrest for thirty minutes was reported by Bailey and Rubenstein (13), in which anuria and uremia developed but recovery occurred.

It is to be expected that, with the idea that a period of hypotension was the prime factor in initiating the renal dysfunction, much experimental work with circulatory shock in animals and functional studies in patients suffering shock were reported. Corcoran and Page (84), working with haemorrhagic hypotension in dogs, showed that the renal blood flow fell and filtration decreased; that renal blood flow was distributed unequally; that renal denervation showed restoration of the renal blood flow; and that a humoral vasoconstrictor was responsible for the failure of the kidney to respond to transfusion. Olson et al (273) used dogs subjected to haemorrhage, burns or

crush and were able to produce renal damage which they believe was a result of low blood pressure, low blood volume, hemoglobinemia and an unknown substance, myoglobin or a toxin from ischemic muscle. Keele and Slome (191), using cats, found that the renal blood flow reduction was greater, in proportion, than the lowering of blood pressure by crushing the limb. They therefore believed that reduction of renal blood flow was not due only to reduction in blood pressure. Selkurt (322) confirmed this finding in dogs in haemorrhagic shock, measuring renal blood flow both directly and by plasma extraction of PAH and diodrast. Although the kidney changes (described by Goldblatt) were minimal, it seems likely that a true acute tubular necrosis was obtained, in view of Oliver's recent work (271). Selkurt concluded that in shock the kidney received proportionally less of the cardiac output (5% instead of the usual 20%) due to intrarenal vascular resistance which may be of humoral or nervous origin. The gradual onset suggested a humoral agent; the restoration suggested a nervous mechanism.

The work of Van Slyke and his group (291, 355, 356) has perhaps been the most complete along these lines. They produced shock in dogs by haemorrhage or by blows on the thigh with a mallet to hold blood pressures below 70 to 80 mm. of mercury. They concluded that with a sudden massive haemorrhage there was an immediate drop in blood pressure with renal arteriolar constriction. If blood pressure dropped below 60 to 100 mm., renal blood flow and function ceased. If the initial loss of

blood was not too great, the blood pressure was restored by peripheral vasoconstriction, which is slower than the renal response and kidney function was restored to less than the pre-haemorrhagic level. This cycle could be repeated to the tolerance of the animal and even then partial renal function could be restored by transfusion. But if the depletion of blood was too great, transfusion became useless because peripheral constriction was replaced by dilatation and function failed. Eventually also efferent arteriolar construction, which maintained glomerular filtration, failed, and complete failure ensued even though blood pressure was maintained at 60 to 100 mm. They found that trauma produced a similar series of events and concluded that, while in man it appeared possible to restore the circulation by transfusion to prevent death by shock without restoring enough kidney function to maintain life and so allow death in uremia, in dogs deaths appeared to be almost consistently from shock. Though they believed it almost impossible to get uremia in these animals, it will be seen that, in Oliver's examination of kidneys from these dogs (271) characteristic tubular lesions were indeed seen.

Functional studies in clinical cases tend to show the same reduction of renal blood flow in shock. The work of Cournand and Lauson (93, 208) is again classical in the clinical field. Determinations of glomerular filtration rates and renal plasma flows in cases of trauma, haemorrhage, peritonitis, burns and head injuries with and without shock showed that "the rate

of glomerular filtration and effective renal plasma flow are significantly reduced in nearly every patient suffering from shock, the degree of reduction being roughly proportional to the severity of shock". Again, because renal blood flow decreased more than did arterial pressure, they concluded that "a considerable degree of renal vasoconstriction must have been present", and because glomerular filtration rate fell more than did the arterial pressure, they concluded that there must have been increased afferent arteriolar constriction. They noted that tubular damage apparently persisted for longer than impaired renal blood flow. In later work Van Slyke (355) summarized results of this work by describing an ischemic phase of shock kidney in which there is renal vasoconstriction as compensation for the low blood pressure, and a renal damage phase of shock kidney in which reversible to irreversible renal failure is seen. That is, there must be a degree of shock sufficient to result in renal failure, but not enough to cause death from shock. These workers (111) also point out that normally the kidney extracts less oxygen than do other tissues, and the increased renal extraction of oxygen in shock is much less than the increased extraction in other tissues.

A second experimental approach to the problem has naturally been the production of definite renal ischemia by occlusion of the kidney blood supply. As long ago as 1923 Marshall and Crane (234) noted that temporary closure of the

renal artery resulted in anuria for a period longer than the closure, presumably because the tubules were more sensitive to anoxia and function was interfered with. Starr (337) produced albuminuria in animals and man by renal artery constriction and by adrenaline and ephedrine injection and emotional upset. He was unable to recognize renal structural damage in the animal experiments. McEnery et al (217) reported elevated blood urea levels, oliguria and anuria and cortical pallor with medullary congestion in kidneys following temporary clamping of the renal blood supply.

The more recent era of renal artery occlusion began soon after the crush syndrome received so much attention. Scarff and Keele (312) describe uremia and renal pathology similar to the crush kidney (but with proximal tubule degeneration) after clamping the renal pedicle for up to two hours. They conclude, "Thus there is a possibility that the kidney lesion in cases of crush injury might be due to renal ischemia ... ". Selkurt (320), with shorter periods of ischemia, records reduced inulin, diodrast and PAH clearances and tubule damage "similar to mild uranium poisoning"; he later confirmed these findings by direct determinations of renal blood flow and believed that afferent arteriolar constriction decreased the glomerular filtration rate (321). Badenoch and Darmady (11), in occluding the renal artery of rabbits temporarily, obtained elevated blood urea levels and renal tubular damage which appeared similar to that seen in human traumatic uremia. Koletsky and Gustafson (195) pointed out that the renal

lesion obtained by clamping the renal pedicle 1½ to 2 hours in rats was not the same as seen in human or experimental crush syndrome or in rats with tourniquet shock; here the lesion was proximal tubule degeneration whereas in crush it was lower nephron nephrosis. Later work (194) demonstrated that healing of the necrotic epithelium was possible. Scheibe et al (313), in clamping the renal pedicle or vein alone, concluded also that the proximal convolution was more sensitive to anoxia.

Again, the work of Phillips et al (291, 292) is outstanding. In noting that renal artery compression depressed urea clearance, they suggested that three possibilities were apparent: decreased renal blood flow, decreased plasma filtration or increased reabsorption of urea by devitalized tubules. They clamped the left renal artery of right nephrectomied dogs for two hours, then determined PAH and creatinine clearances. They observed that blood flow was soon re-established after two hours occlusion, but that PAH and creatinine extraction decreased progressively, indicating progressive tubular damage, and concluded that resultant urea retention was probably due to back diffusion. They emphasized in this work the fact that PAH and diodrast clearances can be used as an indication of plasma flow only when the tubules are undamaged. More recent work (308, 41) has confirmed and elaborated on these conclusions based on renal artery occlusion in dogs.

These investigations leave little doubt that renal

anoxia plays a prominent role in the production of renal tubular damage in many of the clinical entities presenting a picture of acute renal failure. They also indicate that active renal arteriolar constriction is a prominent factor in the development of the anoxia, despite the statement of Schroeder and Steele (316) that there is little renal vasoconstriction in shock. The agent causing that vasoconstriction has not been identified. Two mechanisms (146, 50) have been suggested, the nervous and the humoral (including endocrines and pigments). Darmady (99) reported that as long ago as 1859, Bernard observed that stimulation of peripheral and splanchnic nerves produced renal vasoconstriction. Results of controlled experiments in dogs in which the nerve tracts were interrupted at various levels and in various ways, prior to muscle trauma shock, led Swingle, Kleinberg et al (346) to conclude that pain impulses travelling in the dorsal spinothalamic tracts may be responsible for the various phenomena of shock. Wolff (366) in discussing the mechanism of reflex anuria, believed that the pain or discomfort of cystoscopy produced stimuli which reduced renal blood flow and resulted in anuria. Donnelly (113) also was led to conclude that there was some factor which allowed rapid cessation and resumption of kidney function, and Brod and Sirota (52) remarked on a reflex renal ischemia from manipulating excitable rabbits. It was Trueta's work, begun in 1942 (20) and published in full in 1946 (353), that emphasized the importance of neurogenic renal vasoconstriction originating in damaged limbs. They believed that "noxious agents" stimulated nerves peripherally or centrally to divert

renal blood flow to save the cortex from the toxin. That is, these workers suggested that there was neurogenic initiation of a corticomedullary "shunt" of renal blood. This concept will be discussed fully below. Several other reports (200,210, 361) appeared to support the idea that chronic stimulation of the renal nerves was responsible for renal vasoconstriction, and Cort (92, 91) stated that trauma produced a sciatic-splanchnic reflex which could be interrupted by sympathetic block to allow diuresis. Smith (333), however, summarized opinion on nervous control of renal hemodynamics by stating categorically, "the physiological control of the renal circulation remains almost a complete mystery," and points out that, in animals under local anesthesia, spinal anesthesia or denervation of the kidney does not produce renal hyperemia and that renal blood flow is normally determined by "autonomous intrinsic activity of the renal arterioles and is not dependent upon tonic activity in sympathetic pathways." Nevertheless it would appear probable that the immediate response of the kidneys to drastic blood loss is neurogenic in nature (329).

Several humoral agents are known to constrict renal arterioles but whether they are responsible for this phenomenon in the traumatic anuria syndrome is not known. Smith (333) considers a number of these (adrenalin (316, 260), renin or angiotonin (321, 42) and histamine) and notes that fright, exercise and pain all decrease the renal plasma flow. Corcoran and Page (84) consider that humoral vasoconstriction is responsible,

and eventually Rapport, Green and Page (297) isolated from beef serum this agent, serotonin, which had a vasoconstrictive power twice that of epinephrine. In later work, Taylor and Page (348) concluded that the primary stimulus to renal vasoconstriction in tourniquet shock was neurogenic, but because the denervated kidney also showed vasoconstriction, believed that the ultimate effector mechanism was humoral. Adenosine triphosphate, released from crushed muscle, was thought by Stoner and Green (342) to play a possible role in the production of renal ischemia, while Moyer and Handley (260) showed that norepinephrine had the same action as adrenaline, increasing renal resistance by efferent arteriolar constriction. Finally, as was mentioned in the discussion of the role of pigments in the production of anuria, it has been suggested that hematin (85) and hemoglobin itself (62, 216, 241) have a vasoconstrictive effect and may even produce "ischemic alteration of the glomerular capillaries".

Whatever the cause, neurogenic or humoral, renal vasoconstriction appears to be a phenomenon, accepted by most observers, which is part of a general response to shock. The immediacy of this response would suggest that neural pathways are at least early responsible, while prolongation of vasoconstriction may be due to a humoral agent. Investigations into the pathogenesis of shock itself, such as carried out by Shorr et al (329) and Frank et al (140, 139) may produce progress in

this direction.

Trueta's corticomedullary shunt of renal blood flow:

It was in 1947 that Trueta et al (353) published in full results of experiments begun in 1942 (20, 141) to study the problem of arterial spasm in response to trauma. A complete review of this very thorough work is impossible at this point, but in essence the theory is based on morphological differences between cortical and juxtamedullary glomeruli (Figure 2), which, under stimulus of trauma, shock and so on,

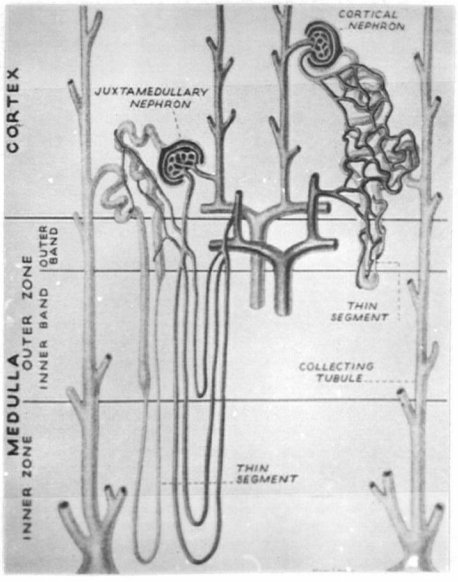

Figure 2

allow the renal blood flow to be shunted through the juxtamedullary glomeruli, bypassing the cortical ones and the main mass of tubules. Trueta et al believe that a reflex neurovascular

mechanism is responsible for these profound alterations of intra-renal circulation. They observed this change by angiographs, by direct observation of cortical pallor and "stream-lines" and by injection masses (neoprene latex and india ink) in response to tourniquets, sciatic nerve stimulation, haemorrhage and various drugs (adrenalin, pituitrin, pitressin, ephedrine and staphylococcus toxin); they made observations on dogs, cats, rats, guinea pigs but chiefly in rabbits. Because of this shunt, cortical glomeruli (which constitute 85% of renal glomeruli) are rendered ischemic which in turn renders at least an equal percentage of renal tubules ischemic (see Figure 2), accounting for the renal dysfunction seen in traumatic uremia, crush syndrome, bilateral cortical necrosis and other renal diseases.

Trueta's shunt theory implies the following: (1) that so-called juxta-medullary glomerular differences are present in species other than rabbits. Trueta states that these differences are seen most often in rabbits, but less so in the dog, cat, guinea pig and rat, and that the differences are less evident in man; (2) that these juxta-medullary glomeruli function differently from the cortical glomeruli. Trueta suggested that they constitute a virtual arterio-venuous anastomosis and pointed out that the close relationship of the vasa recta and loop of Henle may have some significance in water reabsorption; (3) that the shunt, when in operation, allows blood to bypass tubules whose function is essential for normal kidney function with the result that less oxygen is utilized by the kidney. Trueta observed streams of red,

oxygenated blood in the renal vein when the shunt was said to be in operation and therefore implied that the arterio-venous oxygen difference was decreased in this syndrome; (4) that, essentially, the total renal blood flow was not reduced but that this total flow was merely short-circuited through the "lesser circulation of the kidney". In his early work, however, Trueta himself contradicted this implication by his observation that the renal blood supply was reduced by neurogenic renal vasoconstriction. It is on these four points that Trueta's shunt theory has been most severely criticized.

It is interesting that, many years before Trueta suggested his corticomedullary shunt, observations were made on kidney pathologies which support his ideas. Renal pathology in fatal blood transfusions was frequently described as an enlarged kidney with pale cortex and congested, red-brown medulla; microscopically the glomeruli were bloodless and interlobular capillaries (vasa recta) were engorged (365). And Trueta himself pointed to a strong support of his ideas in the kidneys described as bilateral cortical necrosis (115, 336) in which the entire cortex becomes necrotic because of interference with its blood supply. This would appear to be the extreme instance of Trueta's shunt, as suggested by Heggie (179). It is interesting, too, that in 1944, Maegraith and Findley (223) predicted Trueta's theory when they described the kidneys of Blackwater fever as having an anemic cortex but congested medulla and

suggested that the renal glomerular flow was short-circuited. Again in 1946 (225) they pointed out that the renal blood flow must be redistributed in the kidney and from the histological appearance suggested a corticomedullary diversion.

The shunt theory received some early support from the work of Simken et al (330) who injected into the renal artery glass spheres of such a size that, when they were recovered, these workers were forced to conclude that arterio-venous by-passes existed in the kidney or its capsule. Experiments were carried out in rabbits, dogs and human kidneys. The controversy of arterio-venous anastomoses has been apparent for many years and is reviewed adequately in Smith's book (333). Arcadi and Farman (8) were able to duplicate Trueta's work by india ink injections in rabbits, as were Goodwin et al (154), using tourniquets or sciatic nerve stimulation in rabbits, dogs, cats and monkeys. They observed kidneys directly, used india ink and evans blue injections and visualized the renal vasculature with thorotrast, and believed they demonstrated a neurovascular control of the renal circulation in which renal ischemia started in the cortex and spread to the medulla. They saw the possibility of a true ischemia here, however, and questioned whether the phenomenon was a shunt or rather a progressive peripheral vasoconstriction. Black and Saunders (35) also supported Trueta's observations with the reservation that, before the shunt is accepted, three criteria must be satisfied: (1) low inulin and PAH clearances with increased C_{in} to C_{PAH} ratio, (2) PAH

extraction less than 80% and (3) absence of gross changes in general circulation, since efferent arteriolar constriction, rise in renal vein pressure or fall in systemic pressure could produce a picture simulating the shunt.

Evidence against the shunt also appeared early and has continued to accumulate since Barclay et al (17) expressed their unhappiness that clearance methods had not been used by Trueta. Most criticism has been from this point of view. Trueta's observations imply that total renal blood flow is not reduced in the syndrome, the change being merely a short-circuiting of blood rather than a reduction in flow; he did not measure the renal blood flow, however. Based on many reports of functional studies carried out in animals (257, 302a, 258, 183, 259, 260) and in man (347, 302a, 331, 18) it appears conclusive that in the kidneys of sciatic stimulation (347, 257, 258, 259), adrenalin injection (302a, 183, 258, 259, 260), carbon tetrachloride poisoning (331) and incompatible transfusion (18), there is in fact a true reduction of renal blood flow. Moyer et al (258) substantiated this claim by direct measurement of renal blood flow. Further inroads into Trueta's theory have been made in attempts to demonstrate the shunt by injection methods. Maluf (232) got no shunting of blood from cortex to medulla as shown by india ink injections in dogs dehydrated and receiving hemoglobin injections. Kahn, Sheggs and Shumway (189) injected india ink under pressure into kidneys of rabbits treated with epinephrine, pitressin, amyl nitrate, haemorrhage, central sciatic stimulation and renin and saw no

evidence of a bypass. Schlegal and Moses (314) formed the same conclusions using a fluorescent dye to visualize renal blood vessels of rabbits in tourniquet shock, and Block et al (40), using neoprene, state that "the only consistently existing vessels which directly communicate between the renal arteries and veins ... are situated at the hilum of the kidney."

Trueta's implication that oxygen utilization by the kidney was reduced has also been discounted. Repeated determinations of arterio-venous oxygen differences by various workers (258, 259, 260, 257, 302A, 331, 18, 183) have shown that this difference, normally very small, has remained the same or has increased, rather than decreased as implied. Trueta's observation of arterialization of renal venous blood is not substantiated by direct measurement of oxygen levels.

A fourth interesting contradiction of Trueta's work is reported by Mukherjee (263), who states that in dogs subjected to tourniquet shock, radioactive isotopes indicate that renal anoxia is diffuse, not localized as Trueta suggests. He notes that the proximal nephron is affected as well as distal.

No criticism of the work of Trueta et al can be complete without a consideration of the conclusions reached by Maxwell, Breed and Smith (238) on the significance of the renal juxtamedullary circulation in man. These men point out (1) that, since the proximal segment is responsible for excretion, when blood is perfusing the vasa recta which are in contact only with

distal and thin segments PAH clearance should be low; (2) that with the bypass, filtering surface is reduced and therefore inulin extraction should be reduced; (3) that renal arterio-venous oxygen difference should be decreased with shunt and (4) that reduction in PAH and inulin extractions alone would not indicate finally a shunt, because proximal convolution damage could do it; but reduction of these with a normal renal blood flow would be good evidence of a shunt. They found that none of these criteria were satisfied in cases of old age, pitressin or adrenalin injection, hypertension, congestive heart failure and shock anuria. They concluded as follows: (1) juxtamedullary glomerular function does not differ from cortical; there is the essential relationship between tubules and vasa recta which allows usual kidney function; (2) if diversion did occur, to produce cortical ischemia, renal function would continue by way of juxta-medullary glomeruli; (3) evidence is against diversion of blood through uncleared channels; (4) juxtamedullary circulation in man has no unique functional significance. It is seen in the rabbit only as a species difference.

On the basis of evidence cited above, the conclusions of Maxwell et al are justified. Observations made on renal blood flow and arterio-venous oxygen differences are not compatible with Trueta's concept of a corticomedullary shunt of renal blood in the traumatic anuria syndrome. The thoroughness of Trueta's experiments, however, convince one that the phenomenon is of frequent occurrence at least in rabbits, and the occurrence

of clinical cases of bilateral cortical necrosis adds to one's conviction that the Trueta shunt may indeed have a place in the scheme of things as an extreme in a series which includes anything from undisturbed renal blood flow to complete cessation of that flow.

Such a survey of the pertinent literature leads on inevitably to the conclusion that renal ischemia is of prime pathogenetic importance in the development of acute renal failure in shock, burns and crush. The work of Cournand's group (93, 208) showed conclusively that in shock from trauma with or without haemorrhage, peritonitis, abdominal injury and burns, "the rates of glomerular filtration and effective renal plasma flow are significantly reduced ... the degree of reduction being roughly proportional to the severity of shock." The work, too, of Van Slyke's group (355, 356, 291, 111) has substantiated these observations in dog experiments in which shock was induced by haemorrhage and by trauma. Phillips and Hamilton (292) and others (312, 320, 11, 195, 194, 313) completed the cycle of information when, by clamping the renal artery to produce ischemia, they produced renal dysfunction as measured by clearance techniques and tubular lesions described by Oliver (271) as being identical to those seen in shock or crush kidneys. Both the functional and structural changes seen in the kidney in human cases of shock from any cause, therefore, have been observed in animals subjected to experimental shock and in animal kidneys with obvious ischemia induced by clamping of the renal artery.

The pathogenetic significance was apparent to Block et al (41), when they undertook to carry out this complete cycle of experiments in dogs. They subjected 28 dogs to periods of hypotension of 70 mm. of mercury for periods of from six to 26 hours and studied kidney function and later histological changes in fifteen animals, the early histology in thirteen. They also studied the effect of renal artery occlusion from three to six hours on 24 dogs and 23 rats (one to three hours occlusion), as well as the effect of epinephrine on eight dogs. In all cases, they observed changes in renal tubules from degeneration to complete cortical necrosis, but found that death in renal failure was rare; it required almost complete ischemic destruction of the kidney. They state that these results were independent of the nerve supply and that there was no evidence for Trueta's shunt.

It is also of importance to consider the means by which renal ischemia is produced. In cases of shock it is obvious that an initial period of hypotension is responsible for the cessation of renal blood flow (291). Renal vasoconstriction follows and appears to be primarily neurogenic, secondarily humoral (348).

A third possible source of anoxia is a decreased oxygen extraction by the kidney; most investigators point out that, on the contrary, arterio-venous oxygen differences are increased -- that is, that oxygen extraction by the kidney in shock is increased rather than decreased (258, 259, 302a, 183, 111).

Normal renal oxygen extraction, however, is singularly inefficient and arterio-venous oxygen differences are so slight that they may be within the error of methods of determination (39, 238).

Summary of Pathogenesis

In crystallizing an opinion on the pathogenesis of acute renal failure due to acute tubular necrosis, one is impressed by the conclusions reached by Oliver et al (271) in 1951 and by the work of Phillips, Van Slyke, et al, on which to a large extent, Oliver's opinions were based. Observations made clinically by Lauson, Cournand et al (208, 93) are also convincing and the confirmation of experimental conclusions afforded by the work of Block, et al, in September 1952 (41) appears to complete the picture of pathogenesis.

It is at first essential to emphasize the existence of two pathogenetic mechanisms of prime importance. Firstly, there appears little doubt that in certain clinical entities, specific extrinsic toxic agents are responsible for disruption of tubular cell metabolism, producing the acute tubular necrosis. Common among these agents are bichloride of mercury, carbon tetrachloride and uranium salts. Oliver (271) has named the lesions they produce "nephrotoxic tubular necrosis" and described convincingly the characteristic pathological lesion seen in these poisonings and the differences from the second type of tubule damage.

Secondly, there is a large group of clinical conditions in which shock is a common factor and which frequently (146, 114, 335) give rise to the syndrome which we prefer to call "acute renal failure due to acute tubular necrosis". Such conditions have been described earlier in this thesis but can be listed briefly (252): trauma, haemorrhage, crush, burns, peritonitis, hepatorenal syndrome, retroplacental haemorrhage and others. There is no doubt that this second pathogenetic mechanism, renal ischemia, is of prime importance here. A resume of the probable pathophysiological phenomena may well be as follows: in shock induced from any cause the insult may be so severe or the resistance to it so low that the individual progresses to the so-called irreversible stage and dies in peripheral circulatory failure; or the individual may respond well to the stress and recover uneventfully from the hypotensive shock period; or he may make an apparent recovery from the acute shock period only to pass into what might be called a late effect of shock, a state of acute renal failure with oliguria, anuria and, once again, either recovery or death in uremia. This third possibility, acute renal failure, is typified by cases in which massive haemorrhage initiates the renal failure. With the haemorrhage there is an immediate fall in systemic blood pressure which, if slight, may still allow glomerular filtration due to the compensatory renal efferent arteriolar constriction. But if the systemic blood pressure falls below 60 to 100 mm. of mercury (291) renal blood flow and function cease. Accompanying this early hypo-

tensive period there is renal vasoconstriction which is part of a generalized compensatory vasoconstriction which may result in the individual compensating enough to survive the acute shock period following the haemorrhage. Blood pressure can be restored either by this compensation or by transfusion and the kidney circulation returned to something less than pre-haemorrhagic levels. In spite of this apparent return to normal, some cases go on to tubular degeneration and necrosis with clinical acute renal failure. Because in both clinical and experimental cases clearance techniques and direct measurements show renal blood flow to be diminished, and because experimental occlusion of the renal artery in animals can produce lesions identical with those seen in clinical acute tubular necrosis, the damage has been blamed primarily on ischemia. Whether the initial period of hypotension, when prolonged, is sufficient in itself to produce the damage, or whether the early (probably neurogenic) renal vasoconstriction is prolonged either by nerve impulses or by humoral agents to prolong the anoxemia cannot be stated definitely.

