STUDIES ON THE MECHANISM OF RENAL FAILURE ASSOCIATED WITH THE RELEASE OF HAEMOGLOBIN OR RELATED PIGMENTS INTO THE BLOOD PLASMA.

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ABSTRACT

The renal lesion which is seen in certain fatal transfusion reactions, the crush syndrome, blackwater fever, haemolytic sulphonamide reactions, eclampsia, and severe burns, has been found to be of a characteristic type, invariably showing degenerative changes in tubular epithelium along with the presence of pigment casts low in the nephron. These casts were, undoubtedly, derived from the haemoglobin or allied pigments circulating in the blood plasma in all these disorders. For this reason many investigators have sought a common mechanism for the renal failure accompanying this morbid picture; some holding tubular blockage by pigment casts to be the underlying factor; some claiming that the pigments injure tubular epithelium, the casts being merely a sequel; some prefering the concept of a new or newly released substance which causes tubular degeneration, and others stating that the tubules are damaged by anaemia, low blood pressure, or peripheral circulatory collapse. A group of other workers feel that the renal failure seen in these illnesses is not related to the anatomical alterations but is a physiological uraemia. Thus, all the work resolves itself into three main theories, one of which blames the kidney failure on tubular blockage, another laying stress on tubular degeneration, discrediting the blockage theory altogether, and the third saying that neither of these holds true.

The present experimental investigation is the first work in which an attempt is made to evaluate the relative importance of tubular injury and tubular blockage by pigments, being also the first in which a method is devised to readily reproduce the characteristic lesion. The renal tubules of domestic white rabbits were first injured:-

(a) by temporary production of renal ischaemia, and

(b) by the injection of a specific chemical poison, sodium tartrate.

This injury was followed by the injection of pure haemoglobin intravenously. Histological and blood non-protein nitrogen studies were carried out. By means of control animals, and by delaying the haemoglobin injections until the animals were recovering from the initial injury, the importance of the two components was assayed.

It was found that the factor of primary importance was tubular damage for, without it, the animals were unaffected by the injection of haemoglobin and, moreover, if severe enough, it alone could cause death of the animals. However, when initial injury was not severe, haemoglobin precipitation did occur and did, under certain conditions, cause suppression of urine and progressive nitrogen retention. The factors influencing this precipitation were found to be,

(a) the pH of the urine, and

(b) an adequate amount of circulating haemoglobin.

Thus, it was shown that the final outcome in any given instance depends on a fine balance being struck between the degree of injury, the urinary pH, and the level of haemoglobinemia.

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INTRODUCTION

Failure of the kidney develops in many conditions in which the primary pathology is not renal. Among these disorders is a large group, the members of which have one outstanding feature in common, namely, the presence of haemoglobin or haemoglobin-like compounds circulating in the blood plasma. That they vary considerably in their fundamental character is evident, yet the combination of renal failure and haemoglobinaemia compels one to group them together and to look for a common mechanism to explain the renal failure. It seems quite logical to assume that the two features are causally related: that is to say, that in some way or other, it is the pigments that disturb renal function sufficiently to cause a suppression of urine and death in uraemia; the greater part of the investigation in this problem has been undertaken along these lines. However, it is possible that the very factor which causes the pigment to be set free also brings about the changes which result in failure of the kidney; or it may be that the actual phenomenon of breakdown of red blood cells is in itself sufficient disturbance to adversely upset the equilibrium of the kidney. Again, it may be held that the renal failure is absolutely independent of the presence of pigment in the plasma, the factor that caused liberation of this pigment, or the process of liberation itself, and is due entirely to the pre-existing disturbance. Such a concept is supported by the occurrence of renal failure in many instances in which pigment is never seen. Despite the work of many observers, the mechanism of the renal failure remains a mystery.

The work to be described in this thesis was designed first to reproduce the structural changes seen in the kidneys in these disorders, and then to study the functional disturbances associated

with this picture in an attempt to throw some light on the manner in which the morbid anatomy is produced and on the factors which influence its production.

It is first necessary to examine each of the conditions characterized by haemoglobinemia and renal failure in order to establish their common features and determine whether or not these features are sufficiently alike to warrant the search for a single explanation suitable for all. Having accomplished this, it will then be of value to review the experiments and ideas of those who strove for such an explanation.

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PART 1: REVIEW OF THE LITERATURE

THE RELEVANT CONDITIONS

Haemoglobinemia or the presence of haemoglobin-like compounds, is associated with uraemic manifestations in many post transfusion reactions, in the 'crush syndrome', in 'blackwater fever', in haemolytic crises following administration of sulphonamide drugs, in 'eclampsia', and in the haemolysis following severe burns.