The role of intrinsic nephrotoxic agents, presumably released from damaged or ischemic tissues, in the pathogenesis of this syndrome can be less convincingly stated. The status of the various extrinsic chemical agents has been mentioned previously. However, the presence of a protein breakdown product of ischemic muscle (125) or a toxin from massively destroyed tissues, to disrupt the metabolism of tubular cells would

appear to be unnecessary to explain the renal damage since in most of these cases, shock and reduced renal blood flow are accompaniments. Other humoral agents (renin, VDM, serotonin, adenosine triphosphate), if they prove to be of some importance, probably are so by virtue of their shock-producing or renal vasoconstrictive properties. Hemoglobin pigments as well play at least only a minor nephrotoxic role either by an unproven cytotoxic action (6, 309) or by a renal vasospastic action.

It is therefore implied that cellular anoxia is responsible for the tubular damage, which in turn accounts for the renal dysfunction. The mechanism of this dysfunction is probably "back diffusion" (303, 117), in which the dead tubule cells act as a membrane through which tubular fluid, urea and other wastes diffuse back to the interstitial fluid and thence into the circulation.

The place of pigment cast obstruction in the development of renal dysfunction, so evident in the intravascular hemolyses, has been given its proper place by Oliver et al (271) and Block et al (41). Pigment cast obstruction is at least unnecessary. Clinical lower nephron nephrosis is seen in cases in which no pigment release is involved and acute tubular necrosis can be produced experimentally without appearance of hemoglobin or related substances. Again, clinically, intravascular release of pigments usually occurs in cases in which there is associated shock -- transfusions, Blackwater fever, post-operative

transurethral prostatectomy, crush, burns -- so that ischemia is probably contributing more to the renal failure than is tubule obstruction. But, as Oliver has pointed out, in cases in which pigment casts are prominent, one cannot ignore the fact that the involved tubules are plugged and will not carry urine. If there is back pressure in these plugged tubules and tubule dilatation occurs, or if tubular fluid escapes into the interstitium, then occlusion of adjacent, otherwise patent tubules might well occur, adding to the obstruction or damage by increased intrarenal pressure (289, 364). Although it appears possible to produce renal shutdown from induced hemoglobinemia (232, 168, 241), it is always easier to induce the renal damage when dehydration (202-206) or renal anoxia (309, 41) are present. It would therefore appear that the presence of circulating pigments merely adds to renal damage induced by renal ischemia originating in shock or severe dehydration.

Associated with pigment cast obstruction is the problem of precipitation of hemoglobin, myoglobin or related pigments. It is probable that a variety of factors (15, 376, 240), urine pH, glomerular filtration, urine salt content, tubular reabsorption, combine in the lower nephron and collecting tubules to produce conditions favorable for heme pigment precipitation.

EXPERIMENTAL

AIM

As was stated earlier, it was thought advisable to repeat in a systematic way some of the work done by other investigators in order to determine the role of certain factors in the production of traumatic anuria. Preliminary experiments, then, were carried out with the aim of producing "lower nephron nephrosis" in a standard way in a substantial proportion of test rats. Once this standardization was accomplished, further experiments were designed to test the efficacy of certain hormonal agents in the alleviation of the kidney damage.

A sampling of the experimental literature on this subject has shown that three factors play a prominent role in the pathogenesis of acute tubular damage. The pigment cast obstruction theory can be associated closely with the nephrotoxic theory of pathogenesis and so the first variable factor chosen was the role of myoglobin (muscle hemoglobin) in the production of kidney damage. It was not considered crucial to this work whether the pigment produced its effect by a toxic action or by obstruction, though observations on this problem will be made. The second factor considered was what may be called clinical shock, and here

a crush injury to the limb of the test animal was the means of production. Again, whether the pathogenetic mechanism was one of release of nephrotoxic materials from damaged tissues or simply one of production of prolonged hypotension was not considered, though some conclusions will be drawn. The third factor controlled in the following experiments was the hydration of the animals, since dehydration (202, 203, 232) has been shown to emphasize, in some way, the tubular damage to the kidney.

It can be seen that the aim of the experiments reported below has not been primarily to investigate the pathogenesis of acute tubular necrosis, but rather to standardize the production of the syndrome in the white rat, and to investigate the therapeutic possibilities of various hormones. Statements as to the mechanism involved, based on these experiments, will therefore be impressions rather than conclusions drawn from controlled experiments.

METHODS AND MATERIALS

Both male and female white rats of the Sprague-Dawley and Wistar strains, weighing from 200 to 350 grams, were used in order to bring out any sex difference. These animals are convenient generally because of their size, relative economy, availability and hardiness; specifically, they are of use because the skeletal muscle contains little, if any, myoglobin so that in experimental work designed to test the role of

myoglobin release and of crush injury, these two factors can be conveniently separated.

Dehydration was accomplished merely by withdrawing water for periods ranging from 24 to 72 hours. The effectiveness of this method was easily ascertained by the massive weight loss recorded (up to 20% of body weight in 48 to 72 hours) and by the production of urine which was low in volume and high in concentration, of feces which were small, hard and dry as well as reduced in amount. Urine specific gravities were not determined, but gross observation of color, and in some cases viscosity, gave a rough index of that factor.

Myoglobin was obtained commercially in a purified crystalline form and was administered intravenously into the femoral vein after dissolving it in a phosphate buffer of pH = 7.35 (170). Difficulty was encountered in dissolving the protein which had been dehydrated so completely to this crystallized form. It was not thought advisable to use solutions of pH too far removed from its isoelectric point of 6.78 (7), since injecting a solution of very acid or alkaline pH would introduce a complicating factor. The possibility of dissolving the pigment in rat serum was considered but again it was thought advisable to avoid the added question of sensitivity reactions to necessarily heterologous serum as well as the difficulty in keeping such a solution sterile. It was found fairly satisfactory to make up a solution of 25 mg. per cc. of buffer which was

kept 24 to 48 hours in an oven at 65% C, with frequent shaking. Complete dissolution was not obtained, but it was estimated that 60 to 70 per cent of the protein dissolved and the remainder could be suspended by vigorous shaking prior to injection.

Dosage administered ranged from 0.1 to 0.15 mg. per gram of body weight, a figure based on that calculated by Bywaters (75), and used also by Corcoran and Page (85). This amount necessitated the injection of a volume up to 1.6 cc. in a 250 gm. rat, but if given slowly, no ill effects were observed. Injections were made with a #25 hypodermic needle with the rat under ether anesthesia, at times considered to simulate as closely as possible the time relationships encountered in human "crush syndrome" -- i.e., immediately on release of the crushing ligature.

Ligation was carried out on the hind limb of the animal, either left or both, in the manner illustrated in Figure 3.

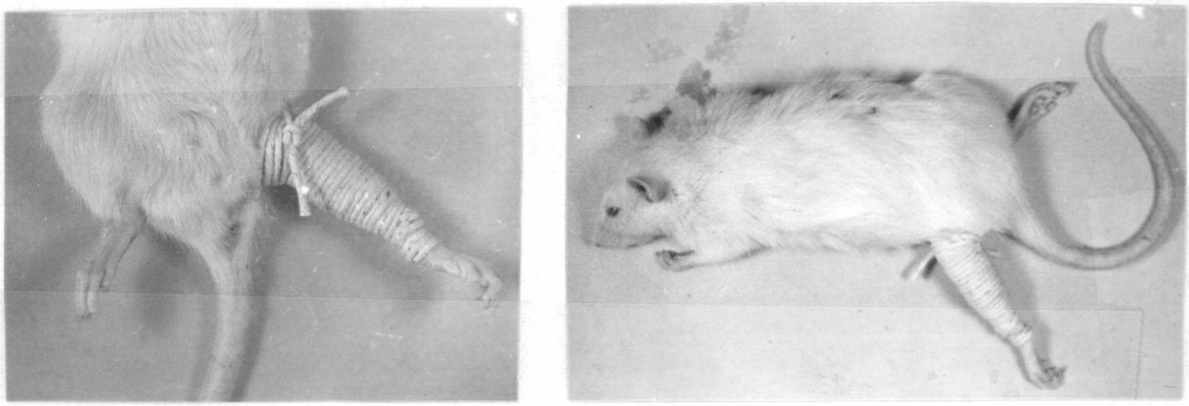

Figure 3

Under ether anesthesia, the limb was clipped of hair from ankle to groin in order that the ligature would not slip. Using heavy twine, the limb was then wrapped tightly from ankle to as high on the limb as possible without interfering with the urethral outlet or incurring the risk of loosening. This meant that the ligature was usually tied at what was essentially the "mid-thigh" level. The ligature was left in place for intervals of four to five and one half hours, being protected by adhesive tape wrapping in case the animal attempted to bite loose the string. During this time the rat was sedated with pentobarbital 2.5 mg. per 100 gm. of body weight (5 mg. per cc.) given intraperitoneally as needed. The five hour period was spent in large glass funnels which allowed for more easy observation and handling as well as for convenient collecting of urine. With the period of crush completed, animals were removed from funnels, the ligature was removed under the remaining nembutal sedation or ether anesthesia, the injured limb was massaged until it lost its "doughy" consistency and the foot appeared bright red, and the animal was then placed in a metabolism cage for observation. The bladder was not emptied by compression at the completion of the five hours crush because only the total 24 hour urine volume was to be recorded. In animals which were normally hydrated, difficulty was encountered in that they would bite the injured limb, resulting in haemorrhage and an at times severe anemia. Attempts were made to protect the limb in some way to prevent the biting but not restrict the swelling. Loosely applied adhesive tape, which could be added as necessary, was found to be most satisfactory in

this regard. Dehydration animals were observed to not bite the crushed limb and frequently these were left unwrapped. The effectiveness of the ligature in producing a typical crush injury was observed in the almost immediate swelling of the limb (Figure 4) and at autopsy by the appearance of subcutaneous and muscle edema as well as discoloration of the muscle (Figure 5).

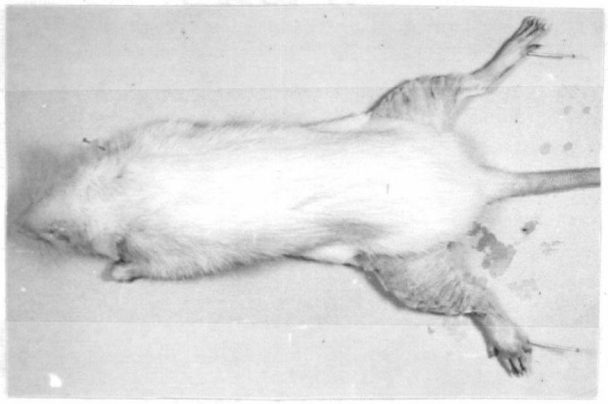

Figure 4

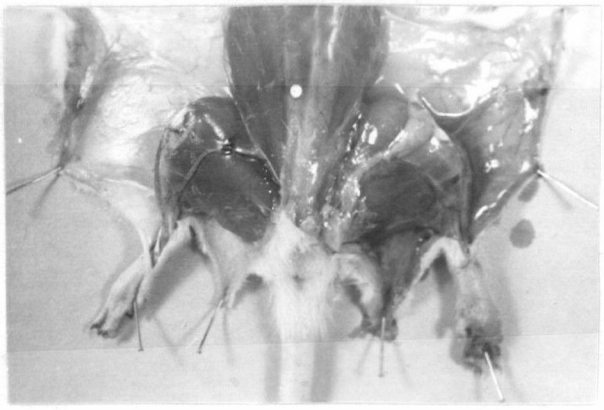

Figure 5

Because production of the syndrome was not completely satisfactory in the intact animal, and because the rat is known

to have a formidable renal reserve, it was decided to reduce this reserve in as physiological a way as possible. Right nephrectomy was therefore carried out in later experiments. Under ether anesthesia, the right kidney was approached posteriorly and decapsulated in order to assure that the associated suprarenal gland remained in situ; the pedicle was tied, the kidney removed and the wound closed with a single black silk suture and skin clips. A single subcutaneous injection of 9,000 units of penicillin in oil was given post-operatively. Animals under 200 gms. weight were given four days in which to recover before the stress of experiment began; those over 200 gms. were allowed only three days. Too long a period of recovery allowed for compensatory hypertrophy of the remaining kidney, while the period allotted them allowed for complete recovery as signified by gain in weight.

Test hormones used were a crystalline preparation of testosterone propionate, a saline suspension of cortisone acetate a saline suspension of compound F (17 hydroxycorticosterone -- 21 - acetate) and a watery suspension of desoxycorticosterone acetate (DCA). Testosterone was given either as a saline suspension or dissolved in sesame oil, 10 to 20 mgs. per cc. Two or three doses of 5 mgs. each were given subcutaneously, the initial dose being given 48 hours before the initial stress of experiment to assure adequate blood levels. Cortisone was given as 0.4 to 0.5 cc. of a 5 mg. per cc. saline suspension (2 to 2.5 mgs.) subcutaneously. This was a daily dose, begun

24 hours before ligature of the limb. Compound F was given as 0.4 to 0.6 cc. of a 5 mg. per cc. suspension (2 to 3 mgs.) subcutaneously, a daily dose started 48 to 72 hours prior to ligation. DCA doses were 0.1 cc. of a 25 mg. per cc. suspension (2.5 mgs.) given subcutaneously each day beginning two days prior to crush injury.

During the 72 hours of observation, the animals were kept in individual metabolism cages designed for collecting urine and screening feces. Urine was collected for 24 hour periods in small, open-mouthed jars placed close to the funnel outlet. Loss by evaporation was minimal. Cages were equipped with deep food troughs in which measured amounts of powdered standard feed were placed. Animals could eat this form of food easily without interfering with the urine collection. If water was to be supplied to the animals during the test, it was done so with the usual bottle arranged so that it did not drop spontaneously and any water which did drop was caught in a small pan which could be easily emptied. The method allowed a check of water intake and avoided interference with the urine collection.

Observations made were: animal weight before and after experiment, amounts of water and food consumed, urine volume, urine pH, blood urea nitrogen levels and kidney histology. Later it was thought that a correlation of kidney weight and body surface area would be of value, so that in later experiments kidney weights were recorded. Urine sediments were examined in

early experiments but this procedure proved to be laborious and unrewarding and so was discarded.

Urine volumes were recorded in 24 hour periods, 9.00 a.m. to 9.00 a.m., and at the same time the gross appearance of the urine was noted. Urine pH was determined with nitrazine paper.

Blood urea nitrogen levels (B.U.N.) were determined every 24 hours or immediately after death. Tail blood samples were taken earlier, but this method was found to be impossible when dehydration was a factor. Cardiac punctures were therefore done every 24 hours for three days, the blood being drawn into a citrated syringe and measured in a citrated pipette. Occasional difficulty was encountered in the dehydrated animals and the occasional death resulted from hemopericardium and cardiac tamponade, but in general the animals stood the procedure well when sharp one and one-half inch No. 25 needles were used and only 0.3 cc. of blood were withdrawn at a time. For the urea determination, a modification (19) of Ormsby's diacetyl monoxime method (274) was used. By this method, a Folin-Wu filtrate of blood is treated with diacetyl monoxime and concentrated sulphuric acid, heated in a boiling water bath, the color emphasized by addition of potassium persulfate and the resulting yellow solution read in a photoelectric colorimeter. Urea nitrogen levels in mg. per cc. can be easily calculated by constructing a standard curve and reading off the appropriate levels.

Three difficulties were encountered with this method. It was found that solutions to be read were occasionally cloudy, so that readings were falsely high. This fault was found to be incomplete precipitation by sulphuric acid in preparation of the Folin-Wu blood filtrate and when one drop of 10 per cent sulphuric acid was added to each blood sample, the final cloudiness no longer occurred. A second trouble arose when the final solutions appeared red in color, rather than yellow, their readings also being falsely high. It was determined that saliva blown into solutions in expelling the contents of pipettes, produced this pink discoloration. Henceforth, pipettes were plugged with absorbent cotton. The third problem was that of accuracy of the method. As in most procedures using small amounts of test substance, results allowed a wide range of error and normal figures for rat blood urea nitrogen levels can only be stated as 50 to 100 mg. per cent. It follows that differences of 10 to 20 mg. percent in B.U.N. levels cannot be significant, but this flexibility was nevertheless felt to be adequate for the purpose of these experiments.

Histological sections of the left kidneys were examined after immediate post-mortem fixation in Zenker's or in Herlant's fixative, paraffin embedding and haemalum-phloxine staining. Kidneys of animals which died overnight were treated similarly but a kidney of any animal known to have died more than one hour prior to fixation was not considered valid. The hour of death for the first six hours could be estimated roughly from the extent

of rigor mortis and other post-mortem findings. In all cases ether was used for killing animals remaining alive at the termination of an experiment.

Kidney weights were taken in the following way. At post-mortem, the entire decapsulated left kidney was incised and placed in fixative for 18 to 24 hours, at which time it was briefly blotted dry and weighed in milligrams. Surface area in square centimeters was obtained from tables based on the animal weight in grams at time of death. The relationship was then calculated in milligrams of kidney tissue per square centimeter of surface area.

REPORT OF EXPERIMENTS

Experiment 1:

Experiment 1A considers the effect of 48 hours dehydration, with and without nembutal anesthesia, on the urea nitrogen, urine output and kidney histology of the intact rat. Of twelve rats four were subjected to dehydration alone, four to dehydration plus nembutal and four were normal controls. Preliminary "test runs" of the procedure were carried out on these rats for 24 hour periods but they were allowed sufficient time for recovery before the 48 hours experiment. Observations appear in Table 1A.

Figures for food and water are totals for 48 hours. It is evident that, deprived of water, the rats' intake of solid

food is markedly diminished. Weight change indicates that in 48 hours of dehydration, animals lose about 10 per cent of their body weight. Figures for urine volume show that the dehydration is not felt significantly until the second 24 hour period; there appeared to be no trend in pH values for urine. B.U.N. figures are here low and show only a slight rise in those animals dehydrated; blood was taken by cardiac puncture and no apparent errors were encountered during the analyses. Histological examination revealed no significant changes in kidney structure in these animals.

It can be concluded that dehydration by removal of water source is accompanied by reduction in solid food intake and is effective in reducing animal weight and urine output over a 48 hour period. Urea nitrogen levels rise slightly, probably accountable for by hemoconcentration. There was no alteration of kidney histology.

At a later date, the above experiment was repeated on twelve rats which had been right nephrectomied three days previously (Experiment 1B); four animals allowed free water were controls, while eight were dehydrated for 72 hours. Food available was 21 gms. each and kidney weights were recorded in addition to the usual observations which appear in Table 1B.

Results are essentially the same as those seen in intact animals in Experiment 1A. Animals allowed water ate solid food well; previously dehydrated animals had an increased

water intake when allowed water (following the 72 hour readings) but handled this water well; all animals lost weight. Urine output figures revealed normal figures for control animals except at 96 hours, when figures were unaccountably low. Dehydrated animals experienced a dehydration oliguria. Blood urea nitrogen figures after 24 hours dehydration are high (average 125 mg %, range 110 to 140, for controls; 145 and 110 to 170 mg % for test animals) and continue high at 48 hours (average 150 mg %, range 140 to 180, for controls; 155 and 140 to 170 mg % for test animals). At 72 hours, a marked drop in B.U.N. levels is recorded in spite of the fact that dehydration continued in test animals (average 70 mg %, range 60 to 80, for controls; 100 and 80 to 120 for dehydrated). At 96 hours, levels rose in control animals but fell slightly in test animals (average 115 mg %, range 100 to 140, for controls; 95 and 70 to 130 for dehydrated).

It is apparent that these urea nitrogen determinations may not be entirely satisfactory. The high figures in control animals at 24 hours may be accounted for by the fact that one kidney had been removed four days previously, with those in test animals slightly higher because of the 24 hours dehydration. At 48 hours, however, control levels continued to rise to essentially the same levels as test animals. The sudden drop observed at 72 hours can be explained only as an error in absolute determination of urea nitrogen figures. The relation

TABLE 1A DEHYDRATION IN INTACT RATS

	RAT	TOTAL FOOD (GMS)	TOTAL WATER (cc)	FINAL WEIGHT AND CHANGE (GMS)	URINE VOLUME (cc)		URINE pH		B.U.N.
					24 hrs	48 hrs	24 hrs.	48 hrs.	48 hrs
CONTROL	D.1	42.3	51.7	318 (+ 14)	8.0	6.7	6.5	6.5	40
	D.2	38.0	44.5	350 (+ 4)	9.0	5.5	6.5	6.5	40
	D.3	35.3	56.0	330 (+ 12)	12.3	11.4	7.0	6.5	40
	D.4	30.2	31.5	282 (0)	7.9	3.5	6.5	6.5	40
DEHYDRATED	D.5	0	--	290 (-34)	8.4	3.4	6.5	6.0	50 mg %
	D.6	0	--	290 (-32)	7.0	1.6	6.5	6.5	50
	D.7	0	--	294 (-30)	4.8	2.1	6.0	6.0	50
	D.8	6.0	--	322 (-36)	13.2	3.2	6.5	6.5	50
DEHYD. + NEMB.	D.9	16.5	--	270 (-28)	7.3	2.6	7.0	6.5	50
	D.10	15.0	--	310 (-22)	6.4	2.2	6.5	6.5	60
	D.11	16.5	--	322 (-28)	6.4	2.5	7.0	6.5	50
	D.12	16.3	--	295 (-25)	8.6	3.0	7.0	6.5	50

TABLE 1B DEHYDRATION IN RIGHT NEPHRECTOMIED RATS

	RAT	TOTAL FOOD	TOTAL WATER	FINAL WEIGHT AND CHANGE (GMS)	URINE VOL. (cc)				URINE pH				B.U.N.				KIDNEY WEIGHT (GMS.)
					24	48	72	96	24	48	72	96	24	48	72	96	
CONTROL	265	21	97	190(-30)	10.2	6.0	10.2	3.6	6.5	6.5	7.0	7.5	150	180	80	110	214.6
	266	21	89	208(-42)	10.8	14.2	12.2	2.2	7.0	6.5	7.0	7.0	110	140	60	110	208.1
	267	21	54	176(-30)	10.0	7.2	6.8	1.0	6.5	7.0	7.0	7.5	120	140	70	140	196.0
	268	21	92	180(-30)	10.8	13.4	30.4	1.0	7.0	7.5	7.0	6.0	140	150	70	100	201.7
DEHYDRATED	269	21	41	214(-44)	4.8	1.2	0.6	12.8	6.5	6.5	6.0	6.5	140	160	80	110	213.9
	270	21	47	234(-34)	6.4	1.6	1.0	10.8	7.0	6.0	6.0	6.5	110	150	80	80	223.0
	271	21	46	230(-20)	6.2	1.2	1.2	3.2	7.0	6.0	6.0	6.0	150	140	120	90	220.9
	272	21	42	226(-16)	3.0	0.4	0.2	2.5	7.0	6.0	6.0	6.0	150	160	100	80	225.9
	273	21	42	198(-22)	5.0	2.0	0.8	5.0	7.0	5.5	6.0	6.5	140	150	90	70	233.6
	274	21	39	220(-28)	5.4	1.8	1.5	7.0	7.0	6.0	6.0	7.5	150	160	100	90	217.6
	275	15	19	224(-30)	6.4	1.4	1.0	4.5	6.5	6.0	6.0	7.0	150	150	100	130	207.7
	276	21	37	184(-36)	4.0	0.8	0.8	5.2	7.0	6.0	6.0	7.5	170	170	110	120	209.9

between controls and dehydrated animals remains satisfactory. The rise of B.U.N. in controls at 96 hours, to exceed levels in test animals, correlates with the decrease in urine output of controls at this time and it can be concluded that some degree of dehydration must have occurred accidentally. 96 hour figures for test animals fell satisfactorily following the full 24 hours of hydration.

Examined microscopically, kidney structure remained unaltered after 72 hours dehydration; kidney weights showed no significant alteration after the stress.

It is apparent that uninephrectomy results in a temporarily elevated B.U.N. level which is accentuated moderately by a period of dehydration lasting 72 hours.

Experiment 2:

Experiment 2 is concerned with the effect of myoglobin injected intravenously on B.U.N., urine output and kidney histology in the rat. Experiment 2A consisted of four rats as normal controls, four receiving intravenous injection of physiologic saline and four injected with myoglobin dissolved and suspended in 1 cc. of saline, 0.1 mg. per gram of body weight. Experiment 2B duplicated this procedure, while Experiment 2C used an increased dose of myoglobin in saline, 0.15 mg. per gm. of body weight. B.U.N's were determined on tail blood samples in Experiment 2A at 24 and 48 hours, but all other determinations were on

cardiac blood samples. Observations appear in Tables 2A, 2B and 2C.

Difficulties were encountered with the methods and procedures of this experiment so that figures in red are considered not valid. These errors are considered in the section on "Discussion and Conclusions".

In normal controls, food and water intake remained fairly constant and all animals gained weight. Urine output in the first measured 24 hour period averaged 7.3 cc. per rat (range 3.2 to 10.5 cc.) and in the second 24 hour period averaged 5.9 cc. (range 3.5 to 9.4 cc.). pH figures were not significant; urea nitrogen levels were within normal limits.

Saline controls in all three experiments had less constant food and water intakes but figures are essentially the same as for normal controls; most animals gained weight. Urine volumes were comparable to the normal group, as were urine pH determinations. Urea nitrogen levels remained within the normal range.

No significant difference was detected in animals given the increased dose of myoglobin (Experiment 2C). All test animals showed food and water intakes comparable to those of all saline controls; five animals lost weight and three gained weight, of the eight animals weighed. Urine volumes also were comparable, though in two instances (P.22 and P.23) urine output

was increased. This increase may represent an osmotic diuresis. Urine pH figures ranged from 6.5 to 7.5, but tended to the acid side at the time of myoglobin injection. B.U.N. figures again were within normal limits.

Renal histology was not essentially altered and though casts could be seen in the cortical tubules with myoglobin injection (Figure 6), these were also present in saline injected animals (Figure 7).

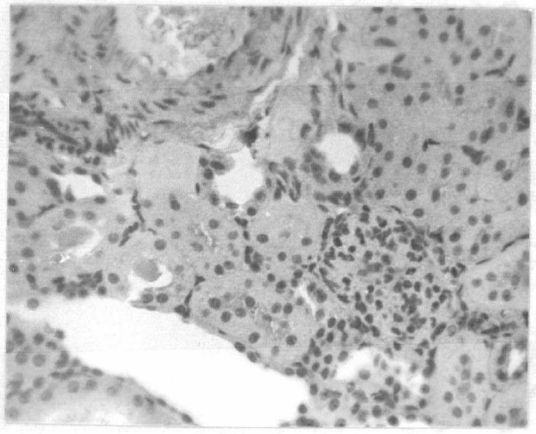

Figure 6

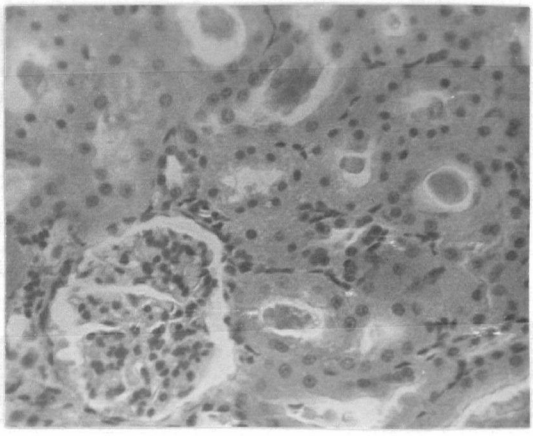

Figure 7

Myoglobin injected intravenously appears to have no effect on the kidneys of intact rats as measured by urine output, blood urea nitrogen and kidney histology. In two cases, there may have been an "osmotic diuresis". The procedure of anesthetizing the animal and injecting it with saline or myoglobin may well account for the greater variation in food and water intakes seen in these animals.

TABLE 2A MYOGLOBIN IN INTACT RATS

	RAT	TOTAL FOOD (GMS)	TOTAL WATER (cc.)	FINAL WEIGHT AND CHANGE (GMS)	URINE VOLUME		URINE pH.		B.U.N.		
					48 hrs.	72 hrs.	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
NORMAL CONT:	P.1	41.5	64.0	394(+12)	9.1	9.4	6.0	7.0	120	90	70
	P.2	42.0	49.0	358(+12)	7.4	4.8	6.5	7.0	60	80	40
	P.3	45.5	58.5	380(+18)	10.5	8.9	6.5	6.5	80	70	60
	P.4	44.5	51.0	304(+4)	8.1	8.3	6.5	7.0	100	90	50
SALINE CONTROL	P.5	0	1.5	342(-16)	11.6	2.8	7.5	7.0	70	80	60
	P.6	19.0	32.5	424(-24)	12.6	10.3	7.0	6.5	110	90	70
	P.7	0	2.0	324(-40)	6.4	2.4	6.0	6.0	180	70	50
	P.8	23.0	26.5	370 (0)	2.2	7.2	6.5	6.0	70	90	60
MYOGLOBIN	P.9	24.0	36.0	360(-8)	4.2	2.8	7.0	6.5	100	--	60
	P.10	24.5	24.5	340(-12)	6.0	4.4	7.0	6.5	--	90	60
	P.11	28.0	29.0	352(-14)	6.2	5.0	7.0	7.0	--	--	50
	P.12	20.0	18.5	330(-16)	6.0	3.5	6.5	7.0	190	--	70

TABLE 2B MYOGLOBIN IN INTACT RATS

	RAT	TOTAL FOOD (GMS)	TOTAL WATER (cc)	FINAL WEIGHT AND CHANGE (GMS)	URINE VOLUME 48 hrs.	URINE VOLUME 72 hrs.	URINE pH 48 hrs	URINE pH 72 hrs	B.U.N. 24 hrs	B.U.N. 48 hrs	B.U.N. 72 hrs
SALINE CONTROL NORMAL CONTROL	P.13	42.0	57.0	-	7.0	4.6	6.5	6.5	60	60	60
	P.14	38.0	58.0	-	7.6	5.8	6.5	7.5	60	70	60
	P.15	41.5	57.0	-	10.1	9.0	7.0	7.5	60	70	50
	P.16	41.5	50.5	-	7.0	3.8	7.0	7.0	70	60	60
	P.17	47.5	94.5	-	9.1	23.8	6.5	7.5	70	80	70
	P.18	45.0	69.5	-	9.6	6.0	7.0	7.5	50	50	80
	P.19	39.0	60.5	-	6.6	6.1	6.0	7.5	60	60	50
	P.20	45.5	57.0	-	4.4	3.0	6.0	6.5	70	60	60
MYOGLOBIN	P.21	43.5	59.5	-	7.4	6.6	6.5	7.0	60	60	50
	P.22	26.5	64.0	-	10.4	15.4	6.5	7.5	70	60	60
	P.23	44.5	87.0	-	11.5	15.4	7.0	7.5	70	70	50
	P.24	43.5	54.0	-	6.6	7.2	6.5	7.5	60	70	50

TABLE 2C MYOGLOBIN IN INTACT RATS

	RAT	TOTAL FOOD (GMS)	TOTAL WATER (cc)	FINAL WEIGHT AND CHANGE (GMS)	URINE VOLUME 48 hrs	URINE VOLUME 72 hrs	URINE pH 48 hrs	URINE pH 72 hrs	B.U.N. 24 hrs	B.U.N. 48 hrs	B.U.N. 72 hrs
NORMAL CONTROL	P.25	41.0	59.0	248 (+8)	9.2	4.6	6.5	7.0	70	-	50
	P.26	37.0	47.0	238 (+18)	4.4	3.8	6.5	7.5	60	70	50
	P.27	41.0	54.0	246 (+14)	3.2	3.5	6.0	6.5	60	60	60
	P.28	41.0	58.5	252 (+14)	4.4	3.6	6.5	6.5	60	70	60
SALINE CONT:	P.29	35.0	53.0	252 (+8)	4.2	4.0	6.5	6.5	70	60	60
	P.30	40.0	66.0	248 (+10	10.8	8.6	7.0	7.5	60	70	50
	P.31	40.0	63.5	256 (+10)	7.5	5.0	6.5	7.5	70	70	60
	P.32	34.0	73.5	240 (+8)	14.2	9.0	6.5	7.5	70	80	70
MYOGLOBIN	P.33	40.0	58.0	254 (+14)	5.0	4.9	6.5	7.5	60	60	50
	P.34	39.0	60.0	240 (+8)	9.6	7.2	7.0	7.5	70	60	60
	P.35	32.0	63.0	244 (-1)	6.6	8.6	6.5	6.5	70	70	50
	P.36	25.0	40.0	232 (+4)	4.6	3.8	7.0	7.5	60	70	50

TABLE 3 MYOGLOBIN AND DEHYDRATION IN INTACT RATS

	RAT	TOTAL FOOD (GMS)	TOTAL WATER (cc)	FINAL WEIGHT AND CHANGE (GMS)	URINE VOLUME 48 hrs	URINE VOLUME 72 hrs	URINE pH 48 hrs	URINE pH 72 hrs	B.U.N. 24 hrs	B.U.N. 48 hrs	B.U.N. 72 hrs
DEHYDRATED CONT:	M.13	24.0	54	276 (+6)	0.15	2.6	6.0	6.0	70	70	60
	M.14	11.0	36	250 (-10)	0.2	2.2	6.0	6.0	70	70	60
	M.15	19.0	47	240 (+4)	0.1	2.0	6.0	6.0	90	70	70
	M.16	23.0	49	286 (-14)	0.1	1.0	6.0	6.0	120	90	70
MYOGLOBIN + DEHYDRATION	M.17	29.0	51	262 (+4)	0.1	1.4	6.0	6.5	80	90	70
	M.18	24.0	48	250 (-4)	0.2	1.6	6.5	6.5	90	60	70
	M.19	17.0	46	238 (0)	1.0	3.1	6.0	6.0	70	90	70
	M.20	21.0	49	290 (0)	0.6	2.1	6.0	6.5	50	90	70
	M.21	22.0	54	264 (+6)	0.15	2.5	6.5	6.0	120	100	70
	M.22	23.0	55	260 (+10)	0.5	3.8	6.0	6.0	90	70	70
	M.23	16.0	43	250 (0)	0.1	2.4	6.0	6.0	140	80	70
	M.24	24.0	54	284 (-2)	drop	0.8	-	5.5	80	80	60

Experiment 3:

Experiment 3 considers the effect of 72 hours dehydration plus myoglobin injection on the urine output, B.U.N. and kidney histology in eight rats, with an additional four animals, subjected to dehydration alone, as controls. Animals were dehydrated for 24 hours before the injection, which was followed by a further 48 hours without water. Dosage was again 0.15 mg of myoglobin (in saline) per gram of body weight and all blood samples were by cardiac puncture. Table 3 lists the observations.

Figures in red are again not valid. Food and water intakes did not differ in control and test groups; weight change was also essentially the same in each group. Figures for urine volume were recorded for the 24 to 48 hour period following injection -- i.e., for the last 24 hours of dehydration, -- and for the subsequent 24 hours in which free water was allowed. Urine output during dehydration was markedly diminished in all cases, as little as 0.1 cc as recorded here. In one case (M.19) an osmotic diuresis may have been seen. Urine was noticeably more acid in both groups here, being usually pH 6.0, and at no time was it observed to be discolored by the injected pigment. Figures for urea nitrogen show the effect of dehydration (24 and 48 hour figures), with a fall after water intake was allowed (72 hour figures). These first two series of figures also are slightly higher in the test animals (average

80 mg %, range 70 to 120 mg %). No essential differences were seen in the kidney histology of the two groups. There were no casts.

Intravenous myoglobin therefore would appear to have no specific effect on urine output, urea nitrogen level or kidney histology even in the presence of dehydration. As in Experiment 1, there was a diminution of urine volume almost to the point of anuria, a slight elevation of B. U. N. and a slightly more acid urine. These changes appear to be due to dehydration alone and are not significantly changed by the addition of myoglobin. There was no pigmentation of the urine seen and no tubular casts even though urine was consistently acid.

Experiment 4:

The role of crush injury in the genesis of "lower nephron nephrosis" was investigated here by ligation of the left hind limb for five hours. Eight rats were so tested, with another four animals acting as normal controls. Since it was assumed that the test animals would not drink freely after the crush injury, attempts were made to match the water intake of the control animals to that of the test animals. Observations of water intake were therefore made at 24 hour intervals. In order that the kidney histology might be viewed temporally, animals M.32 and M.35 were sacrificed 24 hours after ligation, animals M.30 and M.34 after 48 hours and the remaining four

72 hours after crush injury, at which time control animals were also killed. Observations are recorded in Table 4.

It can be seen from this Table that animals suffering crush injury did not eat as much solid food as did control animals in spite of the fact that they drank more water. It is apparent that the matching of water intake was not accomplished. As a result, the 'normal' controls were in effect dehydrated to some extent, as evidenced by their consistent weight loss. Weight loss in test animals was nevertheless greater. The most remarkable observations were, however, of the urine output. Test animals experienced a consistent, immediate and marked diuresis, the average output for the first 24 hours being 23.1 cc. compared to control average of 3.4 cc. The polyuria was evident during the five hours of ligation itself, the test animals putting out an average of 10.3 cc of urine in that time, the control animals averaging 0.6 cc. This last figure is low because volumes were recorded with animals in metabolism cages rather than in funnels. The diuresis persisted to the 48 hour observation and was even evident 72 hours after the initial stress of crush.

Urine acidity again varied irregularly between 6.0 and 7.5. Urea nitrogen figures exhibited some elevation above normal limits but cannot be said to be significantly elevated as measured here in test animals. The occasional high value seen in the control group can be accounted for by partial dehydration. Also, some of the lower figures seen in test animals at 48 and

TABLE 4 LEFT HIND LIMB LIGATION IN INTACT RATS

	RAT	TOTAL FOOD (GMS)	WATER INTAKE (cc)				FINAL WEIGHT AND CHANGE (GMS)	URINE VOLUME			URINE pH			B.U.N. (mg. %)		
			24	48	72	Tot.		24	48	72	24	48	72	24	48	72
CONTROL	M.25	37	9	24	3	36	298 (-30)	4.7	4.6	4.6	7.0	7.0	6.5	70	110	80
	M.26	36	13	21	14	48	320 (-28)	2.1	1.4	2.6	6.5	6.5	6.0	80	80	60
	M.27	43	15	20	31	66	322 (-14)	3.0	4.7	4.8	7.0	6.5	7.0	90	70	70
	M.28	35	7	12	24	43	302 (-24)	3.8	2.8	2.4	7.6	6.5	7.0	90	100	60
LIMB LIGATION	M.29	29	39	18	42	99	292 (-22)	28.6	7.5	6.6	6.5	7.5	7.0	80	60	50
	M.30	3	20	11	-	38	300 (-40)	24.4	13.2	-	6.5	7.5	-	110	100	-
	M.31	7	33	15	20	68	270 (-34)	12.2	12.8	6.4	6.5	7.5	7.5	100	70	70
	M.32	0	33	-	-	39	-	25.2	-	-	6.5	-	-	90	-	-
	M.33	17	48	14	34	98	324 (-48)	29.4	21.0	17.2	6.5	7.5	7.5	90	100	80
	M.34	0	36	32	-	68	314 (-22)	31.2	26.5	-	6.7	7.0	-	80	80	-
	M.35	0	28	-	-	31	-	19.4	-	-	6.0	-	-	110	-	-
	M.36	13	22	6	37	65	296 (-48)	14.1	11.6	7.8	6.0	7.0	7.0	110	90	70

72 hours may be explained by the hemodilution following haemorrhage from self-amputated crushed limbs. This habit of the hydrated animals biting its injured limb gave some difficulty in obtaining valid observations.

Post mortem findings were essentially negative - there was no gross evidence of kidney change - except for the injured limb, which was swollen, cold, pale to blue and edematous. Histological changes were not remarkable at any stage except for slight granular changes in the cytoplasm of tubules of Zone 3, with swelling and vacuolization of the nuclei (see Fig. 8 and compare with Fig. 9). An occasional cast was seen in

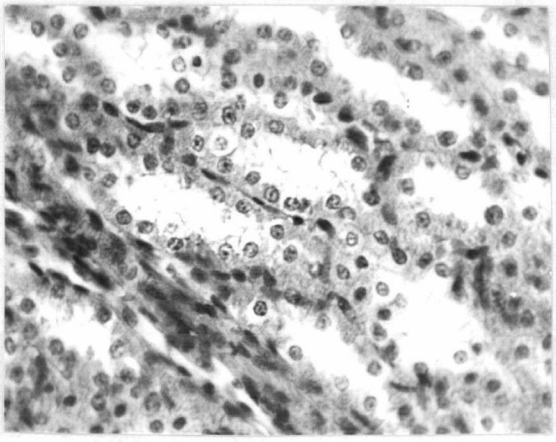

Figure 8

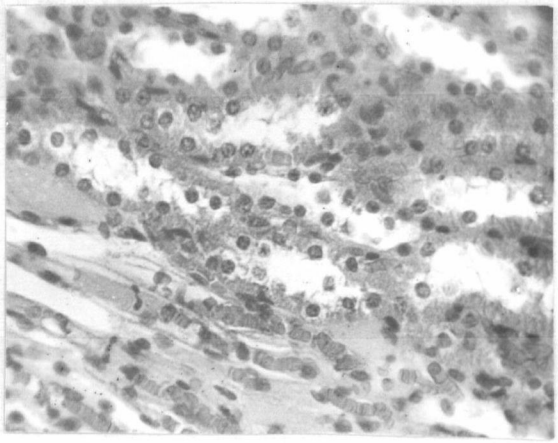

Figure 9

medullary tubules (Figure 10).

Ligation of one hind limb for five hours in otherwise intact hydrated rats appears to have no permanent damaging effect on the kidneys. There was no significant elevation of blood urea nitrogen and no remarkable renal tubular damage

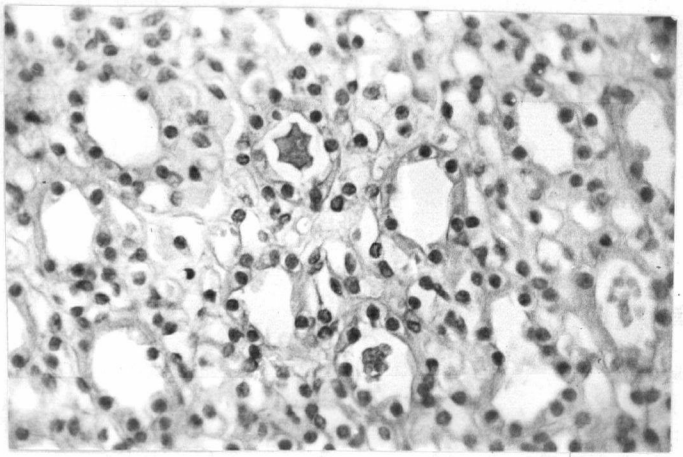

Figure 10

histologically. There was also no oliguria, and on the contrary, a marked diuretic effect in response to the injury was observed, indicating some alteration in kidney function.

Experiment 5:

In Experiment 5A, eight male rats were subjected to the stress of left hind limb ligation for five hours together with 72 hours dehydration (24 hours pre-ligation, 48 hours post-ligation). Four additional animals acted as controls, being only dehydrated. Experiment 5B repeated this procedure except that the ligation period was lengthened to five and one half hours. Observations appear in Tables 5A and 5B.

It is apparent that there is no essential difference between results of the five and the five and one-half hour ligation periods so these experiments will be considered together.

TABLE 5A FIVE HOURS LIGATION PLUS DEHYDRATION

	RAT	TOTAL FOOD (GMS)	TOTAL WATER (cc) 24 hrs.	FINAL WEIGHT AND CHANGE (GMS)	URINE VOLUME			URINE pH			B. U. N.		
					24	48	72	24	48	72	24	48	72
DEHYDRATED CONT:	M.37	11	33	338 (+2)	1.2	1.0	5.6	6.0	6.0	6.0	80	50	50
	M.38	12	33	346 (+2)	0.8	0.6	4.3	6.5	6.5	6.0	100	60	50
	M.39	11	33	330 (-2)	2.5	0.7	5.4	6.5	6.0	6.0	100	70	60
	M.40	7	22	342 (-2)	0.4	1.2	1.6	6.0	6.0	6.0	80	90	60
LIGATED AND DEHYDRATED	M.41	4	25	350 (-12)	2.8	7.3	6.9	6.0	6.5	6.5	170	110	110
	M.42	0	-	244 (-26)	2.6	7.3	-	6.0	6.0	-	140	230	-
	M.43	0	31	328 (-18)	1.5	8.7	15.4	6.0	6.0	7.0	190	180	160
	M.44	0	31	329 (-15)	2.5	6.0	14.4	6.0	6.0	7.5	160	130	110
	M.45	0	-	300 (-34)	2.2	-	-	6.0	-	-	-	-	-
	M.46	0	0	270 (-34)	1.4	4.3	-	6.0	6.0	-	220	230	-
	M.47	0	-	298 (-30)	1.2	-	-	5.5	-	-	280	-	-
	M.48	0	18	310 (-26)	3.4	8.0	15.1	6.0	6.0	6.5	250	-	130

TABLE 5B FIVE AND ONE-HALF HOURS LIGATION PLUS DEHYDRATION

	RAT	TOTAL FOOD (GMS)	TOTAL WATER (cc) 24 hrs	FINAL WEIGHT AND CHANGE (GMS)	URINE VOLUME			URINE pH			B. U. N.		
					24	48	72	24	48	72	24	48	72
DEHYDR: CONTROLS	M.49	9	29	254 (-30)	1.6	0.2	2.6	6.0	6.0	6.0	80	110	70
	M.50	9	29	298 (-32)	1.8	dried	1.8	6.0	-	5.5	100	100	70
	M.51	0	20	304 (-50)	1.8	0.8	4.0	6.0	6.0	6.5	80	100	80
	M.52	6	11	226 (-44)	0.6	dried	0.6	6.0	-	6.0	80	120	90
LIGATED AND DEHYDRATED	M.53	0	-	330 (-32)	0	-	-	-	-	-	-	-	-
	M.54	0	35	318 (-62)	2.6	4.4	24.5	6.0	6.0	7.5	170	220	180
	M.55	1	30	292 (-52)	2.8	4.0	8.0	6.0	6.0	6.5	140	110	90
	M.56	1	31	240 (-46)	2.0	4.7	11.0	6.0	6.0	7.0	130	140	90
	M.57	0	28	274 (-48)	2.8	1.0	10.0	6.0	5.5	7.5	230	220	250
	M.58	0	0	272 (-50)	2.6	1.0	-	6.0	5.5	-	360	270	-
	M.59	0	-	310 (-36)	2.5	-	-	6.0	-	-	440	-	-
	M.60	0	0	270 (-52)	2.1	3.4	-	6.0	5.5	-	-	280	-

The difficulty in matching food intakes and water intakes is evident here. Animals not allowed water will eat little solid food, and those also ligated will eat virtually no solids at all. For this reason it was thought wise to limit solid food available to all animals in further experiments to 10 grams each. Similarly, it was difficult to control water intakes; some of the ligated animals - those obviously ill - would not drink water when it was made available, thereby hastening their deaths.

In Experiment 5A the control group managed to retain its weight well, while all other animals (including controls of Experiment 5B) showed a satisfactory weight loss (up to 16% of original body weight).

Urine volumes in both experiments again showed the diuretic response, apparent particularly at 48 hours and amplified at 72 hours by allowing water for the preceding 24 hours. Eight control animals at the 48 hour reading averaged 0.6 cc of urine, while twelve test animals averaged 5.0 cc for that 24 hour period - almost ten times as much. pH figures showed a slight tendency towards acidity after 72 hours but no definite trend can be stated.

With regard to B. U. N. figures, definite uremic levels were reached for the first time. In three instances cardiac puncture was unsuccessful because the heart could not be located

by palpation or with the needle and in all instances blood withdrawn was very thick and dark. In spite of the fact that the volume of urine excreted in 24 hours by control animals was as little as 0.2 cc, their B.U.N. levels did not rise above 120 mg %. On the other hand, test animals whose urine output was always above 1.0 cc per 24 hours and rose as high as 8.7 cc. had B. U. N. levels consistently over 100 mg % and at times rising as high as 440 mg % during the dehydration period. These facts would presumably necessitate a dilute urine in test animals, a condition which was verified by the appearance of very pale, watery urine in test animals while that of controls was dark amber, almost syrupy.