Transfusion Reactions

The first record of unfavorable reaction to the administration of blood was made in 1667 by Denys[33] who gave sheep erythrocytes to man, and until the beginning of the twentieth century, the hazard with which the transfusion of blood was accompanied, made its universal use impossible. The work of Landsteiner,[62] Jansky[60] and Moss[78] by demonstrating the different agglutinogens and agglutinins of normal human blood, and by establishment of the four blood types, made possible the wide therapeutic use of whole blood which began during World War I. Despite the use of blood typing and cross-matching, however, reactions to blood transfusion continued to occur. Reports of such reactions dot the literature up to the present day. The causes of these untoward responses are discussed by many[81,96, 85,27,87,8,79,46,12,28,55,11] and the types of reaction are well classified by Ham[55]. Review of this work shows that the most important cause of reactions is the presence or development of some incompatibility, be it the formation of haemolysins,[81,27] minor or abnormal agglutinins,[8,79] or the presence of Rh agglutinins[11]. This incompatibility commonly manifests itself in the most important type of reaction, namely, haemolysis of donor cells with haemoglobinemia, haemoglobinuria, and, later, a progressive renal insufficiency with death in uraemia. Many of the deaths from blood

transfusion (variously estimated at 0.01 to 0.2%[29] and 0.2%[30] of all transfusions) are of this nature.

What is the common clinical course in a patient who receives incompatible blood? De Gowin[30] discussed all types of grave sequelae from a clinical standpoint. Bordley[12] reported 17 cases in which there was a haemolytic reaction and associated renal disturbance. He described an immediate reaction with chills, fever, back pain and vomiting. This is an allergic type of response, and in fact death may be immediate, and due to allergic shock. However, this is rare and, usually, the early symptoms persist for about two hours and are followed by the passage of dark, smoky urine; occasionally jaundice is seen. The initial stage is followed by an interval of one to seven days during which time there is apparent improvement, but persistent oliguria and a rising non-protein nitrogen level in the blood oracle the storm to come. The delayed reaction begins with agitation, drowsiness and soon all the symptoms of uraemia become manifest. In people who recover the peak is reached eight to twelve days after the transfusion and recovery is usually sudden, beginning with marked diuresis. Fatal cases reach the peak on the fourth to the eighteenth day.

The kidneys in the fatal reactions have been described by Bordley[12], by Daniels et al[26], by De Gowin et al[31], by Ayer and Gauld[4] and by numerous other investigators. When death occurs in the first few days, only minor structural abnormalities, consisting of glomerular epithelial swelling, protein material in Bowman's capsule, and cloudy swelling of the proximal convoluted tubules, can be seen. Along with these, the descending and ascending limbs of Henle's loop show small droplets of pale brown to brick red pigment inside epithelial cells. Mild degenerative changes are

noted in the distal convoluted and collecting tubules, the epithelium of which also contains pigment droplets, and the lumina of many show the same pigment in granular masses or condensed to form casts. If death has taken place after an interval of about one week a modified picture is seen. The distal convoluted and collecting tubules are the seat of a severe degenerative process, frequently exhibiting complete necrosis, while in the interstitial tissue oedema, cellular proliferation and leukocytic infiltration are seen. Polymorphonuclear leukocytes may invade the necrotic tubules, and often their identity is hard to make out. Pigment casts are often abundant, often scarce. They are sometimes coarsely granular, or may be well compacted. They were first described by Ponfik[86] and have been considered the outstanding feature of the transfusion kidney, their peculiar staining properties (negative iron reaction, color in haematoxylin-eosin) strongly indicating that the pigment is haemoglobin or one of its derivatives.

Ayer and Gauld[4] decided that the only progressive renal changes are the interstitial oedema and infiltration, and the tubular necrosis. They admit the possibility that the amount of cast formation may depend on the amount of blood injected, but state that the casts do not increase as time goes on. It is of interest to note that Bordley[12] observed that "except for the occurrence of the pigment, the changes are much like those found after poisoning with mercuric chloride".

Thus, in an individual who suffers such a transfusion reaction, there appear to be certain cardinal points which are worthy of note. First, there is the debilitation which necessitated transfusion. Next, the disturbance attendant on the allergic type of reaction, and on the phenomenon of haemolysis. Third, the presence

of haemoglobinemia and haemoglobinuria, and, last, failure of the kidney associated with a characteristic lesion in which the presence of haemoglobin casts is a feature.

The Crush Syndrome

The bombing of London in the early days of World War II caused attention to be focused on a "new" disease. The British Medical Journal in 1941 carried three papers[7,72,20], two of which [7,72] were case reports and the third[20] a discussion of the clinical and pathological findings in such cases. It was noticed that people who were buried under debris and suffered a crushing injury with the crushed member under compression seemed quite well for a while after release and then gradually developed renal failure and died. It was thought that such a condition must have been seen before, but at first no report could be found in the literature. Later, those interested in the disease found that the ischaemic muscle necrosis seen here was described by Frankenthal[49] in soldiers buried after mine explosions, and Minami's article[75] states that in 1917 Hockradt was the first to describe structural alterations in the kidney. It seems then that this condition was first noticed by the Germans in World War I, but disappeared from the "public eye" in the interval between wars.