Histological evidence of renal tubular damage was present definitely for the first time. Sections were examined under low and high powers, dividing the renal tissue into four zones for facility of examination. These were :

Zone 1 - cortex proper, containing glomeruli and convoluted tubules, proximal and distal, with their appropriate vasculature. This first zone corresponds to Smith's division "cortex". (See Fig.11).

Zone 2 - corticomedullary region, in which there is the thick descending limb of Henle's loop. (Smith's "Medulla - outer band of outer zone.")

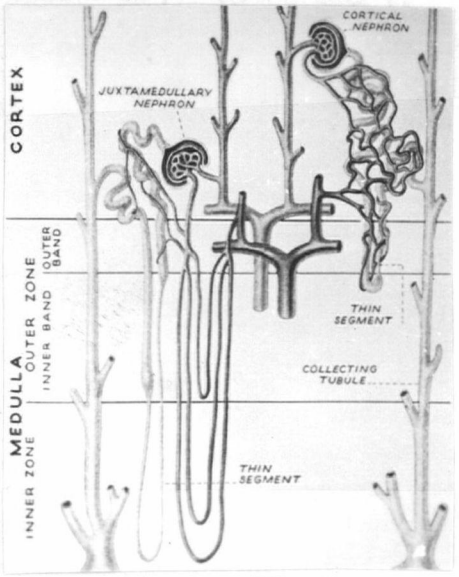

Figure 11.
From Smith: "The Kidney" 1951 (333).

Zone 3 - outer medullary portion containing sections of both thick and thin limbs of Henle's loop together with bundles of venae rectae. (Smith's "Medulla - inner band of outer zone.").

Zone 4 - medulla proper, containing sections of Henle's thin loop, collecting tubules and vasa recta. (Smith's "Medulla - inner zone").

Zones 1, 3 and 4 are more easily defined in the rat kidney and received more attention in the examinations. Zone 3, consisting exclusively of "lower nephron" received most attention and was found on examination to exhibit the characteristic pathologic damage seen throughout these experiments.

Control animals showed no histological alteration in kidney structure, a fact which would be expected from observations recorded in Experiment 1. (See Figure 12).

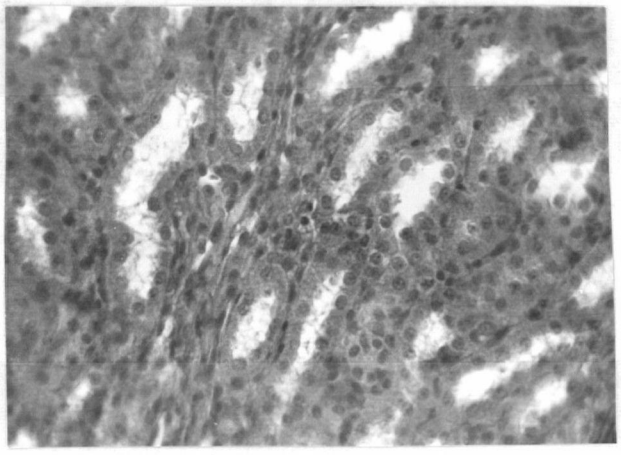

Figure 12

Of the 16 test animals, eight showed minimal to moderate changes in renal tubules, to be described below; two others had questionable changes. Two animals died several hours before their kidneys could be fixed, thereby exhibiting post-mortem change; these sections could not be considered valid. The remaining four animals showed no histological evidence of renal tubular damage in spite of the fact that two had urea nitrogens of over 280 mg %. Typical changes appear in Figure 13, most marked in Zones 2 and 3, but extending into Zone 1 as well. Proximal tubule cells remain intact, whereas distal tubules (chiefly the thick portion of Henle's loop) are in an early stage of degeneration with granular, vacuolated cytoplasm, swollen, pale and vacuolated nuclei and the occasional pyknotic nucleus. A desquamated epithelial cell can be seen in one lumen. These changes accurred in an animal putting out 3.4, 8.0 and 15.1 cc. of urine per 24 hours and whose B.U.N. rose to at least 250 mg %. Similar changes appear in the distal

tubules of Zone 3 in Figure 14. Here the degenerating tubules are furthest removed from the venae rectae, which appear at the upper and lower margins of the figure. This animal (M. 59) died at 24 hours with a postmortem value for B. U. N. of 440 mg % and a urine volume for 24 hours of 2.5 cc.

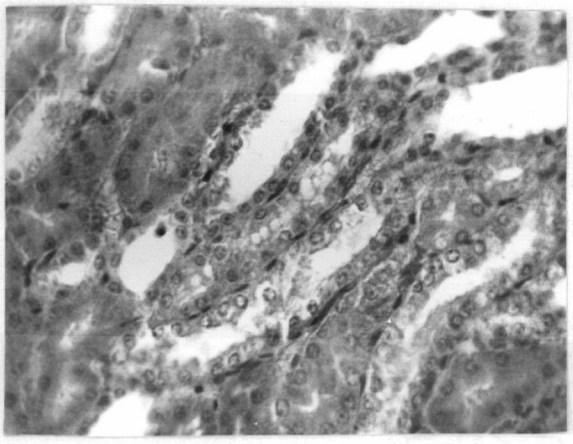

Figure 13

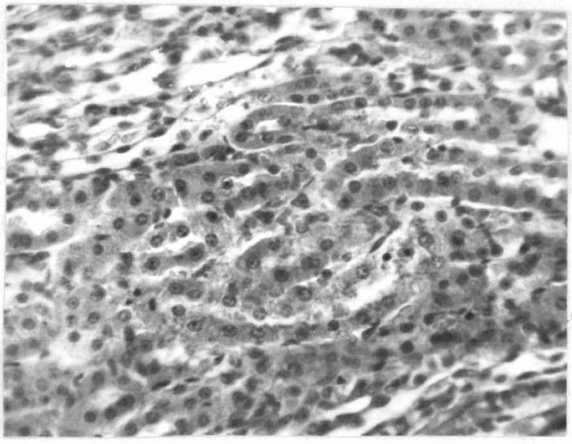

Figure 14

From this experiment it appears that crush injury (left hind limb ligation) when coupled with severe dehydration can produce renal tubular damage in a fair proportion (50% of 16 rats) of otherwise intact male albino rats. This damage is indicated by disordered function of the kidney (elevated blood urea nitrogen, increased volume of dilute urine) and disordered structure as well (tubular degeneration). The diuretic response observed is not one of the criteria stated for renal damage, being in fact the opposite of oliguria or anuria, but it nevertheless indicates a disorder of renal function.

Experiment 6:

The effect of crush injury in the presence of intravenously injected myoglobin in normally hydrated animals was observed in this experiment. Water intake of four normal control animals was matched to that of a test group of four animals and a control group of four animals in which the buffer solution alone, here used as a solvent for myoglobin, was injected. Table 6 lists the complete data on these animals. Animal M.71 died at time of injection and therefore is not included in the analysis of observations.

In spite of the fact that animals were allowed free solids, the normal control group did not eat freely. The amounts consumed, however, compared favourably with the remaining animals. Water intakes and weight changes also compared favorably in the three groups. The diuretic response to the trauma of ligation was again seen in the seven ligated animals, no significant difference between the myoglobin-injected and the buffer-injected groups being evident. Two control animals did, however, show a pronounced diuresis at 72 hours which is unexplained. B. U. N. levels cannot be said to be elevated significantly in any of the groups; a high figure in one control animal is unexplained and must be presumed to be an error. Urea nitrogen figures may be low owing to hemodilution which was observed in many animals during cardiac puncture at 24 hours. This was thought in early experiments to be the result of

TABLE 6 LIGATION PLUS MYOGLOBIN INJECTION IN INTACT RATS

	RAT	TOTAL FOOD (GMS)	TOTAL WATER (cc.)	FINAL WEIGHT AND CHANGE (GMS)	URINE VOLUME			URINE pH			B.U.N.		
					24	48	72	24	48	72	24	48	72
NORMAL CONTROL	M.61	28	57	202 (-16)	3.8	5.0	22.2	6.5	6.0	6.0	90	70	90
	M.62	28	51	220 (-6)	4.6	5.4	20.5	7.0	6.0	6.0	100	70	70
	M.63	25	39	224 (-12)	4.4	6.8	8.6	6.5	6.0	6.0	110	70	60
	M.64	26	40	206 (+6)	6.5	2.3	3.6	6.0	6.0	7.0	150	80	90
LIGATION AND BUFFER	M.65	19	66	242 (-20)	16.4	16.5	12.4	6.5	7.0	6.0	100	80	80
	M.66	4	18	224 (-24)	6.0	8.0	5.4	6.0	7.0	7.0	120	80	80
	M.67	13	75	238 (-2)	23.4	10.5	9.7	6.0	7.0	6.0	110	90	100
	M.68	12	37	228 (-14)	11.2	8.8	4.2	6.5	7.5	6.0	120	90	80
LIGATION AND MYOGLOBIN	M.69	23	62	215 (-5)	10.2	12.0	4.8	6.0	7.5	6.0	120	80	80
	M.70	19	58	250 (-2)	13.6	6.4	4.2	6.0	6.5	7.0	120	80	80
	M.71	-	-	- -	-	-	-	-	-	-	-	-	-
	M.72	21	55	236 (-14)	14.4	8.5	8.7	6.0	7.0	7.5	120	90	80

TABLE 7 DEHYDRATION, LIGATION AND MYOGLOBIN IN INTACT RATS

	RAT	TOTAL FOOD (GMS)	TOTAL WATER (cc)	FINAL WEIGHT AND CHANGE (GMS)	URINE VOLUME			URINE pH			B. U. N. (mg. %)		
					24	48	72	24	48	72	24	48	72
DEHYDRATED CONTROL	M.73	10	23	185 (-29)	2.4	0.6	1.2	6.0	6.0	6.0	80	110	60
	M.74	10	27	200 (-30)	1.9	1.5	2.4	6.0	6.0	5.5	90	100	70
	M.75	10	21	180 (-16)	1.1	0.6	1.5	6.0	6.0	6.0	60	90	70
	M.76	10	39	214 (-26)	2.3	1.6	10.1	6.0	6.0	6.0	80	140	70
DEHYDRATION + LIGATION	M.77	10	35	220 (-30)	1.6	3.4	6.4	6.0	6.0	7.0	-	110	90
	M.78	9	43	224 (-20)	3.2	7.2	10.4	6.0	7.0	6.0	170	150	80
	M.79	7.5	44	216 (-42)	2.4	4.0	8.6	6.0	6.0	7.5	160	120	80
	M.80	5.0	33	196 (-40)	2.0	5.0	9.0	6.0	6.5	7.5	130	130	90
DEHYDRATION + LIG'N + MYOGL'N	M.81	1.0	41	224 (-44)	2.9	5.0	14.0	6.0	6.0	7.0	290	150	100
	M.82	0	34	208 (-42)	2.3	7.4	15.8	6.0	7.0	7.5	160	220	160
	M.83	7.0	59	224 (-28)	4.9	9.8	15.1	6.0	6.0	7.0	-	200	110
	M.84	2.0	25	218 (-46)	3.0	7.8	9.2	6.0	6.0	7.0	220	170	120

haemorrhage from chewed limbs, but these animals had not attacked their limbs. The response appears to be one of hemodilution to the shock of limb ligation, when hydration is adequate. Constantinides (81a) states that in the production of severe shock in rats by pinching exposed intestines in several places, marked and rapidly developing hemodilution rather than the expected hemoconcentration, appeared. Histologically, the kidneys showed no definite tubular damage when animals were killed at 72 hours.

In Experiment 2 it was shown that myoglobin injection alone had no nephropathic effect; in Experiment 4, in which ligation of a limb was carried out on normally hydrated animals, again no lethal renal damage resulted, although a diuretic effect was noticed. In the present experiment these two factors together (myoglobin and crush injury) also produced no lethal kidney damage but again resulted in a marked and definite diuresis. In addition, an apparent hemodilution response to the trauma was observed.

Experiment 7:

The three factors, limb ligation, dehydration and myoglobin injection were combined in this experiment. Four animals were tested thus and compared with four additional rats subjected to dehydration and ligation alone. The control group of four animals was dehydrated similarly but was otherwise

untouched. Dehydration and myoglobin injection were accomplished as in Experiment 3, with a phosphate buffer as the solvent. The dehydrated-ligated group received no injection of buffer. Observations are in Table 7.

Intakes of solids and water were fairly well matched in the three groups, as was the weight loss. The ligated animals, however, did lose more weight with one animal losing 17% of its original body weight. Urine volume figures again show the diuretic response without any significant difference between the two ligated groups. Urea nitrogen figures in ligated animals were raised to uremic levels and although at first glance there seems to be a significant elevation of the myoglobin-injected group over the non-injected one, it is necessary because of the wide range of figures and the small group of statistics to apply statistical methods to these figures in order to reach accurate conclusions. When this is done (see Table 7A) it is found that, at the 24 hour reading the difference between these two groups is not significant and could have occurred by chance. On the other hand, 48 and 72 hour readings are found to differ significantly in the two groups at the 5% level - i.e., in 95% of cases this difference would not occur by chance.

The normal histology of the kidney of animal M. 76 is shown in Figure 15, contrasting well with a similar area in Zone 3 of animal M.80 (Figure 16) which shows various degrees of degeneration in the renal tubules. This animal was subjected

to ligation and dehydration without pigment injection. A similar type of damage is apparent in animal M.82 (Fig. 17)

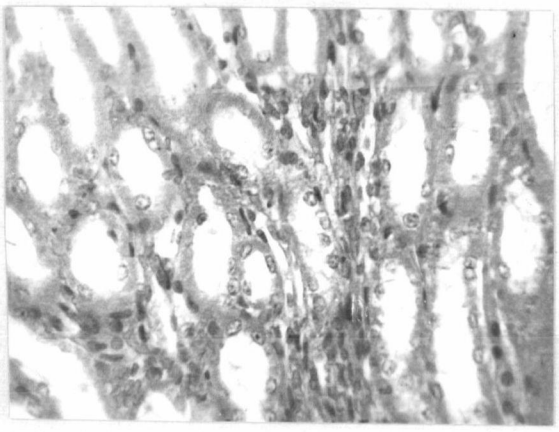

Figure 15

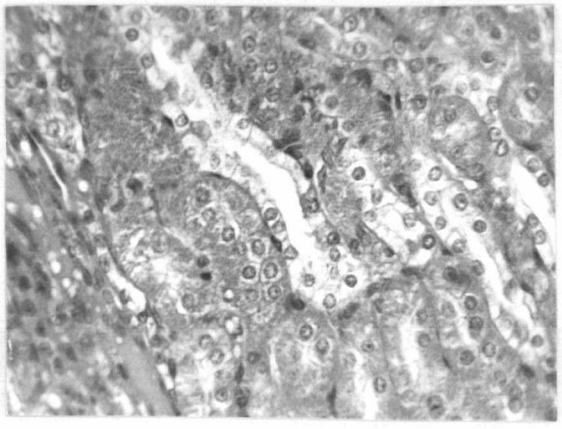

Figure 16

which received myoglobin and here a rare cast is shown. In addition, distal tubules in the cortical region of this kidney also were degenerating (Figure 18).

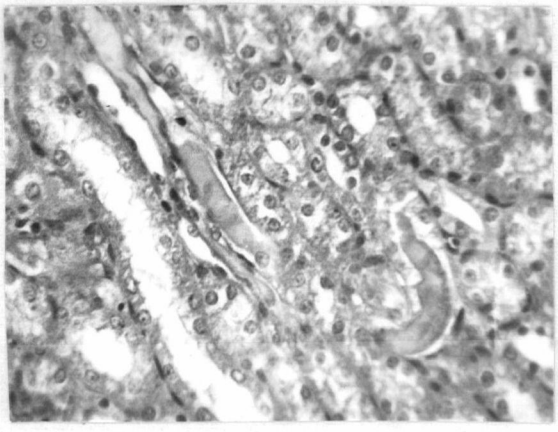

Figure 17

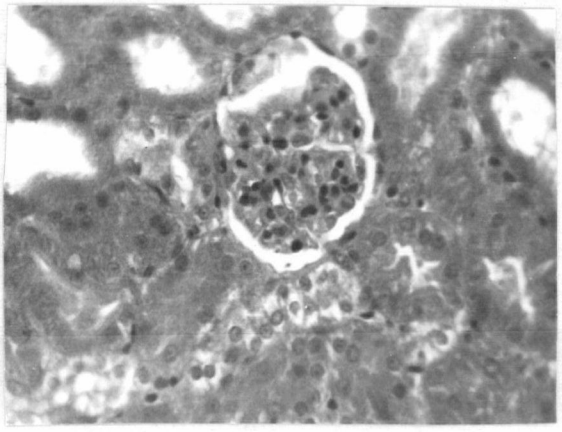

Figure 18

Results of this experiment (ligation + dehydration + myoglobin) should be compared to those of Experiments 5 (ligation + dehydration) and 6 (ligation + myglobin) as well as with the control animals. There were no deaths and there was very little

elevation of B.U.N. with ligation and myoglobin as the stress; with ligation and dehydration, uremic levels were reached in twelve of sixteen test animals and there were no renal deaths. It would appear, then, that dehydration is an essential factor in the development of acute tubular necrosis from crush injury and that injection of myoglobin though not essential for the development of the syndrome, adds significantly to the tubular damage as indicated by urea nitrogen levels and kidney histology.

Experiment 8:

The effect of increasing the area of crush was investigated in four rats subjected to bilateral hind limb ligation for four hours after being dehydrated for 24 hours previously. Dehydration was continued for the subsequent 48 hours. Control groups of four animals were run on dehydration alone as well as dehydration plus left hind limb ligation for five hours. Table 8 classifies the pertinent data.

The difficulty in controlling food intake and to a lesser extent water intake in the last 24 hours is again evident, but all animals apparently suffered similarly as gauged by their losses of weight. Figures for urine volumes, though incomplete because of deaths, again show polyuria at 24 hours which is amplified by the water intake at 72 hours. In addition it was noted that in two dehydrated control animals wine-coloured urine was excreted though there was no evidence of external bleeding.

This was apparently a true hematuria. Urine pH figures again show the general trend towards alkalinity with diuresis.

Urea nitrogen figures were among the highest yet recorded. Dehydrated control figures were elevated at 24 hours and maintained that level at 48 hours, though one animal rose to 180 mg %. That this elevation was due to dehydration alone is shown by the prompt return to normal in all cases at 72 hours, after the animals had been allowed water for 24 hours. In the unilaterally ligated dehydrated control group, B. U. N. levels were generally higher and persisted at an elevated level for a longer time. Only one animal reached an excessively high level and that animal died apparently of renal damage.

In the bilaterally ligated dehydrated test group, the results appeared to be not essentially different from the above group except that the damage appeared more fatal. Three animals died in this group, one (M.95) from the shock of haemorrhage from a lacerated foot and from excessive withdrawal of blood at cardiac puncture. Two others (M.93 and M.96) died uremic deaths with elevated B.U.N's and periods of anuria of from six to eight hours. The fourth animal of the group survived but maintained an elevated B. U. N. level.

Histological sections showed essentially normal kidneys in the dehydrated controls in spite of the hematuria observed in two of the four animals (Figure 19). Typical degenerative changes were seen in Zone 3 of animals M.89, M.90, M.92, M.94,

TABLE 7A STATISTICAL ANALYSIS OF FIGURES IN TABLE 7

	DEHYDR. + LIG'N "X"	DEHYDR. LIG'N, + MYOGLOBIN "Y"	DIFF. FROM MEAN "x"	DIFF. FROM MEAN "y"	x^2	y^2	
24 HOURS	170	290	+17	+67	289	4489	$\sigma_x = 17$ $\sigma M_x = 12.06$ $\sigma d_M = 39.48$
	160	160	+7	-63	49	3969	$\sigma_y = 53.12$ $\sigma M_y = 37.60$ $D_M = 70$
	130	220	-23	-3	529	9	$t = 70/39.48 = 1.77$
	M=153	M=223			$\Sigma x^2 = 867$	$\Sigma y^2 = 8467$	For significance at 5% level, need 2.78
48 HOURS	110	150	-18	-35	324	1225	$\sigma_x = 14.76$ $\sigma M_x = 8.53$ $\sigma d_M = 17.74$
	150	220	+22	+35	484	1225	$\sigma_y = 26.92$ $\sigma M_y = 15.56$ $D_M = 57$
	120	200	-8	+15	64	225	$t = 3.21$
	130	170	+2	-15	4	225	For significance at 5%, need 2.45; at 1%, 3.71.
	M=128	M=185			$\Sigma x^2 = 872$	2900	
72 HOURS	90	100	+5	-22	25	484	$\sigma_x = 5$ $\sigma M_x = 2.89$ $\sigma d_m = 13.47$
	80	160	-5	+38	25	1444	$\sigma_y = 22.76$ $\sigma M_y = 13.16$ $D_M = 37$
	80	110	-5	-12	25	144	$t = 2.75$
	90	120	+5	-2	25	4	For significance at 5%, need 2.45; at 1%, 3.71.
	M=85	M=122			$\Sigma x^2 = 100$	2072	

TABLE 8 DEHYDRATION AND BILATERAL LIGATION IN INTACT RATS

	RAT	TOTAL FOOD (GMS)	TOTAL WATER (cc)	FINAL WEIGHT AND CHANGE (GMS)	URINE VOL. cc.			URINE pH			B. U. N. mg %			DIED (HRS)
					24	48	72	24	48	72	24	48	72	
DEHYDRATION CONTROLS	M.85	10	36	222 (-38)	2.2	1.8	3.3	6.0	6.0	6.0	110	110	70	
	M.86	10	34	244 (-28)	0.5	0.3	2.8	7.0	6.0	6.5	90	110	70	
	M.87	10	24	234 (-34)	1.6	1.0	3.4	6.0	6.0	7.0	100	100	90	
	M.88	10	28	216 (-28)	1.3	0.3	1.9	6.0	6.0	6.5	130	180	80	
DEHYDRATION + L. Limb LIG 'N	M.89	10	39	252 (-32)	4.1	5.4	8.2	6.0	7.0	7.0	140	120	110	
	M.90	6.5	36	230 (-30)	3.6	7.2	11.3	6.0	7.0	7.5	190	140	90	
	M.91	0	-	-	2.1	0	-	6.0	-	-	410	-	-	36-48
	M.92	0	15	190 (-46)	1.5	8.1	9.0	6.0	6.5	7.0	210	150	160	
DEHYDRATION + BILATERAL LIG 'N	M.93	0	-	232 (-28)	2.3	0	-	6.0	-	-	210	-	-	36-48
	M.94	0	3.5	224 (-32)	2.8	5.1	13.3	6.0	6.5	7.5	190	220	220	
	M.95	0	-	220 (-30)	0.7	-	-	6.0	-	-	290	-	-	20
	M.96	0	-	222 (-28)	1.9	-	-	6.0	-	-	260	-	-	24-26

M.95 and M.96. (Figures 20 and 21). Animals M.91 and M.93 had been dead more than one hour so sections were not valid. In addition, the medulla of animal M.95 showed numerous hyaline casts in Henle's loop; no hematuria was noted in this animal (Figure 22).

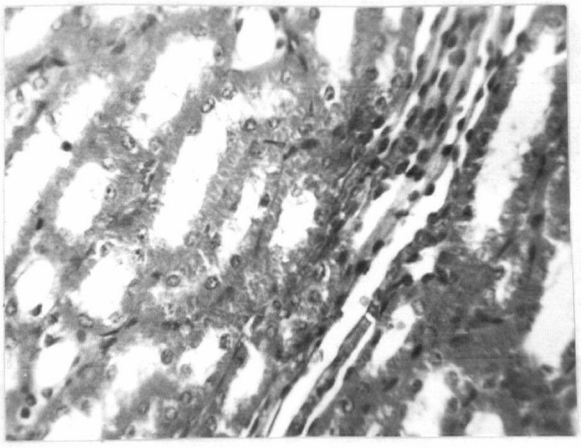

Figure 19

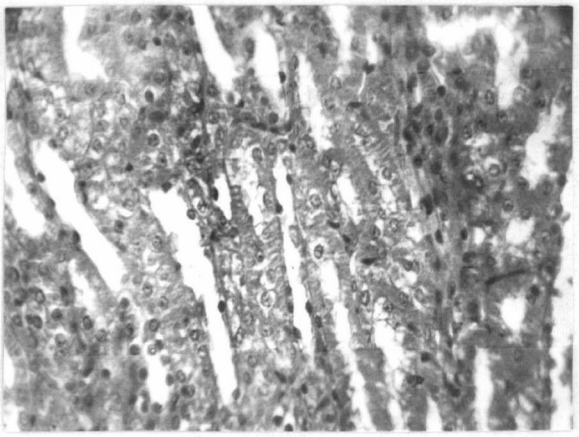

Figure 20

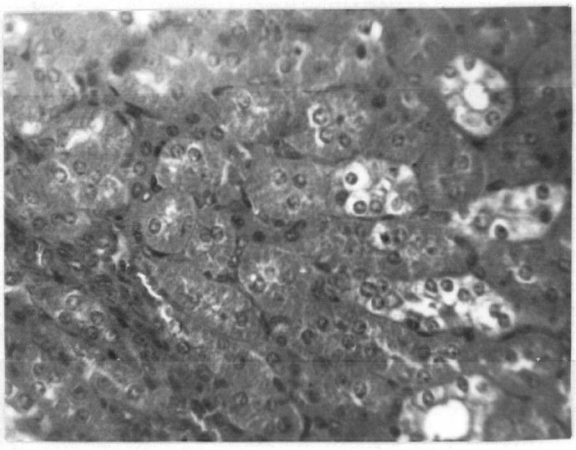

Figure 21

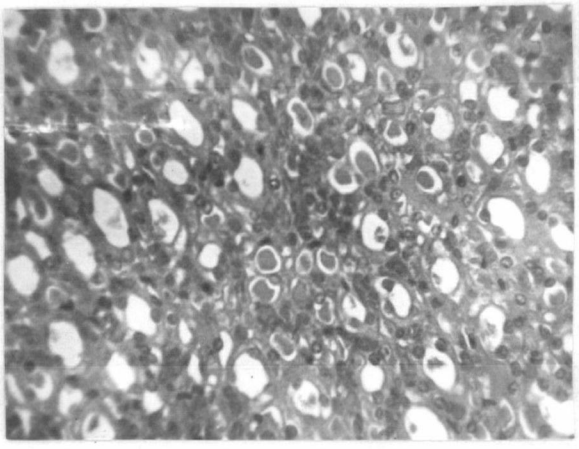

Figure 22

It is apparent that the more drastic trauma of bilateral crush injury coupled with the essential dehydration is capable of producing a more fatal renal damage than is unilateral

ligation and dehydration. However, the improvement gained by this procedure, when balanced against the extra time involved and the impression that the procedure approached the area of shock deaths, was not felt to be sufficient to warrant its use. It was instead felt advisable to approach the problem from the opposite point of view -- i.e., reduce the renal reserve of the animals. Subsequent experiments accomplish this by prior uninephrectomy on all animals.

Also emphasized in this experiment is the fact that dehydrated control animals retain water, when it is supplied, much better than do those animals whose limbs had been ligated.

Experiment 9:

The effect of reducing renal reserve is studied in Experiments 9A and 9B, which deal with male, right-nephrectomied rats subjected to 72 hours dehydration and five hours ligation. In 9A, twelve animals were nephrectomied one week prior to initiation of dehydration; four of these were simply dehydrated, while the remaining eight were ligated as well as dehydrated. In 9B, nephrectomy was done three days prior to dehydration, but the control group of four and test group of eight animals were treated as in 9A. Experiment 9B was designed as a 36 hour experiment so that animals were killed at that time and correspondingly fewer observations are recorded (Tables 9A and 9B).

In Experiment 9A, all animals appeared fully recovered following nephrectomy, having regained or exceeded their pre-operative weights. However, all were noticeably more sensitive to nembutal sedation. Food, water and weight change figures were not unusual. Urine volumes again showed the diuretic response principally at 48 hours, and pH figures the tendency towards alkalinity with diuresis. All figures were higher than comparable figures for dehydrated intact animals.

Two dehydrated control animals showed excessively elevated B.U.N. levels and at autopsy one of these had a granular kidney which showed a chronic interstitial inflammation histologically. The kidney of the other animal was not examined.

Of the remaining eight test animals, six reached and held high B.U.N. levels, two of these as high as 470 mg % and 530 mg %. The remaining two behaved in a way much like the dehydrated controls.

Animals M.105 and M.107 died at 48 and 72 hours respectively, as a result of hemopericardium following heart puncture. Animal M.106 was killed at 53 hours post-ligation because it had been moribund for the entire day, was anuric and suffering severe rigors. Animal M.104 was carried beyond the 72 hour period but was killed at 100 hours because it was moribund; its post-mortem B.U.N. was 280 mg % and it was noticed that its bowel was filled with tarry material like old blood. This

TABLE 9A LIGATION AND DEHYDRATION IN RIGHT NEPHRECTOMIED RATS

	RAT	FOOD (GMS)	WATER (cc)	FINAL WEIGHT AND CHANGE (GMS)	URINE VOLUME cc.			URINE pH			B. U. N.			DIED
					24	48	72	24	48	72	24	48	72	(HRS)
DEHYDRATED CONTROL	M.97	5	30	184 (-26)	2.2	1.7	8.8	6.0	6.0	7.0	240	170	100	
	M.98	5	27	220 (-22)	1.5	1.2	6.0	6.0	6.0	7.0	110	100	90	
	M.99	5	30	226 (-14)	1.5	0.6	7.6	6.0	6.0	7.5	180	90	110	
	M.100	5	31	210 (-20)	1.2	0.8	9.8	6.0	5.5	7.0	120	90	80	
DEHYDRATION AND LIGATION	M.101	5	31	226 (-14)	1.8	4.3	17.0	6.5	6.5	7.5	260	130	100	
	M.102	5	36	226 (-16)	2.0	1.9	9.1	6.5	7.0	7.5	230	130	90	
	M.103	0	46	196 (-28)	1.3	2.7	29.3	7.0	7.0	7.5	410	230	140	
	M.104	0	9	192 (-26)	1.4	1.2	5.1	6.5	7.5	7.5	200	240	300	
	M.105	0	-	180 (-44)	1.8	7.0	-	6.0	7.0	-	410	230	-	48
	M.106	0	3	212 (-28)	1.0	0.6	-	6.0	6.5	-	270	220	310	53
	M.107	0	41	222 (-34)	2.2	7.4	20.8	6.0	7.0	7.5	530	220	160	72
	M.108	0	23	220 (-40)	1.2	0.8	16.5	6.5	6.0	7.5	220	230	250	

TABLE 9B LIGATION AND DEHYDRATION IN RIGHT NEPHRECTOMIED RATS

	RAT	FOOD (GMS)	WATER (cc)	FINAL WEIGHT AND CHANGE	URINE VOL.		URINE pH		B.U.N.		DIED (HR)	KIDNEY WEIGHT mg per $cm.^2$
					24	36	24	36	24	36		
DEHYDRATED CONTROL	M.109	4	-	200 (-34)	1.1	dried	7.0	-	100	80		271.5
	M.110	5	-	238 (-36)	1.9	0.2	6.0	6.0	100	80		269.1
	M.111	4	-	254 (-40)	1.6	dried	6.0	-	-	140		280.0
	M.112	4	-	230 (-42)	1.7	dried	7.5	-	110	90		258.9
DEHYDRATION AND LIGATION	M.113	0	-	258 (-24)	1.7	0	6.0	-	190	290	32-36	254.9
	M.114	0	-	256 (-32)	2.2	0	6.0	-	400	-	26	276.5
	M.115	0	-	248 (-24)	1.7	-	6.0	-	600	-		304.7
	M.116	0	-	242 (-18)	2.9	0.7	6.0	6.0	210	310		293.6
	M.117	0	-	248 (-40)	2.6	1.4	6.0	6.5	230	270		261.9
	M.118	0	-	248 (-38)	2.2	2.3	6.0	6.5	220	200		275.4
	M.119	0	-	264 (-38)	3.1	2.3	6.0	6.0	210	220		-
	M.120	0	-	258 (-34)	2.8	1.0	6.0	6.0	220	260		248.4

observation had been made before but not recorded and apparently illustrates one of the three classical reactions to stress, that of gastro-intestinal haemorrhage. This animal was grossly anemic at post-mortem. In addition, true hematuria was again observed, this time in a test animal (M.108).

Of eight test animals, then, six had elevated B.U.N's which were maintained. Six were actually polyuric while one was oliguric and one anuric for the ten hours preceding death. Histologically, three showed definite Zone 3 lower nephron degeneration (Figure 23, compared Figure 24); one showed questionable changes; the remaining four did not show histological evidence of renal damage when killed at 72 to 144 hours.

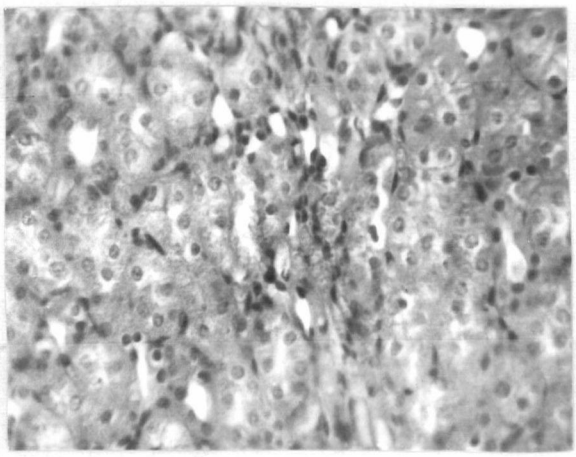

Figure 23

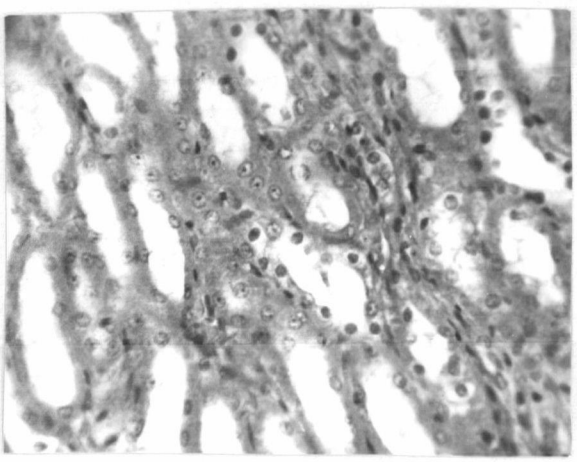

Figure 24

An interesting series of observations was made on animal M. 104. This dehydrated ligated animal exhibited oliguria, progressive uremia and gastro-intestinal but not

bladder haemorrhage. Figures 25, 26, 27, 28, and 29 show dilated tubules and ischemic glomeruli of the cortex and Zones 3 and 4 (medulla) loaded with hyalin, granular and yellowish (pigment?) casts. This was the only observation, in this series of experiments, in which oliguria was so apparently associated with frequent tubular casts and dilatation.

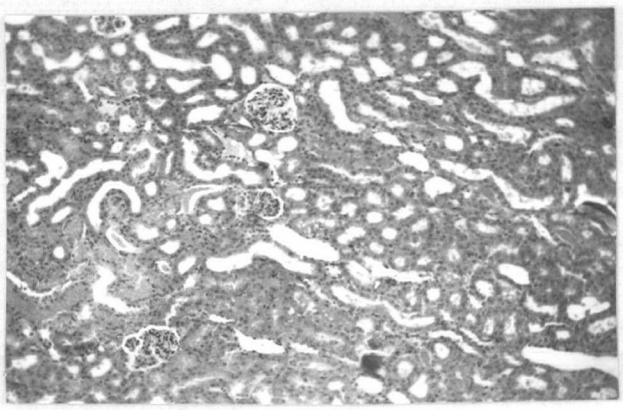

Figure 25

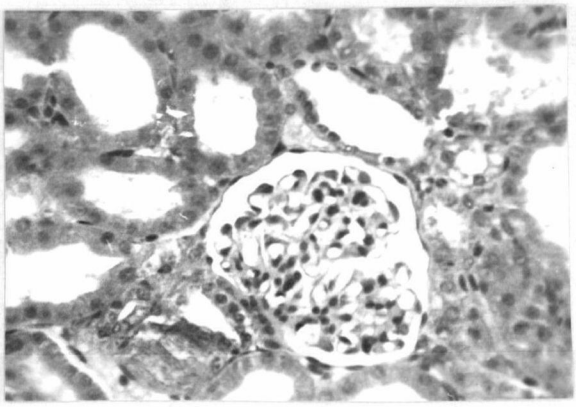

Figure 26

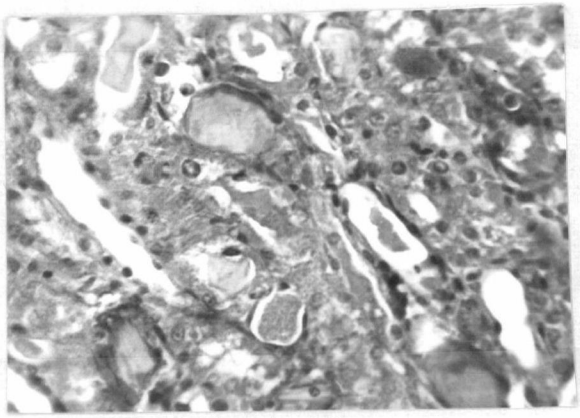

Figure 27

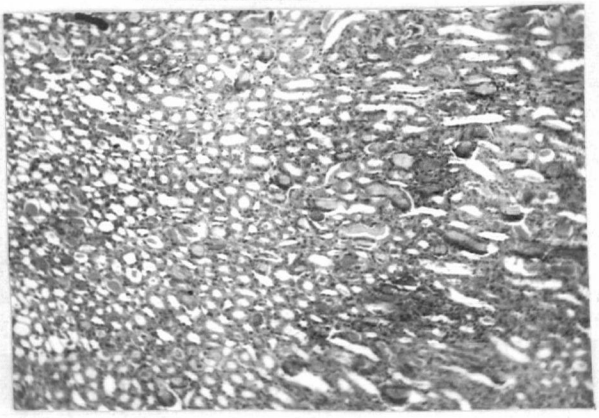

Figure 28

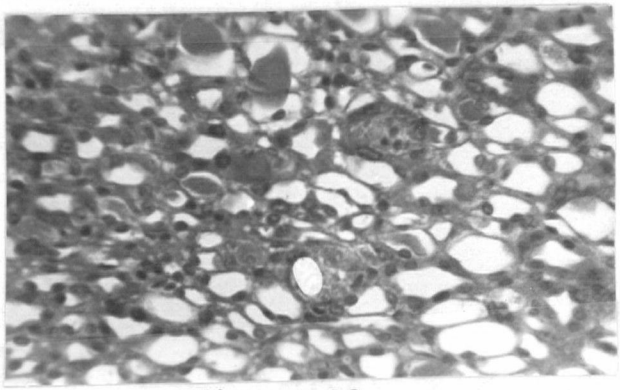

Figure 29

Results of Experiment 9B were essentially the same as in Experiment 9A. One control animal reached a B.U.N. level of 140 mg % while all test animals were over 190 mg. %. Two of eight test animals died 24 to 36 hours following ligation, one being anuric for about 20 hours prior to death. All remaining animals retained (in most cases at higher levels) their elevated B.U.N's until killed at 36 hours. Histological examination of these kidneys revealed that five of eight animals died with typical degenerative changes in Zone 3; one kidney was normal, one showed questionable change and the eighth illustrated postmortem changes only. All these five had elevated B.U.N.'s and two died in uremia. Six animals were polyuric and only one was anuric prior to death.

Kidney weights at death were recorded for the first time in Experiment 9B and cannot be said to show any significant difference between the two groups. Controls averaged 269.8 mg. per cm^2 (range 258.9 to 290.0); test animals averaged 273.6 mg. per cm^2 (range 254.9 to 304.7).

Comparing these results to those of Experiments 5A and 5B make it apparent that rats with reduced renal reserve are more susceptible to crush injury with dehydration as indicated by urea nitrogen levels, tubular damage and mortality rate. These results compare favorably with those of Experiment 8, in which the area of crush was increased in intact animals.

Experiment 10:

In an attempt to reduce the number of tests used and to observe the natural course of the syndrome, this experiment was concerned chiefly with the mortality in right nephrectomized rats subjected to 72 hours dehydration and five hours limb ligation. Because cardiac punctures would interfere with spontaneous deaths, only postmortem B.U.N's were done in surviving animals. The weight of kidneys at death in mg. per sq. cm. of surface area was again recorded (Table 10).

Data in the first five columns are not significantly different from previous observations except for the remarkable weight loss seen, especially in dehydrated controls, in which it is commonly 30% of body weight. The diuretic response appears again. B.U.N.'s were not obtained in the first two animals nor the three test animals dying spontaneously. Figures for test animals surviving were not elevated. Kidney weights average 319.4 mg. per $cm.^2$ for controls (range 289.5 to 368.8) and 332.7 mg. per $cm.^2$ for test animals (range 307.4 to 348.4) and though this is a difference of some 13 mg. per $cm.^2$, it cannot be significant because of the wide range seen in control animals. Histological changes were not valid in the three test animals which died, and appeared absent in the remaining animals, including controls.

Three of eight animals died spontaneously. M.139 was found dead at 24 hours and appeared to have died about 11 to 15

hours after removal of ligation; M.143 was also found dead at 24 hours and judging by lack of rigor mortis had died 16 to 17 hours following ligation removal; M.144 died more than 48 hours after the initial stress. These three were felt to be "valid" deaths - i.e. from renal failure and not from shock - and this mortality rate was taken as a standard for subsequent experiments on treatment.

It can be concluded that 72 hours dehydration plus five hours unilateral hind limb ligation in uninephrectomied male albino rats of the Wistar strain produced death in renal failure in three of eight test animals (37%). Disturbance of renal function as indicated by polyuria and hyposthenuria was seen in eight of eight (100%) test animals.

Experiment 11:

Female rats were used at this point in order to determine any sex difference in the response to the various therapeutic agents. In Experiment 11A, twelve uninephrectomied animals were used, six as controls and the remaining six (alternate animals) were treated with testosterone 5mg. in oil and 5 mg. in saline subcutaneously at the time of ligation. All animals were subjected to 24 hours pre-ligation dehydration followed by five hours left hind limb ligation and 48 hours post-ligation dehydration. Again, mortality was the main factor observed in order to determine the effect of testosterone in protecting

against acute renal failure. Observations are recorded in Table 11A.

Because it was felt that the testosterone may not have had sufficient time in which to act, Experiment 11A was repeated (Experiment 11B) giving 5 mg. of testosterone in oil 48 and 24 hours prior to ligation. The procedure followed was otherwise the same and again mortality was the chief factor observed (Table 11B).

In Experiment 11A, figures for urine output and B.U.N. were comparable to previous results. Kidneys of animals receiving testosterone are not significantly heavier (at the 5% level) than those of control animals (controls averaged 251.2 mg. per $cm.^2$ with a range of 222.8 to 259.9; test animals averaged 266.9 mg.% $cm.^2$, range 238.8 to 302.5). Five animals in the control group of six, and four in the test group of five, died. This increased mortality plagued all subsequent therapy experiments whether in male or female animals and the problem will be dealt with in the discussion to follow. It is, however, apparent that testosterone proprionate given in adequate dosage at the time of the initial stress is not effective in protecting female rats against traumatic uremia and death in acute renal failure.

Histological examination of these kidneys revealed, in test animals, three with typical lower nephron degeneration, one with questionable changes, and the fifth animal illustrated post

TABLE 10 MORTALITY IN DEHYDRATED, LIGATED UNINEPHRECTOMIED RATS

	RAT	FOOD (GM)	WATER (cc)	WEIGHT AND CHANGE (GM)	URINE VOLUME (cc)				URINE pH				B.U.N. at 96 HRS	DIED	KIDNEY WEIGHT$_2$ (mg/cm^2
					24	48	72	96	24	48	72	96			
DEHYDRATED CONTROL	M.133	5	16	128 (-66)	1.6	0.5	7.4	4.8	6.0	6.0	7.0	7.0	-		313.4
	M.134	5	11	126 (-64)	0.8	0.5	5.3	4.3	6.5	6.0	6.0	6.5	-		306.0
	M.135	5	14	114 (-50)	1.2	1.0	6.8	2.0	6.5	6.0	6.5	7.0	100		368.8
	M.136	5	15	116 (-60)	1.4	0.4	5.0	3.8	6.0	5.5	7.5	7.5	80		289.5
DEHYDRATION + LIGATION	M.137	5	66	128 (-46)	1.1	2.2	16.8	29.0	6.0	6.0	7.5	7.5	110		338.0
	M.138	5	42	130 (-66)	2.0	8.8	18.7	7.2	6.0	7.0	7.5	7.5	90		348.4
	M.139	0	--	138 (-26)	0.7	-	-	-	-	-	-	-	-	12-24	337.8
	M.140	5	53	136 (-52)	1.9	6.6	13.9	7.6	6.0	6.0	7.0	7.5	80		307.4
	M.141	5	41	132 (-50)	1.8	5.4	15.2	10.6	6.0	6.5	7.0	7.5	110		310.3
	M.142	5	37	122 (-50)	2.0	4.6	12.8	7.0	6.0	6.5	7.5	7.5	130		341.8
	M.143	0	-	160 (-32)	0.4	-	-	-	7.0	-	-	-	-	12-24	345.5
	M.144	0	3	140 (-24)	1.1	1.0	-	-	6.0	6.0	-	-	-	34-36	-

TABLE 11A TESTOSTERONE IN CRUSH SYNDROME

	RAT	FOOD (GMS)	WATER (cc)	WEIGHT AND CHANGE (GMS)	URINE VOLUME (cc) 24	48	72	URINE pH 24	48	72	B.U.N. at DEATH	DIED (HRS)	KIDNEY WEIGHT Mg/cm^2
T	M.145	0	0	180 (-14)	0.7	-	-	6.0	-	-	300	21	302.5
C	M.146	0	0	188 (-18)	0.7	-	-	6.0	-	-	-	29	258.6
T	M.147	0	0	168 (-6)	0.8	-	-	6.0	-	-	370	24	272.7
C	M.148	1	37	220 (-28)	1.3	0.3	24.5	6.0	6.0	7.0	170		254.3
T	M.149	0	0	200 (-24)	1.8	drop	-	5.5	7.5	-	-	56-72	263.7
C	M.150	0	0	178 (-20)	1.1	0	-	6.0	-	-	-	56-72	259.9
T	M.151	-	-	- -	-	-	-	-	-	-	-	-	-
C	M.152	0	0	200 (-22)	1.0	drop	-	6.0	-	-	-	56-72	253.3
T	M.153	3	50	198 (-24)	1.1	0.6	26.0	6.0	6.5	7.5	90	-	238.8
C	M.154	0	0	200 (-26)	0.7	-	-	6.0	-	-	300	20	258.5
T	M.155	0	0	152 (-21)	drop	-	-	-	-	-	300	17	257.0
C	M.156	0	0	188 (-22)	1.0	-	-	5.5	-	-	-	26	222.8

mortem changes. A feature of the pathology previously mentioned is here very evident. The fact that the degenerative and pyknotic changes in Zone 3 tubules are farthest removed from congested venae rectae is seen in Figure 30 (low power) and Figure 31 (high power).

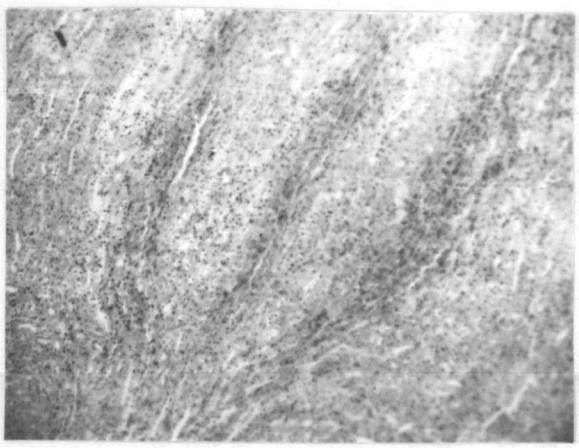

Figure 30

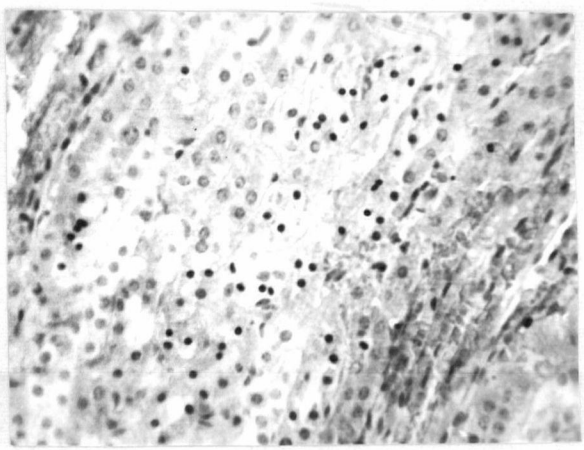

Figure 31

In Experiment 11B, not one of the animals survived to be killed at 72 hours, so that once again the increased fatality rate is illustrated. Eight of the animals were "found dead" 24 hours after ligation was applied and so the problem of shock death rather than uremic death is raised. Four animals (two test and two control) died after this 24 hour period and are taken to be certain uremic deaths; two of these (the test animals) had raised urea nitrogen figures.

Three kidneys could be examined histologically and all three showed changes typical of acute tubular necrosis (see Figure 32) which were also apparent in the distal tubules of the cortex. These kidneys also exhibited the frequently

observed marked congestion of the medulla (Figure 33). Kidney weights in mg. per cm.^{2} at death again show no predictable plan,

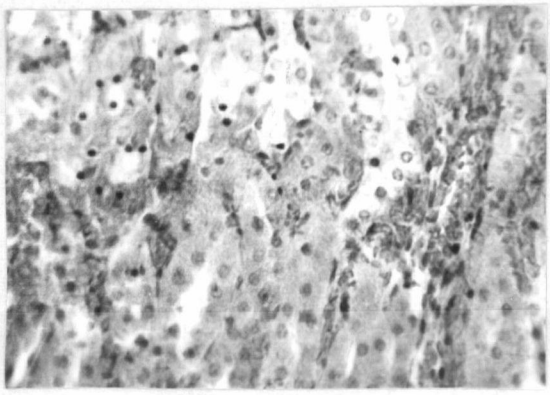

Figure 32

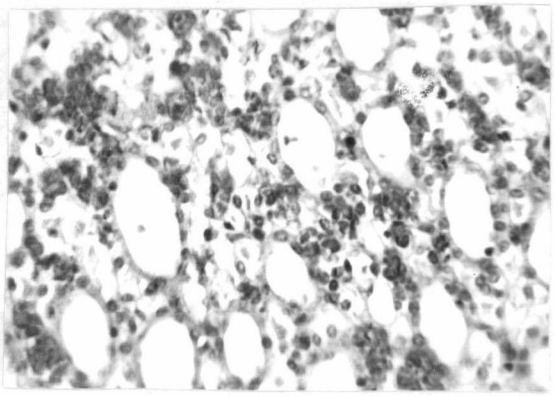

Figure 33

average figures being 271.6 mg. per cm.^{2} for test animals (range 260.5 to 280.0) and 277.2 mg. per cm.^{2} for controls (range 258.0 to 310.8).

In considering results of Experiments 11A and 11B it must be concluded that testosterone proprionate given in adequate doses to ligated, dehydrated, uninephrectomied female rats does not reduce their mortality rate. Whether or not the hormone has some palliative effect as measured by decreased structural damage or lower urea nitrogen levels in test animals cannot be stated.

Experiment 12

Experiment 11 was repeated here using twelve male animals and a dose of testosterone 5 mgs. in oil 72 and 24 hours prior to ligation, as well as at time of ligation, in alternate animals. Table 12 presents the observations.

TABLE 11B TESTOSTERONE IN CRUSHED FEMALE RATS

	RAT	FOOD (GMS)	WATER (cc)	WEIGHT AND CHANGE (GMS)	URINE VOLUME (cc)			URINE pH			B.U.N. at DEATH mg. %	DIED (HRS)	KIDNEY WEIGHT Mg/cm^2
					24	48	72	24	48	72			
T	M.157	0	0	196 (-18)	0.3	-	-	7.0	-	-	-	24	269.8
C	M.158	0	0	190 (-26)	0.8	-	-	7.0	-	-	-	24	310.8
T	M.159	0	0	202 (-18)	0.2	-	-	-	-	-	-	24	269.4
C	M.160	0	0	176 (-24)	0.4	0	-	-	-	-	-	32-48	286.9
T	M.161	0	0	194 (-26)	0.9	0	-	6.0	-	-	290	29	281.9
C	M.162	0	0	194 (-28)	1.4	0	-	6.0	-	-	-	26	258.0
T	M.163	0	0	180 (-18)	0.5	-	-	6.0	-	-	-	24	260.5
T	M.164	0	0	170 (-20)	0.7	-	-	-	-	-	-	24	282.0
T	M.165	0	0	200 (-24)	0.8	0	-	6.0	-	-	300	28	282.0
C	M.166	0	0	185 (-20)	0.2	-	-	-	-	-		24	258.2
T	M.167	0	0	190 (-20)	0.4	-	-	6.0	-	-		24	264.9
C	M.168	0	0	170 (-22)	1.2	-	-	6.0	-	-		24	267.4

TABLE 12 TESTOSTERONE IN CRUSHED MALE RATS

	RAT	FOOD AND WATER	WEIGHT AND CHANGE	URINE VOLUME (cc)			URINE pH			B.U.N. at DEATH	DIED	KIDNEY WEIGHT mg/cm^2
				24	48	72	24	48	72			
T	M.253	0	208 (-14)	0	-	-	-	-	-		24	266.4
C	M.254	0	206 (-20)	0.1	-	-	-	-	-		24	214.8
T	M.255	0	192 (-16)	0	-	-	-	-	-		24	203.7
C	M.256											
T	M.257	0	186 (-16)	0	-	-	-	-	-		24	230.1
C	M.258	0	200 (-24)	0.5	-	-	-	-	-		24	250.6
T	M.259	0	194 (-16)	0.3	-	-	-	-	-		24	226.0
C	M.260	0	202 (-20)	0.3	-	-	-	-	-		24	230.5
T	M.261	0	210 (-18)	0.4	-	-	-	-	-		24	249.3
C	M.262	5 gm 60cc	188 (-24)	0.3	3.4	30.0	-	5.0	6.0	310	-	279.8
T	M.263											
C	M.264	0	196 (-22)	0.2	-	-	-	-	-	-	24	201.8

All animals appeared markedly shocked when ligatures were removed after five hours and nine of ten were dead when seen the following morning (24 hours after ligatures were applied). Two other animals died during the period of ligation. These results were typical of those of later experiments in which death occurred earlier and more frequently. Kidney weights averaged 235.5 mg. per $cm.^2$ (range 201.8 to 279.8) for control and 235.1 mg. per $cm.^2$ (range 203.7 to 266.4) for treated animals.

One untreated control animal ran a 72 hour course showing a typical clinical picture of acute renal failure in the rat, with an eventual diuretic response and a B.U.N., when killed, of 310 mg. %. Histologically, that kidney showed changes taken to be regeneration following tubular damage: areas of flattened, basophilic tubular cells associated with cellular debris in the lumens (see section on "Discussion").

Testosterone propionate therefore would appear to be ineffective in maintaining or prolonging life in male rats experiencing shock and acute renal failure.

Experiment 13:

The effect of cortisone acetate on mortality in uni-nephrectomied, dehydrated and ligated female rats is considered in this experiment. Two mgs. of cortisone in saline were injected subcutaneously 24 hours prior to ligation and the dose repeated daily until death. Twelve animals were used, alternate

ones being treated with the test drug. Observations are in Table 13.

Mortality rate was again high, but most animals lived beyond the 24 hour period. All treated animals died in the 24 to 28 hour period, while two control animals died in the 32 to 48 hour period. Of eight animals in which B.U.N. levels were determined at death, all were elevated to uremic levels and all showed histological changes of acute tubular necrosis. Representative areas of Zone 3 are illustrated in Figures 34 (control) and 35 (test).

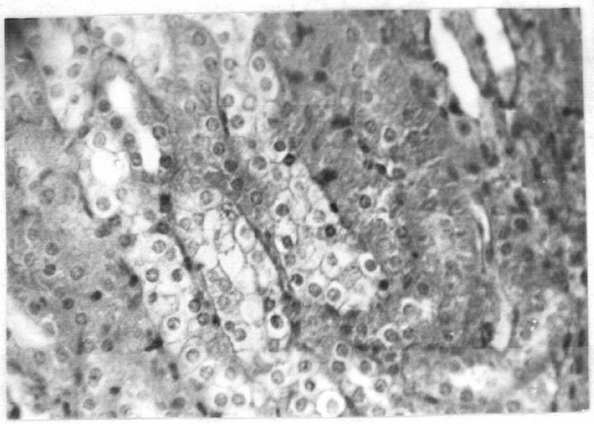

Figure 34

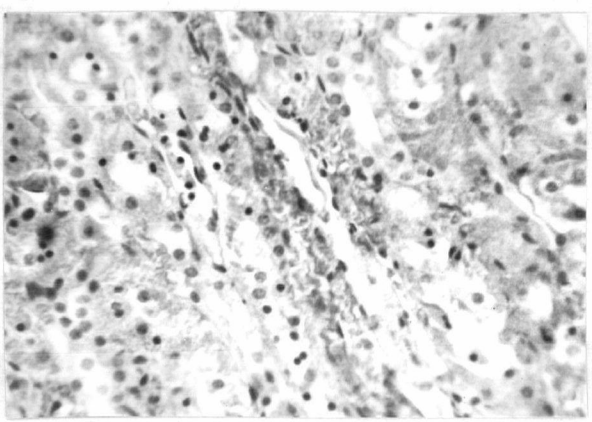

Figure 35

These animals were also anuric for periods up to 24 hours prior to death. Kidney weights again revealed no significant relationship of treated (average 250.7 mg. per $cm.^2$ - range 240.4 to 272.2) to untreated (av. 246.4 mg. per $cm.^2$ - range 235.5 to 260.5).

Cortisone acetate would appear to be ineffective in

reducing the mortality from or severity of acute tubular necrosis in female rats subjected to uninephrectomy, dehydration and crush injury.

Experiment 14:

The above experiment with cortisone is here repeated using, instead, male animals. Procedure and dose schedule of cortisone acetate were essentially the same as in that experiment. Observations are in Table 14.

All twelve animals (six treated, six untreated) were dead when observed 24 hours after ligatures were applied, so that no observations were made, other than weight loss and 24 hour urine volume. No explanation for this exaggerated increase in early mortality was apparent, but the problem is considered in the section on "Discussion". Kidney weights show an isolated example of significance at the 1% level in that treated animals averaged 243.6 mg. per $cm.^2$ (range 220.4 to 260.3), while controls averaged 214.3 mg. per $cm.^2$ (range 191.8 to 236.3). No histological examinations were made because all kidneys had undergone postmortem change.

No conclusions can be drawn from the experiment other than that cortisone appears to have no favorable effect in protecting male rats from death from shock and/or renal failure. It becomes apparent that the standardized production of acute

TABLE 13 CORTISONE IN CRUSHED FEMALE RATS

	RAT	FOOD (GMS)	WATER (cc)	WEIGHT AND CHANGE (GMS)	URINE VOLUME (cc)			URINE pH			B.U.N. at DEATH mg. %	DIED (HRS)	KIDNEY WEIGHT Mg/cm^2
					24	48	72	24	48	72			
T	M.169	0	0	180 (-20)	1.4	-	-	5.5	-	-	190	25	243.7
C	M.170	0	0	180 (-20)	1.0	-	-	-	-	-	400	26	260.5
T	M.171	0	0	180 (-20)	0.5	-	-	5.5	-	-	230	27	240.9
C	M.172	0	0	170 (-30)	1.2	0	-	6.0	-	-	270	48	235.5
T	M.173	0	0	178 (-18)	0.4	0	-	-	-	-	370	27	251.4
C	M.174	0	0	184 (-24)	0.6	-	-	-	-	-	-	12-24	248.6
T	M.175	0	0	174 (-28)	1.3	0	-	6.0	-	-	290	27	272.2
C	M.176	0	0	182 (-30)	0.7	0	-	6.0	-	-	-	32-48	-
T	M.177	0	0	184 (-20)	0.6	0	-	5.5	-	-	500	28	251.4
C	M.178	0	0	210 (-22)	0.6	0	-	5.5	-	-	-	30	236.8
T	M.179	0	0	194 (-34)	0.6	-	-	6.0	-	-	-	12-24	244.7
C	M.180	0	0	212 (-28)	1.1	0	-	6.0	-	-	400	27	250.6

TABLE 14 CORTISONE IN CRUSHED MALE RATS

	RAT	FOOD (GMS)	WATER (cc)	WEIGHT AND CHANGE (GMS)	URINE VOLUME (cc)			URINE pH			B.U.N. at DEATH	DIED (HRS)	KIDNEY WEIGHT Mg/cm^2
					24	48	72	24	48	72			
T	M.241	0	0	190 (-20)	0.3	-	-	-	-	-	-	12-24	248.6
C	M.242	0	0	192 (-22)	0.3	-	-	-	-	-	-	12-24	209.1
T	M.243	0	0	198 (-16)	0.9	-	-	-	-	-	-	12-24	220.4
C	M.244	0	0	232 (-16)	0.5	-	-	-	-	-	-	12-24	231.1
T	M.245	0	0	180 (-16)	0.9	-	-	-	-	-	-	12-24	238.0
C	M.246	0	0	190 (-16)	0.1	-	-	-	-	-	-	12-24	191.8
T	M.247	0	0	204 (-22)	0.4	-	-	-	-	-	-	12-24	260.3
C	M.248	0	0	184 (-14)	0.4	-	-	-	-	-	-	12-24	196.1
T	M.249	0	0	228 (-22)	0.6	-	-	-	-	-	-	12-24	252.9
C	M.250	0	0	190 (-18)	0.2	-	-	-	-	-	-	12-24	221.6
T	M.251	0	0	224 (-18)	0.5	-	-	-	-	-	-	12-24	241.5
C	M.252	0	0	214 (-18)	0.3	-	-	-	-	-	-	12-24	236.3

tubular necrosis and renal failure by these methods is much less possible than was indicated in earlier experiments.

Experiment 15:

A combination of testosterone, with its renotropic (333) and/or protein-sparing actions, and cortisone, with its co-called life-maintaining factor, might conceivably be effective in cases of acute tubular necrosis, where either one of these agents alone would fail. These hormones were therefore used together in this experiment, with dosages arranged as in Experiments 11B and 13. Twelve female animals were again used with alternate animals receiving the test drugs. Table 15 presents the observations.

These animals were smaller than usual, being 165 to 180 gm. in weight, which fact may account in part for the increased mortality at 24 hours. Only four animals lived beyond the 24 hour period; three of these were untreated control animals and one had been treated with hormones; all four had elevated B.U.N.'s; and all showed histological evidence of acute tubular necrosis. Kidney weight figures averaged 284.5 mg. per $cm.^2$ (range 261.0 to 322.9) for test animals, 277.4 mg. per cm^2 (range 251.7 to 287.5) for controls, hardly a significant difference.

Though these animals were perhaps too small for an

TABLE 15 TESTOSTERONE PLUS CORTISONE IN CRUSHED FEMALE RATS

	RAT	FOOD (GM)	WATER (cc)	WEIGHT AND CHANGE (GM)	URINE VOLUME			URINE pH			B.U.N. at DEATH Mg. %	DIED	KIDNEY WEIGHT Mg/cm.^{2}
					24	48	72	24	48	72			
T	M.181	0	0	168 (-12)	0.5	-	-	-	-	-	-	12-24	261.0
C	M.182	0	0	148 (-20)	0.3	-	-	-	-	-	-	12-24	287.5
T	M.183	0	0	152 (-18)	0.6	-	-	-	-	-	-	12-24	282.1
C	M.184	0	0	162 (-20)	0.9	-	-	6.0	-	-	410	25	273.3
T	M.185	0	0	156 (-18)	1.1	-	-	6.0	-	-	-	12-24	265.4
C	M.186	0	0	146 (-20)	0.6	-	-	6.5	-	-	-	12-24	283.9
T	M.187	0	0	152 (-16)	0.6	-	-	5.5	-	-	-	12-24	322.9
C	M.188	0	0	140 (-14)	0.7	-	-	5.5	-	-	230	25	251.7
T	M.189	0	0	146 (-20)	0.8	-	-	6.0	-	-	440	26	296.8
C	M.190	0	0	144 (-14)	1.6	-	-	6.0	-	-	320	27	285.7
T	M.191	0	0	160 (-14)	0.8	-	-	6.0	-	-	-	12-24	278.8
C	M.192	0	0	154 (-18)	0.6	-	-	6.0	-	-	-	12-24	282.6

TABLE 16 TESTOSTERONE AND CORTISONE IN CRUSHED MALE RATS

	RAT	FOOD (GM)	WATER (cc)	WEIGHT AND CHANGE (GM)	URINE VOLUME (cc.) 24	48	72	URINE pH 24	48	72	B.U.N. at DEATH Mg.%	DIED (hrs)	KIDNEY WEIGHT $Mg/cm.^2$
T	M.193	0	0	200 (-28)	0.15	-	-	-	-	-	-	12-24	281.9
C	M.194												
T	M.195	0	11	220 (-34)	0.95	-	-	5.5	-	-	-	36-48	261.6
C	M.196	0	0	212 (-40)	0.3	-	-	-	-	-	-	12-24	-
T	M.197	0	0	224 (-34)	0.4	-	-	-	-	-	-	12-24	285.0
C	M.198	0	0	210 (-36)	0.5	-	-	-	-	-	-	12-24	269.5
T	M.199	0	0	214 (-42)	0.5	-	-	-	-	-	-	12-24	318.4
C	M.200	5	55	228 (-38)	1.9	17.2	9.2	5.5	6.5	7.0	180	-	295.9
T	M.201	0	0	222 (-38)	0.1	-	-	-	-	-	-	12-24	286.4
C	M.202	0	7	194 (-36)	0.4	0	-	5.5	-	-	-	30	281.9
T	M.203	0	0	208 (-32)	0.5	-	-	-	-	-	-	12-24	296.9
C	M.204	3	57	200 (-58)	0.55	14.7	20.8	5.5	6.5	7.0	200	-	276.7

adequate test, it would appear that testosterone and cortisone together afford no protection against the lower nephron damage produced by uninephrectomy, dehydration and crush injury in female rats.

Experiment 16:

Experiment 16 considers the effect of testosterone and cortisone on the mortality in uninephrectomied, dehydrated and ligated male albino rats of the Wistar strain. Twelve animals were used, but one control died during the ligation period and was not replaced. Dosage of testosterone was increased to 5 mg. in oil 96, 48 and 24 hours before ligation, as well as 5 mg. at the time of ligation; that of cortisone was, as before, 2 mg. daily starting 24 hours before ligation and continuing as a daily dose. The period of dehydration here, however, was shortened to a total period of 48 hours - 24 before and 24 after ligation - in order to lessen the stress and prolong life to allow determination of whether or not the animals reached a state of acute renal failure. Observations appear in Table 16.

Again, seven animals died within the 24 hours following ligation in spite of being of adequate body weight; one died during the ligation period of massive retroperitoneal haemorrhage following nembutal injection; two animals (one test and one control) survived for 24 to 48 hours; two remaining animals

(both controls) survived for 72 hours and were killed. These had urea nitrogen levels of 180 and 200 mg. % and though the first (M.200) histologically appeared to show signs of healing lower nephron degeneration (epithelial debris in medullary tubules - see Experiments 12 and 17B), the second (M.204) had an essentially normal kidney. Figures 36 and 37 show a typical area of Zone 3 degeneration and of Zone 4 (medulla) with frequent casts. The two animals which recovered showed a

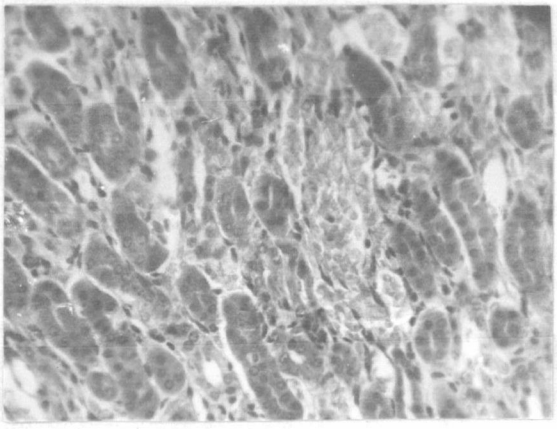

Figure 36

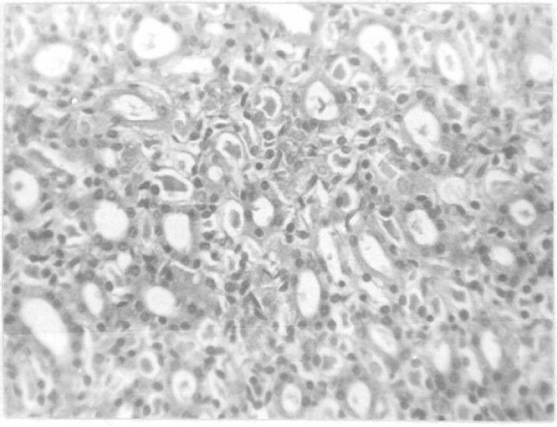

Figure 37

typical diuretic response at 48 and 72 hours. Kidney weights averaged 288.4 mg. per cm^2 for six treated animals (range 261.6 to 318.4), 281.0 mg. per cm^2 for four controls (range 269.5 to 295.9).

As in Experiment 15, it can only be concluded that testosterone and cortisone have no therapeutic value in male rats with acute tubular necrosis.

Experiment 17:

The efficacy of Compound F in the treatment of acute tubular necrosis in uninephrectomied, dehydrated, ligated male rats is tested in Experiment 17A. Observations are made on urine output, blood urea nitrogen, kidney histology and mortality rate. Six animals were so tested, with six untreated controls. Compound F was given as a daily dose of 2 mgs. in saline subcutaneously, starting 48 hours before ligation. Observations appear in Table 17A.

Because of the promise shown by Compound F in this experiment, it was repeated in Experiment 17B using a slightly higher dosage of this substance, 3 mgs. daily starting 72 hours before ligatures were applied. Alternate animals of a group of twelve males were so treated. Observations were made on urine outputs and mortality rate but B.U.N's were determined only postmortem on animals freshly dead. These appear in Table 17B.

By 24 hours following ligation, nine animals in Experiment 17A had died. The three remaining animals survived for 72 hours to be killed at that time for kidney histology. These were treated animals. Urine outputs were typical of the diuretic response, and urea nitrogen levels were elevated at 24 hours but at 72 hours were subsiding. Histological sections of these three kidneys revealed what is considered to be healing acute tubular necrosis (see Figures 38-40 in Experiment 17B). Kidney

TABLE 17A COMPOUND F IN CRUSHED MALE RATS

	RAT	FOOD (MG)	WATER (cc)	WEIGHT AND CHANGE (GM)	URINE VOLUME cc.			URINE pH			B.U.N. mg.%.			DIED (hrs)	KIDNEY WEIGHT Mg/cm^2.
					24	48	72	24	48	72	24	48	72		
T	M.205	9	66	172 (-36)	1.5	5.9	26.4	6.0	6.0	6.5	240	-	170	-	231.2
C	M.206	0	0	224 (-24)	0.4	-	-	-	-	-	-	-	-	12.24	251.2
T	M.207	0	0	188 (-28)	0.0	-	-	-	-	-	-	-	-	12-24	201.0
C	M.208	0	0	172 (-24)	0.5	-	-	6.0	-	-	-	-	-	12-24	202.3
T	M.209	0	0	174 (-24)	0.75	-	-	5.5	-	-	-	-	-	12-24	197.7
C	M.210	0	0	180 (-28)	0.3	-	-	-	-	-	250	-	130	-	260.5
T	M.211	9	63	184 (-44)	1.8	6.7	19.8	6.5	7.0	7.5	-	-	-	12-24	262.4
C	M.212	0	0	188 (-28)	0.4	-	-	-	-	-	-	-	-	12-24	247.2
T	M.213	0	0	186 (-30)	0.3	-	-	-	-	-	-	-	-	12-24	210.9
C	M.214	0	0	170 (-28)	0.1	-	-	-	-	-	-	-	-	12-24	203.4
T	M.215	9	40	172 (-36)	1.1	2.9	7.0	6.5	6.0	6.5	190	-	150	-	225.4
C	M.216	0	0	198 (-24)	0.6	-	-	-	-	-	-	-	-	12-24	212.5

TABLE 17B COMPOUND F IN CRUSHED MALE RATS

	RAT	FOOD (GM)	WATER (cc)	WEIGHT AND CHANGE (GM) (GM)	URINE VOLUME (cc)			URINE pH			B.U.N. Mg.%	DIED (hrs)	KIDNEY WEIGHT mg/cm.^{2}
					24	48	72	24	48	72			
T	M.229	0	99	218 (-38)	2.6	16.6	47.4	5.5	6.0	7.5	200	-	289.9
C	M.230	10	86	276 (-40)	3.4	28.0	11.0	6.0	7.0	7.5	150	-	201.6
T	M.231	9	77	174 (-31)	1.4	21.3	18.6	6.0	6.5	7.0	170	-	229.2
C	M.232	0	0	190 (-20)	0.2	-	-	6.0	-	-	-	12-24	208.1
T	M.233	0	0	226 (-30)	0.9	0	-	6.0	-	-	280	25	218.7
C	M.234	0	3	230 (-38)	1.2	0	-	6.0	-	-	420	26	236.6
T	M.235	0	8	220 (-32)	0.6	0.3	-	6.0	-	-	-	29-33	222.4
C	M.236	0	0	196 (-22)	0.7	-	-	6.0	-	-	-	12-24	201.0
T	M.237	0	0	190 (-28)	0.4	-	-	-	-	-	-	12-24	245.9
C	M.238	0	61	224 (-52)	2.1	8.0	31.0	6.0	6.0	7.5	240	-	246.3
T	M.239	0	0	190 (-24)	0.7	0	-	6.0	-	-	300	25	251.3
C	M.240	0	0	184 (-24)	0.2	-	-	-	-	-	-	12-24	234.8

weights were again not significant: treated animals averaged 221.4 mg. per cm.^{2} (range 197.7 to 262.4), untreated controls 229.5 mg. per cm.^{2} (range 202.3 to 260.5).

On the basis of Experiment 17A with twelve male animals, it can be concluded that Compound F shows some promise in the treatment of acute tubular necrosis induced by dehydration in crushed animals. Three of six treated animals survived while none of six untreated animals survived.

In experiment 17B, water was allowed animals after 48 hours dehydration, instead of the usual 72 hours, in an attempt to prolong life so that more serial observations could be made. It was felt that, if kidney damage was already present, free water intake would not improve the ultimate outlook and so not interfere with comparisons of mortality rate. Kidneys were here and subsequently fixed in Herlant's solution because of technical difficulties with Zenker's fixative and results amply justified the switch.

Urine outputs in those animals surviving 48 hours or more showed a remarkable diuretic response and indicated that these animals with damaged kidneys could not handle water intake satisfactorily. It appeared that the increased oral amount was rapidly flushed out through the kidney and lost, so that animals continued to lose weight.

In the four animals surviving to 72 hours (two treated,

two untreated), B.U.N. levels remained elevated, indicating continuing renal damage, and renal histology showed a picture of what has been described previously as healing acute tubular necrosis. This healing picture is shown in Figure 38 (Zone 2 of animal M. 229) in which new, low basophilic cuboidal cells are seen appearing in disorganized or degenerated areas; Figure 39 (Zone 3 of M. 229) showing epithelial debris with nuclei in tubules; and in Figure 40 (medulla of M. 229) which again shows

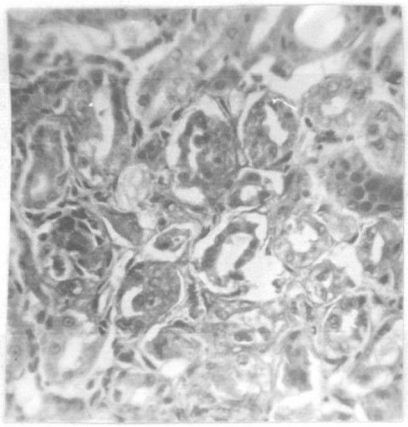

Figure 38

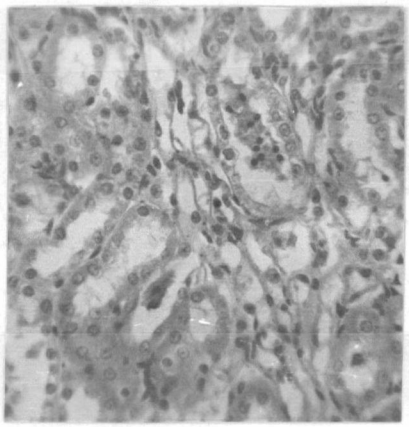

Figure 39

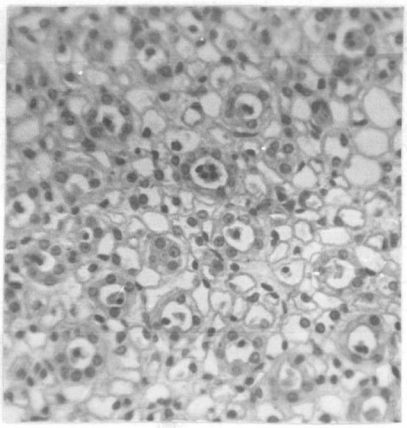

Figure 40

the epithelial casts. Two treated and two untreated animals therefore survived to be killed at 72 hours with elevated urea nitrogen levels, evidence of healing tubular damage and records of diuresis again indicating kidney dysfunction.

Three other animals (two treated, one untreated) died 25 to 36 hours after ligatures were applied, with B. U. N. levels elevated to 280 to 420 mg. %, 24 hour urine volumes at oliguric levels (0.7 to 1.2 cc) and histological acute tubular necrosis.

A total of eight animals died spontaneously, four having been treated with Compound F and four untreated; the remaining four survived to 72 hours.

Kidney weights averaged 242.9 mg. per cm.^{2} (range 218.7 to 289.9) for treated and 221.4 mg. per cm^2 (range 201.0 to 246.3) for untreated control animals.

Conclusions to be drawn from this experiment include the following: 1) Compound F does not appear to prolong the life of or reduce mortality in male rats suffering from experimental acute tubular necrosis. Since this agent did seem to have some palliative effect in Experiment 17A, determination of its true value in treatment of the syndrome must await further experimentation. 2) Animals with evidence of renal damage are seen to handle oral intake of water in an inefficient and disadvantageous manner.

Experiment 18:

A fourth and final hormonal agent, desoxycoricosterone acetate, was tested in male rats in which dehydration and crush injury were used to produce acute tubular necrosis. In particular, mortality rate was noted, but observations on urine output, blood urea nitrogen and kidney histology were also made. Again, twelve animals were used, alternate ones being treated with DCA 2.5 mgs. in water subcutaneously each day beginning 48 hours before ligation of the limb. Table 18 lists the observations.

TABLE 18: DESOXYCORTICOSTERONE IN CRUSHED MALE RATS

	RAT	FOOD (GM)	WATER (cc)	WEIGHT AND CHANGE (GM)	URINE VOLUME (cc)			URINE pH			B.U.N. Mg. %			DIED	KIDNEY WEIGHT (Mg./cm^2)
					24	48	72	24	48	72	24	48	72		
T	M.217	0	0	236 (-24)	1.9	0	-	5.5	-	-	300	-	-	32-48	230.7
C	M.218	0	0	250 (-12)	0.5	-	-	-	-	-	-	-	-	12-24	213.4
T	M.219	0	0	228 (-36)	1.9	2.0	-	6.0	6.0	-	-	-	-	32-48	214.7
C	M.220	5	60	210 (-32)	2.9	2.3	25.9	6.0	5.5	7.5	280	-	190	-	256.9
T	M.221	0	0	224 (-26)	2.3	0.7	-	5.5	5.5	-	420	-	-	32-48	198.0
C	M.222	0	0	224 (-20)	2.1	0.1	-	5.5	5.5	-	240	-	-	32-48	195.6
T	M.223	0	0	182 (-16)	1.0	0	-	5.5	-	-	-	-	-	31	202.7
C	M.224	0	0	194 (-26)	1.2	0	-	6.0	-	-	290	-	-	32-48	223.4
T	M.225	0	0	188 (-24)	0.7	0	-	5.5	-	-	-	-	-	32-48	209.2
C	M.226	0	0	186 (-12)	0	-	-	-	-	-	360	-	-	24	243.8
T	M.227	0	0	174 (-16)	0.9	0	-	5.5	-	-	-	-	-	31-32	200.5
C	M.228	0	0	178 (-26)	1.5	0	-	5.5	-	-	210	-	-	32-48	186.4

Only one animal, M. 220 (a control animal) survived to 72 hours; this animal had an elevated B.U.N. and kidney histology showed foci of regeneration in Zones 2 and 3 as previously described. It has been pointed out before that this change was seen frequently in animals known to have suffered kidney damage but which eventually recovered. A second control animal, M. 226, was observed to die in convulsions 24 hours after the ligature was applied and at that time the B.U.N. was 360 mg. % and kidney histology was typical of "lower nephron nephrosis" (Figure 41) together with frequent proximal tubule vacuolization. From this observation it becomes apparent that acute tubular necrosis with death in uremia can indeed be produced within 17 to 18 hours following removal of the crushing ligature.

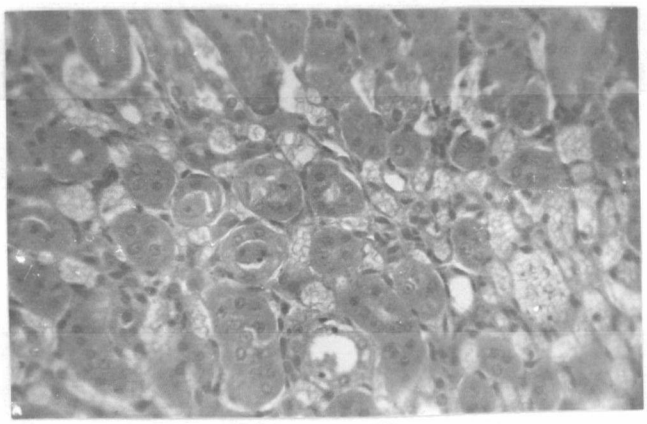

Figure 41

Five other animals had B.U.N's elevated to from 210 to 420 mg. % and died at from 32 to 48 hours following ligation. All apparently died in acute renal failure with a varying number of hours anuria preceding death. Kidney histology of the two

test animals was probably reliable, though the animals could have been dead for one hour and ten minutes when their kidneys were fixed; it showed typical acute tubular necrosis in both cases.

Kidney weights following fixation averaged 209.3 mg. per $cm.^2$ (range 198.0 to 230.7) for treated animals and 219.9 mg. per $cm.^2$ (range 186.4 to 256.9) for untreated.

It can be concluded that DCA, given in adequate dose, to male rats suffering from acute tubular necrosis, does not decrease their mortality rate nor prolong life. It can also be stated that death in uremia from acute tubular necrosis resulting from dehydration and crush injury in uninephrectomied animals can be produced 18 hours following release of ligation.

DISCUSSION AND CONCLUSIONS

From these experiments several conclusions can be drawn which give rise to some discussion; but before presenting these points it is essential to recall the original aim of the work. It was planned to produce a standardized "lower nephron syndrome" in rats by varying three stresses, dehydration, myoglobin injection, and crush injury. This accomplishment was to be followed by therapeutic use of testosterone, cortisone, desoxycorticosterone and Compound F in alleviation of the acute

renal failure. That this initial aim was accomplished is apparent in Experiments 5, 7, 8, 9 and 10.

Several statements can be made about the factors responsible for the production of acute tubular necrosis. Dehydration has been shown to be an essential factor in the production of traumatic uremia in the rat. Even severe dehydration (Experiment 1B), when alone, succeeded in producing only slight uremia and oliguria with almost immediate recovery on re-hydration, without histological evidence of tubule damage. These results are probably adequately explained by simple hemoconcentration; though prolonged dehydration could conceivably produce shock, such a condition was never observed in animals (even uninephrectomied ones) dehydrated as long as 72 hours and therefore could play no part in the urea nitrogen increase and oliguria. The dehydration as employed here anteceded by 24 hours other stresses utilized, and had similar effects in intact animals and in right nephrectomied animals. This finding that the state of hydration is an important factor in the production of the syndrome is in agreement with the works of Lalich (202, 203), Maluf (232) and many others.

It is also obvious (Experiment 5) that there is a second essential factor which in these experiments took the form of a crush injury. Although release of a nephrotoxic agent from the damaged tissue (125, 162, 30, 89) cannot be excluded as the pathogenetic mechanism, shock with renal ischemia was

probably the chief cause of renal damage. That shock actually was present could only be assumed from the appearance of the animals immediately following removal of the crushing mechanism. These animals assumed a crouching position with eyes closed and fur ruffled, an attitude which was occasionally punctuated with attacks of rigors. There was a second observation in favour of anoxia -- i.e. ischemia as a result of shock -- as the damaging agent. It was consistently noted that various degrees of postmortem change could in no way be distinguished from the tubular damage seen in kidneys of animals freshly dead as a result of ligation and dehydration. Since postmortem autolysis must essentially be primarily an anoxic change, then it is probable that the degenerative changes seen in test animals is also anoxic (see Figures 51 - 62).

The method of leg compression was used first by Bywaters and Popjak (74) in order to simulate as closely as possible the clinical crush injury. They early noted that shock occurred following the occlusive period, and used the method later in experiments with myoglobin (75). Duncan and Blalock (116) also used clamping of a limb to produce experimental shock in dogs and noted the similarity to crush syndrome. Eggleton et al (125,126), using cats and dogs, recorded low blood pressures following elastic rubber tube binding of limbs but was of the opinion that renal damage resulted from a released nephrotoxic agent. Corcoran and Page (85) also used a method of limb ligation in their studies of the relationship of myoglobin to crush syndrome, and Keele and Slome (191) noted a marked reduction in blood pressure following release of a

wrapped limb in cats. There would appear to be little doubt, therefore, that this method of limb compression can indeed produce shock in experimental animals.

It is apparent therefore that the combination of severe dehydration and crush injury is capable of producing renal tubular damage as evidenced by elevated blood urea nitrogen levels, altered urine output and histological changes. In experiments designed to test the mortality rate, this damage was sufficient to be fatal to 40 to 50% of test animals. These were the essential factors, such added refinements as prolongation of ligation, bilateral ligation, myoglobin injection and uninephrectomy being merely attempts to produce a more predictable and standardized result. In the case of prolongation of ligation and of bilateral ligation, these procedures either increased the early mortality so that animals died in the shock phase or produced no more satisfactory tubule damage than did the simpler unilateral ligation. In reducing the known high renal reserve of the rat in as physiological a way as possible by surgical removal of one kidney, it was found that acute renal failure could be produced far more readily (Experiment 9).

In examining the syndrome as produced experimentally in the rat and comparing it to that in the human (in which it commonly runs a 7 to 14 day course) it is apparent that it runs a fore-shortened course. The corresponding events in the rat appeared to occur within one to three days following trauma,

those animals surviving for three days being clinically fully recovered. This foreshortening gave rise to difficulties on two accounts. First, it was often difficult to obtain serial observations since affected animals often died within 24 hours; second, it was often difficult to decide whether an animal died of shock itself or of renal failure, when it succumbed within 15 - 18 hours of the initial trauma. It was felt, however, that a fairly definite sequence of events occurred, as observed clinically in the first 24 hours. During the five hour ligation period, though animals were sedated they nevertheless behaved vigorously and in a wide-awake fashion when that sedation subsided. But following removal of the ligature they fell immediately into a period which we called "shock". They crouched far back in their cages, eyes closed and fur ruffled, often developing marked tremor. They remained in this state for from two to four hours at which time their condition could be described as "improved". That is, there appeared to be a definite recovery from the initial trauma which occurred at the time of ligature removal. One encouraging and conclusive observation was made in Experiment 18. This observation proved that it was possible for a rat (animal M.226) to die in acute renal failure with acute tubular necrosis 17½ hours following removal of a five hour unilateral ligation.

It can be stated categorically that the pigment myoglobin is not essential to the production of acute tubular necrosis from crush injury in the rat (Experiments 5, 6 and 7). This

statement is in contrast to the original work of Bywaters and Stead (75) who, though unable to produce renal failure by injecting myoglobin alone or by leg compression alone, produced the syndrome by myoglobin injection following leg compression or following acidification of the urine to pH 4.5 to 6.1 with ammonium chloride. In our experiments, it can be noted that though urine was consistently of pH 5.0 to 6.5 and animals were dehydrated, myoglobin injection did not produce detectable renal damage (Experiment 3). It is interesting to note that, although Bing (31, 32) found that 80 to 120 gm. of ammonium chloride given to dogs to acidify urine did itself produce no renal damage, Govan and Parkes (160, 161) found that both ammonium and calcium chloride produced renal lesions and death in rabbits. It would therefore appear that Bywaters' work (75) should be considered only with reservations. Corcoran and Page (85, 86) have also reported that crush syndrome is reproducible by intravenous injection of metamyoglobin after release of compression from one crushed hind limb of rats. They reported as well "partially recoverable renal injury" in dogs subjected to myoglobin and metamyoglobin injection in aciduric dogs. Bing's (32) work, however, contrasts with these observations; he failed to produce any significant impairment of renal function by injection of myohaemoglobin into normal or acidotic dogs.

Whether or not myoglobin adds to the damage induced by crush and dehydration should be apparent in Experiment 7.

In this experiment a statistically significant increased elevation of urea nitrogen levels for myoglobin injected animals over non-injected ones was found. It can be stated therefore that intravenously injected myoglobin adds to renal damage induced by dehydration plus crush injury, although by itself or coupled with either one of these factors, the pigment is non-toxic. There was no histological evidence that the aggravation of renal dysfunction was due to obstructive casts.

The possibility that these observations of the effect of myoglobin are not valid should be considered. Corcoran and Page (85), in injecting myoglobin remarked that the urine was colored one to two hours after injection. This change was never seen in our experiments. Also, since the myoglobin was in part injected intravenously as a suspension, there remained the possibility that this particulate matter might have been filtered by the lung capillaries. On the other hand, a good proportion (60 to 70%) of the myoglobin was certainly dissolved so that the effective dose would at least be at the upper end of the range calculated by Bywaters (75) and used also by Corcoran and Page (85). And in myoglobin-injected animals a post-injection polyuria was frequently observed; since the only variable was the presence of the hypertonic solution of myoglobin, this fact is best explained as an osmotic diuresis. That is, the easily filtered myoglobin molecules held water in the tubular fluid to result in an increased urine flow. It is reasonable to conclude therefore that myoglobin in adequate

dosage passed through the kidneys.

Because the clinical picture of acute tubular necrosis includes a very apparent oliguria to anuria, this decreased urine output was thought to be a good standard of measurement in the experimental production of the syndrome. It soon became obvious, however, that not only was it difficult to measure urinary output accurately enough to distinguish dehydration oliguria from that of renal failure, but also the period of oliguria to anuria in experimental acute renal failure was so abbreviated that its observation was barely significant. Fortunately, a new standard was available which was, strangely, exactly the opposite of oliguria. Polyuria was observed to be a striking, immediate and consistent response to the trauma of limb ligation. This diuretic response was most marked in normally hydrated animals but was present also in dehydrated; it became apparent during the five hour ligation period and was continued for as long as 72 hours following ligation release. In dehydrated animals, though a comparative polyuria was present, the marked diuresis became very apparent when these animals were allowed free water; they drank excessive quantities and excreted similarly excessive quantities of urine. Anuria was a feature of only a few hours duration in those animals which died as a result of the trauma and kidney damage.

This diuretic response to trauma has been mentioned seldom in the literature. Eggleton et al (126) points out that

nephrotoxins inhibit water and chloride reabsorption to produce a polyuria at first, but adds that this phenomenon is not observed in the crush syndrome in dogs. Block et al (41) reported that polyuria was a striking feature in dogs following a hypotensive period. These workers suggest an explanation which has long been used in the polyuric phase of chronic glomerulo-nephritis -- decreased functioning renal tissue requires that remaining nephrons eliminate the necessary nitrogenous wastes by increasing the volume of urine. This explanation may account for the late polyuria, but another mechanism must be responsible for the immediate diuresis observed. It seems plausible that only changes in renal hemodynamics and, thereby, changes in glomerular filtration, can account for this immediate response to limb ligation. Generalized renal hyperemia or relative efferent arteriolar constriction can only be suggested, not proven, by this investigation. A third possibility may also be considered: hemodilution. Hemodilution was observed frequently in ligated animals but was usually associated with haemorrhage from bitten limbs. It was, however, also observed occasionally in animals which showed no sign of external haemorrhage and in those in which gastro-intestinal haemorrhage and hematuria were observed. It has been observed (81A) that in producing severe shock in rats by pinching the intestine in several places for short periods, marked and rapidly developing hemodilution appeared.

Perhaps the most probable explanation of the later polyuria is one which accounts for the diuretic phase of human acute tubular necrosis, that of tubular damage to the extent that normal water reabsorption is inhibited. Surely anoxic tubular damage can be such that the normal reabsorptive mechanism is disrupted, just as mercury salts can be used either as diuretics or as poisons producing tubular necrosis and anuria. In any case, this diuretic response to trauma in rats is a consistent observation and can be used as a definite indication of renal dysfunction which does not necessarily indicate recovery but is only one stage in the reaction of a damaged kidney.

In those animals which showed evidence of renal dysfunction either by polyuria, anuria or uremia, histological changes were exclusively in the kidney tubules. The intracapsular granular eosiniphilic granular debris and cubical metaplasia of capsular epithelium were not observed. An occasional glomerulus was observed, however, in which the capsular space appeared to be dilated in such a way as to incorporate the upper extremity of the proximal convoluted tubule, giving the appearance of cubical metaplasia of the capsular epithelium (Figure 42 and Figure 43, which shows a less obvious case of the same phenomenon). This change occurred in control and test animals alike and it is interesting to note that a similar though apparently true cubical metaplasia has been described as an action of DCA (333).

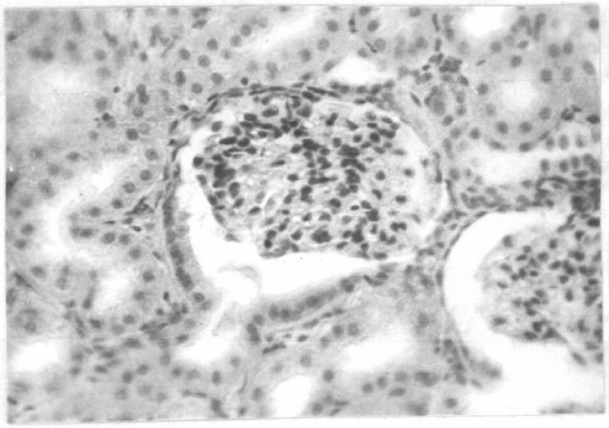

Figure 42

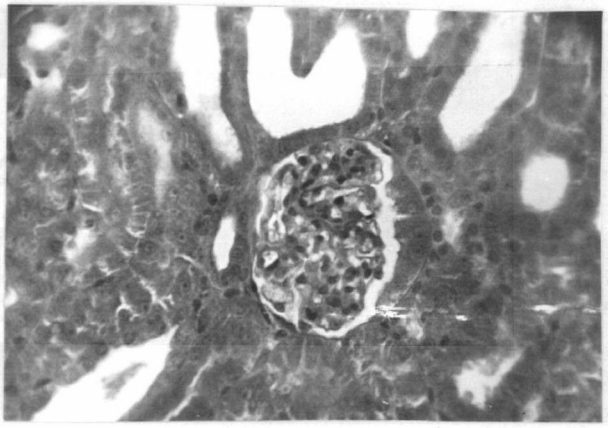

Figure 43

Tubular changes could be recognized at two stages. In animals which died in obvious acute renal failure, tubular cells showed changes ranging from early degeneration to necrosis. Cytoplasm became granular and vacuolated, nuclei swollen, pale and vacuolated and in severe cases, nuclei progressed to the small, dark pyknotic stage of degeneration. In all cases the basement membrane appeared to remain intact (See Figures 31, 32, 34, etc.).

In those animals which showed signs of renal dysfunction -- polyuria and uremia -- but went on to recovery, kidney histology was amazingly normal. However, consistently in these cases there were seen focal areas of bluish, granular degeneration of tubules with desquamation of these cells to form casts, and evidence of regenerating tubular epithelium. In addition, medullary tubules showed occasional casts of epithelial debris including pyknotic nuclei. Such kidneys were taken to be

illustrations of recovered, healing and regenerating phases of the process. (See figures 38 - 40).

The localization of these tubular lesions within the nephron is interesting but not essential. It was first emphasized in the literature that the distal convolution was the involved segment, hence Lucke's (213) term Lower Nephron Nephrosis. Later reports (146) observed degenerative changes often more advanced in the proximal tubule and eventually Oliver et al (271) pointed out that the essential lesion of acute tubular necrosis ("tubulorhexis") could in fact be located at any point in the nephron. Nevertheless, it appears that kidneys of animals subjected to haemorrhagic shock or crush injury more often develop lesions in the lower nephron (59, 60, 88), while those subjected to renal artery occlusion show proximal tubule lesions (194, 195). In the experiments reported herein, in which rats were subjected to the stress of crush and dehydration, the site of the lesion was consistently the distal tubule, chiefly in Zone 3 of the kidney but also (though to a lesser extent) in the cortex. The presence or absence of the brush border in proximal tubules can be used as a very fine index of damage to that unit and in the kidneys examined this structure was consistently present (Figure 44).

There was one exception to this statement in an experiment which was discarded because of a high incidence of chronic

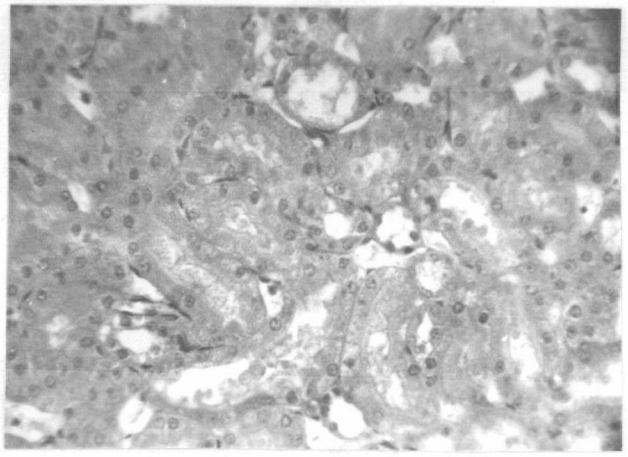

Figure 44

kidney disease in the rats used. In three of four discarded animals which had been subjected only to 72 hours dehydration, proximal tubules showed a high degree of so-called hydropic degeneration (Figures 45 and 46).

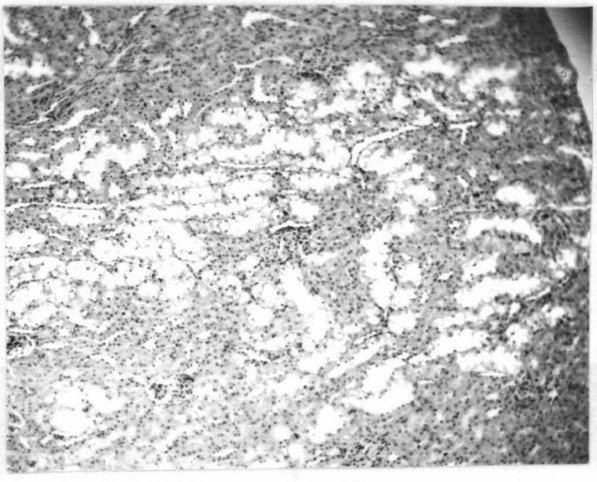

Figure 45

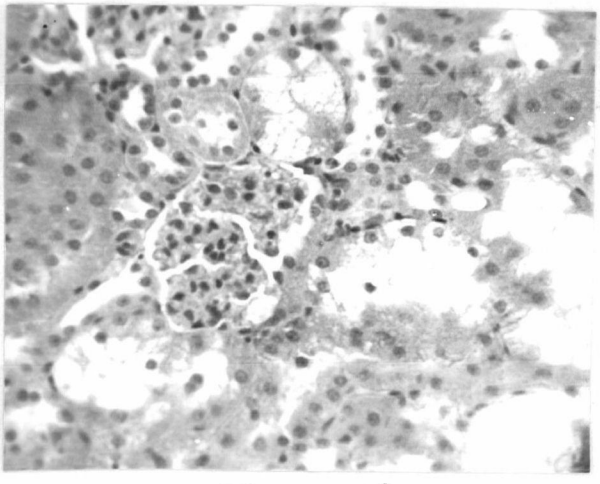

Figure 46

Chronic renal disease was encountered relatively frequently in animals used. Hydronephrosis (Figure 47) and chronic inflammatory changes (Figures 48 and 49) were the chief diseases seen. In one case, this last change appeared to

account for an elevation of the blood urea nitrogen. Almost consistently the hydronephrotic change occurred only in the right kidney, which was of course removed prior to experimentation so that it was felt that this did not interfere with observations. In any case, it is unlikely that chronic disease would interfere with acute experiments such as were carried out.

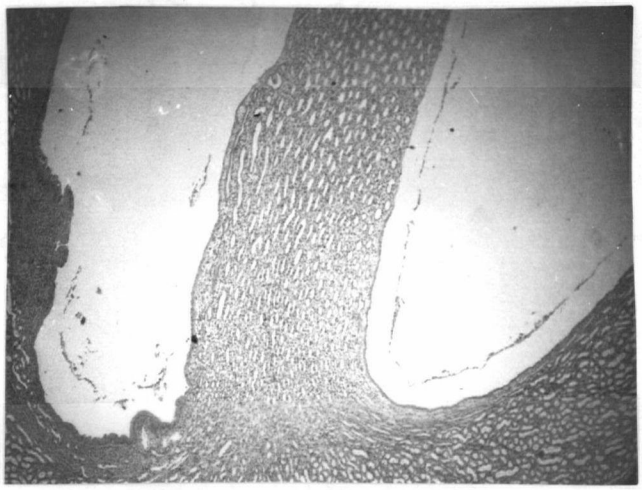

Figure 47

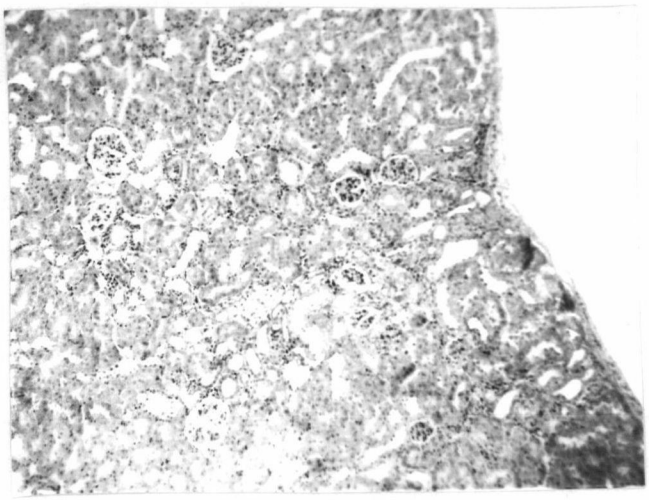

Figure 48

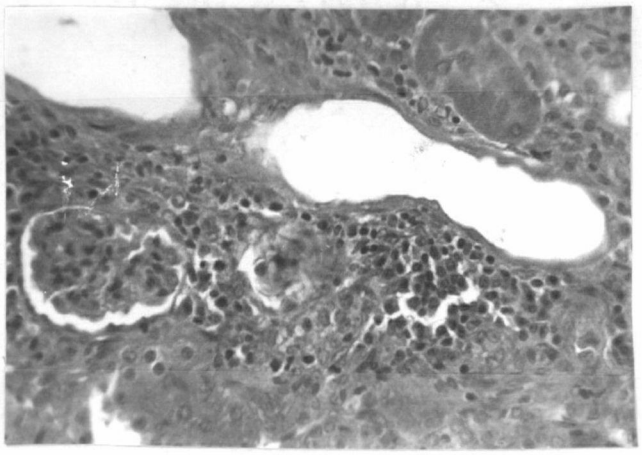

Figure 49

The similarities between early (up to seven hours) postmortem change and acute tubular necrosis due to crush and dehydration have already been referred to. It was noted that the distal tubules quickly and selectively showed degenerative changes appearing very slightly at two hours postmortem (Figures 51, 52 and 53) and markedly by four hours (Figures 54, 55 and 56). Medullary, glomerular and proximal tubule changes occurred only at an advanced stage (Figures 57, 58, 59, 60, 61 and 62). The postmortem distal tubule degeneration was very noticeable in the cortical region as well (Figure 50).

Two of the three classical responses to stress were observed frequently, that of gastro-intestinal haemorrhage and enlarged, brown adrenal glands. The gastro-intestinal haemorrhage was often accompanied by hematuria and a secondary anemia

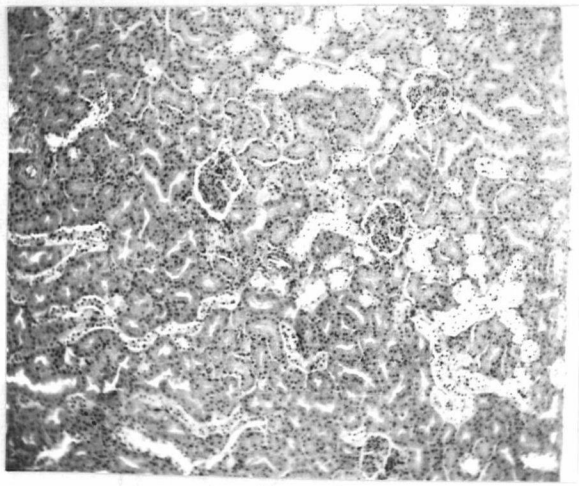

Figure 50

which may have contributed to renal ischemia.

The problem of increased mortality to 72 hours of dehydration and five hours limb ligation encountered in later experiments was a baffling one. Referring to Experiment 10, it will be seen that three of eight animals (37%) subjected to right nephrectomy, dehydration for 72 hours and ligation of left hind limb for five hours died spontaneously within a 72 hour period. In subsequent treatment experiments with female animals this mortality was increased to about 80% and maintained at approximately this figure in male animals also used in

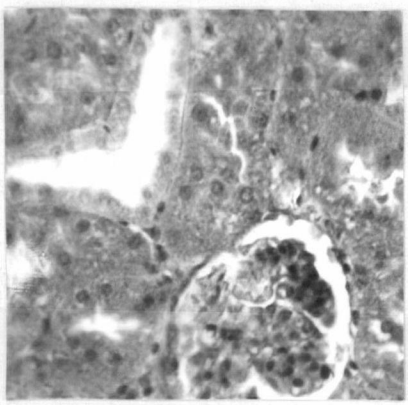

Figure 51

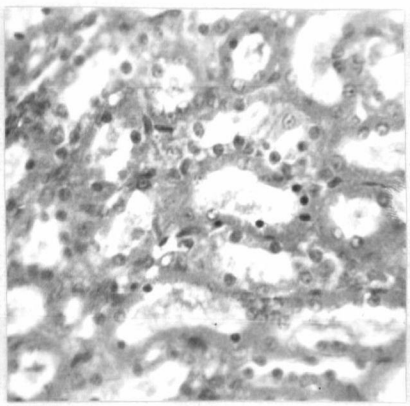

Figure 52

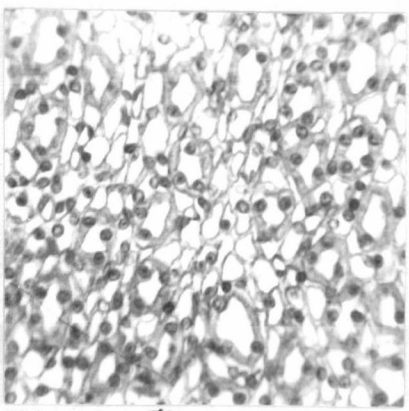

Figure 53

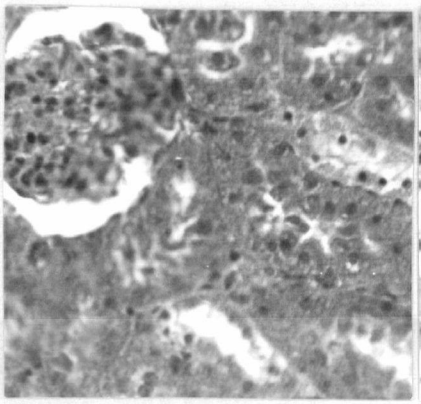

Figure 54

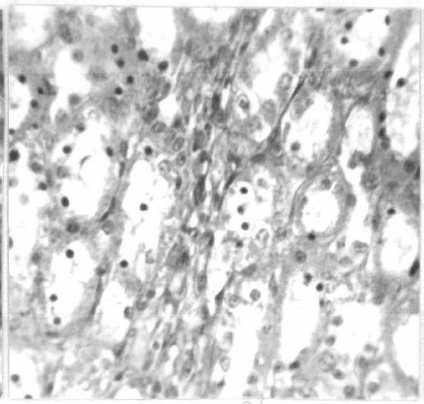

Figure 55

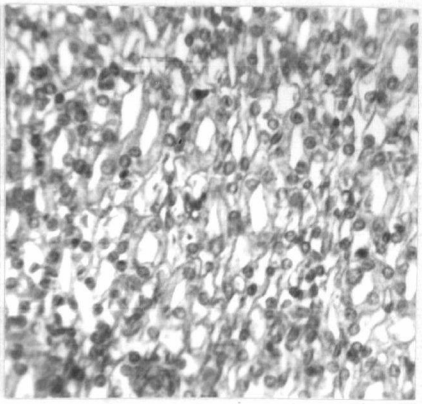

Figure 56

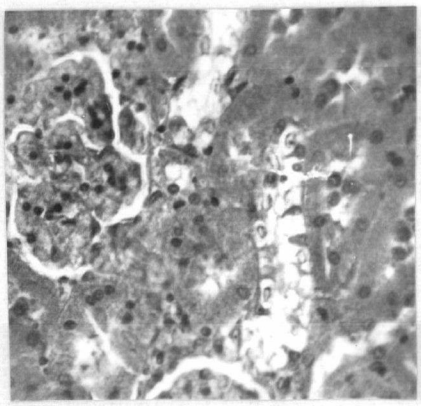

Figure 57

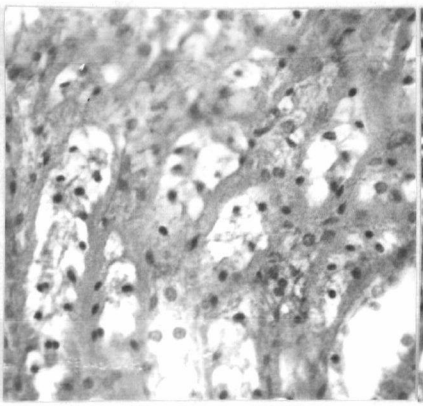

Figure 58

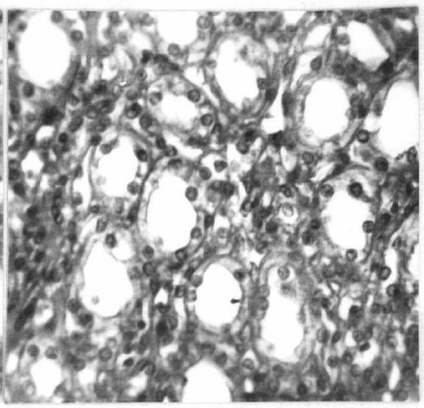

Figure 59

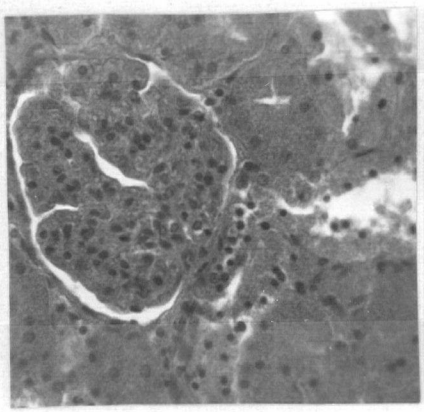

Figure 60

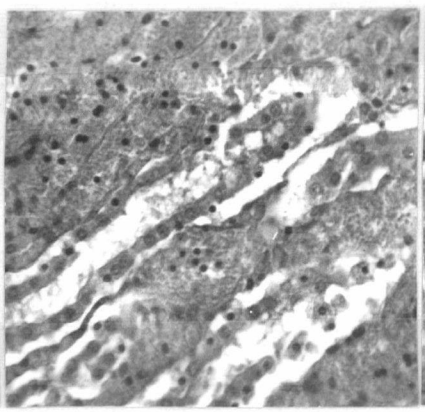

Figure 61

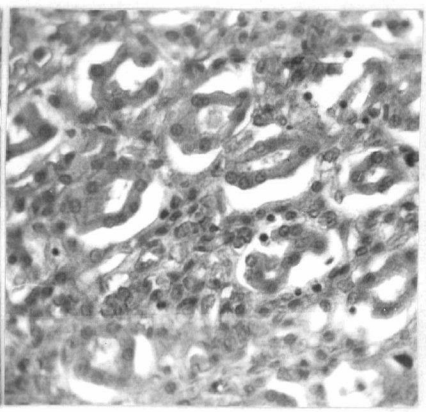

Figure 62

hormonal experiments. In Experiment 14, not only did 100 % of control animals die, but they did so within 24 hours of the crush injury so that the problem of shock deaths is amplified. This problem has been discussed earlier.

Two possibilities can be considered as accounting for this phenomenon. The tension of the string ligature could not be absolutely standardized and with later experiments this was undoubtedly tighter. However, the variation must have indeed been slight and in any case it seems unlikely that simply tightness of occlusion could affect the degree of systemic shock since the mass of tissue damaged and the duration of ligation were the same in all cases. The second apparent factor involved is the time of year -- i.e., during the course of these experiments winter had become spring and summer and the environment was noticeably warmer and more humid. This is a vague but not an unusual effective factor in altering experimental observations and here may have reduced the resistance of the animals by altering their water balance. Interesting is the fact that the increase in mortality was a gradual one. Hamilton, Phillips and Hiller (166) have referred to an increased mortality rate in dogs subjected to renal artery ligation for varying periods when environmental temperature and humidity were increased.

It should be unnecessary to point out that as many

factors as possible were kept constant throughout these experiments. Differences in strain of rat, body weight, sex, duration of ligation, mass of tissue involved or sedatives used could not account for the change in mortality rate.

Results of treatment experiments were not encouraging. It can only be concluded from the observations made that testosterone propionate, desoxycorticosterone acetate, cortisone acetate and Compound F are of no value as therapeutic agents in acute tubular necrosis. In the case of Compound F. some promise was shown in Experiment 17A so that some slight reservations about this agent are held and further experimentation is justified.

Testosterone was used in this work in the hope that its "renotropic" action might lessen the damage induced in the kidney or hasten its recovery; its effect on protein metabolism (a sort of protein-sparing action in the usual stress breakdown of body protein) might also reduce the intrinsic production of nitrogenous wastes. Homer Smith (333) states that testosterone increases the hypertrophy of the remaining kidney after unilateral nephrectomy and this growth is localized in the tubules. The hormone also appears to afford some protection against mercury bichloride poisoning (333). Although it has been shown (333) in the dog that 100 mgs. testosterone per day produces a rapid rise in Tm_D, corresponding doses in man (90 to 300 mg. per day) produce no increase in glomerular filtration rate, renal plasma

flow, Tm_{PAH} or Tm_{G}. Smith also points out that the hormone has been used in the treatment of chronic nephritis and in cholera, in which "better survival" with relief of oliguria and uremia and decrease in albuminuria were reported. In our experiments, the renal hypertrophy was apparent, especially in Experiment 11A, but no reduction in uremia or mortality rate was observed.

Disturbed electrolyte balance, which accompanies acute renal failure, has frequently been named as the cause of death in these cases. In particular, an elevated blood potassium level is said to result in death by cardiac arrest (181). For this reason, DCA would seem to be a useful therapeutic agent. As a mineralocorticoid, it is known to promote sodium and water retention and potassium excretion so that plasma sodium increases while plasma potassium decreases (325). This action of DCA is thought to be a direct action on the renal (distal?) tubules to promote sodium reabsorption (333) but also on the capillary permeability and tissue affinity for water and electrolytes (325). In dogs, DCA has been shown (333) to expand the extracellular fluid space at the expense of the intracellular, to increase the glomerular filtration rate and renal plasma flow and to increase Tm_{PAH}. Plasma potassium concentration is initially decreased. However, whatever the mechanism of death in the rats in our experiments, DCA did not prolong their lives or lessen their uremia. Hoff et al (181) found

that although in surgical anuria (bilateral ureteral ligation or bilateral nephrectomy) the elevation of serum potassium is such that cardiac damage is the cause of death, in mercuric chloride anuria and chronic nephritis, potassium levels do not rise to a fatal level and electrocardiographic changes of potassium intoxication are not seen at death. This work would appear to minimize the role of potassium retention in deaths in acute renal failure and might also explain the failure of DCA to prolong life in rats suffering from the syndrome.

In using cortisone (17 hydroxy - 11 dehydrocorticosterone, Compound E) as a therapeutic agent in the traumatic anuria syndrome (23) it was hoped that the hormone would lessen the mortality by counteracting the shock of the early phase of the alarm reaction (325, 326) seen in response to limb ligation. Selye (326) found that "cortin" was highly effective in this regard, reporting that DCA alone had little effect. Weil et al (358) reported similar findings in rabbits. Ingle (185) on the other hand found that neither DCA, cortisone nor adrenal cortical extract reduced the mortality rate of rats subjected to bilateral hind limb ligation. Ingle (186) reviews the biologic properties of cortisone, pointing out that it can no longer be thought of as a simple glucocorticoid. Its effect on electrolyte and water balance is variable but in acute experiments in rats an increased excretion of sodium, chloride and potassium lasting one to three days has been reported. Cortisone has been reported to maintain adequate circulation in

adrenalectomied dogs subjected to trauma or hemorrhage; to maintain renal function in adrenalectomy; and to increase the PAH secretion in normal males by up to 35% (186, 324). In high doses and prolonged administration it produces hyalinization of glomerular capillaries, hypertension and elevation of plasma chloride and potassium (186, 324, 144, 23).

It can be seen from Experiments 13 and 14 that this hormone was of no value in the alleviation of the renal effects of shock from limb ligation. Life was not prolonged and mortality rate and uremia were not diminished. The combination of testosterone and cortisone (Experiments 15 and 16) also showed no therapeutic effect.

Compound F (17 hydroxycorticosterone - 21 - acetate) was also used as a therapeutic agent, since it has been shown, chiefly clinically, to be of value where cortisone is ineffective. Compound F is in fact very similar to cortisone in chemical composition (325) and in action (287). It is said to be less active in salt and water metabolism, having little influence on these; it induces a slight negative nitrogen balance and like cortisone it also has a hypertensive effect in rats and increases kidney mass (145) Though in Experiment 17A, Compound F appeared to be of some considerable value, this phenomenon was not observed in Experiment 17B. Nevertheless it is apparent that further experimentation with Compound F would be advisable.

In considering the failure of cortisone or Compound F to be of benefit to rats in acute renal failure it is perhaps worthy of note that Selye (326) points out that adrenal cortical hormones in shock are more effective given in divided doses, and that pre-treatment is useless and may be harmful. Pre-treatment may well depress the normal adrenal cortical activity. It is very unlikely that the dosage used in these experiments was sufficiently high or prolonged to produce any of the nephrotoxic actions referred to by Selye (324).

SUMMARY

A brief review of the literature on traumatic anuria (acute tubular necrosis, lower nephron nephrosis) has been presented, including a complete bibliography. Special attention was paid to the pathology and pathogenesis of the syndrome, and it was concluded that Oliver's recent work (271) probably comes closest to presenting the true picture. He described tubular necrotic lesions for which the chemical toxins (mercuric chloride, carbon tetrachloride) were responsible, and tubulorhectic lesions which were characteristic of the shock kidney. These lesions could appear at any level in the renal tubule and were characterized by destruction of the basement membrane. Pigment casts were apparent if intravascular pigment release was associated with the illness. The work of Phillips, Van Slyke and associates (291, 292, 355, 356), of Oliver (271) and of Block et al (41) lead one to conclude that renal ischemia is the chief pathogenetic mechanism, though it is obvious that specific extrinsic renal toxins play a major role in specific cases. The role of hemoglobin appears to be chiefly in the production of obstructive casts later in the course of the disease; these pigments are precipitated in the lower nephron where urine is concentrated and acidified, and dehydration and oliguria contribute to their formation.

Three hundred rats were studied in eighteen experiments concerning crush syndrome. It was concluded that the most important single factor tending to aggravate the renal effects of crushing injury is the antecedent state of dehydration. Myoglobin is not an essential factor in the development of renal damage but tends to aggravate the existing uremia. Acute renal failure was seen to be a late effect of shock; animals developed acute tubular necrosis only if initial shock was severe, but not severe enough to produce death from circulatory failure. Development of this delicate balance of factors was aided by reduction of renal reserve by unilateral nephrectomy. A seldom described but distinct and consistent phenomenon was observed in the development of marked, immediate and persistent diuresis in response to the trauma of limb ligation. This polyuria was of a dilute urine and was taken as an indication of initial increased glomerular filtration followed by decreased reabsorption of water because of tubular damage. It was not an indication of a recovery phase as is recorded in the clinical syndrome.

Testosterone propionate, desoxycorticosterone acetate, cortisone acetate and Compound F did not appear to be promising as therapeutic agents, although in one experiment Compound F showed some promise. Neither did combined therapy with testosterone and cortisone reduce the mortality rate or decrease uremia.

Although there was no doubt that the syndrome of acute renal failure due to acute tubular necrosis could be produced in large numbers of these relatively inexpensive laboratory animals by dehydration and limb ligation, production could not altogether be standardized and the syndrome ran such a short course that serial observations were difficult to obtain and separation of shock deaths was occasionally impossible. It is felt that future work might well make use of some other laboratory animal, perhaps the dog or cat, and that an initial stress of controlled hypotension or renal artery occlusion could be used. It is also our opinion that further investigation into the value of Compound F as a therapeutic agent in this syndrome is justified.

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