The general clinical pattern is always the same. The affected limb shows swelling due to loss of plasma into the tissues. The patient at first feels relatively well, but there is a haemo-concentration, the blood pressure falls, and the limb becomes cold. Oscillometric readings prove the ischaemia in the injured member, and this is thought to be the result of tension interference with venous return and/or arterial spasm. The first urine is acid in reaction and contains a brown sediment of acid hematin, but soon

pigment excretion ceases, and is replaced by the presence of urinary casts. The urine volume becomes markedly decreased exhibiting the composition of a glomerular filtrate, evidence of severe tubular injury. Because of damage to the muscles, there is a rise in serum potassium and creatinine, increase in the former being augmented by kidney damage. Symptoms of renal collapse unfold concomitantly and death is usually sudden about the sixth day. The clinical picture is described in detail by Bywaters[21].

Autopsy in these cases discloses remarkable kidney alterations. These have been carefully described by Bywaters and Dible[22] and by Dunn et al[35]. The glomeruli are not abnormal, except that the capsular spaces often contain granular eosinophilic debris. The proximal convoluted tubules are the seat of a degenerative process which varies in intensity from slight swelling, granularity and vacuolization to intense catarrhal inflammation with debris-filled lumina. Many epithelial cells may be cast off, but the lumen may also contain cell debris and precipitated protein. These changes are seen in diminished severity in the descending limb of Henle's loop. Dunn et al[35] observed no degeneration in proximal convoluted tubules and it is, therefore, likely that this is an early occurrence. The ascending limb of Henle's loop and the distal convoluted tubules suffer severe degeneration and often complete necrosis in certain areas. Later, dilatation, basophilia and more intense nuclear staining are taken to correspond to changes seen in regeneration of tubules after mild experimental tubular nephritis[35]. The damage to tubular epithelium is soon associated with histiocytic proliferation in and about the tubules, the interstitial tissue, which shows oedema, also becomes more cellular. Hyaline casts are seen in these tubules, and in collecting tubules, but the important casts are those

composed of pigment. These are granular masses, or clear strands. They belong to the "heme" group of compounds and Bywaters and Dible[22] assume that they are myohaemoglobin or a derivative of it. They are found chiefly in distal convoluted and collecting tubules, and to a lesser extent in the ascending limb of Henle's loop. In the collecting tubules they are considerably compacted and condensed and appear as dense brown masses. Many casts appear to be partly made up of desquamated epithelial cells, and in later stages cast containing tubules are often surrounded by, and infiltrated with, polymorphonuclear leukocytes.

This type of picture is often seen in other conditions of muscle damage such as meningitis with wasting, and septic abortion; Young[102] noted it in cases of retroplacental haemorrhage with massive uterine muscular damage.

The cardinal points in "crush kidney syndrome" or "traumatic anuria" are first the shock caused by the crushing - lowered blood pressure and haemoconcentration - second, the presence of potassium, creatinine and muscle haemoglobin in the circulation, and last, the characteristic renal lesion which appears to be identical with that in fatal transfusion reactions. In fact, in the early cases, the resemblance was so suspicious that Mayan-White and Solandt[72] reported a case in which no transfusion was given and showed that reactions played no part in the picture.

Blackwater Fever

It has long been realized that haemoglobinuria is a common complication of malaria. The urine is often so strongly tinged that the name 'blackwater fever' has been given to this condition and while it is known that chronic malaria certainly, and large doses of quinine almost certainly, are precipitating factors, why these

factors should produce haemolysis in some instances, and not in others, remains unexplained[95]. When massive haemoglobinuria occurs it is accompanied by a progressive collapse of renal function. The necessity of sending fighting men to the tropics has made malaria, and hence blackwater fever, a problem of special interest in these times.

Clinically, the attack begins with fatigue. Rigors soon become prominent, they are followed by vomiting, and then haemoglobinuria is seen. Pallor, sweating and a thready pulse are associated with shock due to the blood loss. Suppression of urine and impairment of kidney function supervenes, and the malady may terminate in recovery or in uraemic death.

The kidneys are the seat of well marked structural abnormality. Mild degenerative changes are seen in proximal convoluted tubules, with severe alteration in distal convoluted and collecting channels. Pigment casts are seen here and these are surely haemoglobin or its derivative. (The blood pigment in blackwater fever urine is largely methaemoglobin[42].) The pathology is discussed by Price[88], by Lindau[64] and by Maigraith and Findlay[67]. They direct attention to the uniformity of the pathology of transfusion reactions and blackwater fever.

Accordingly, this infirmity shows primary injury from malarial infection and shock, and then haemoglobinuria and renal failure associated with a characteristic lesion of the kidney.

Haemolytic Sulphonamide Reactions

Acute haemolytic anaemia is one of the many complications which accompany administration of sulphonamide drugs. Fox and Ottenberg [47] point out that methaemoglobin is found in the erythrocytes of all patients given sulphonamides and conclude that the drugs pro-

duce oxidizing agents. These do not produce haemolysis because only a few people given sulphonamides show haemolysis. But it is possible, they say, that a special oxidizing agent is produced in some individuals, and these affect red blood cells which contain methaemoglobin. They mention drug allergy as a cause, but recall that there are no reports of haemolysis from drug sensitiveness.

Many cases of haemolytic anaemia have been reported, but massive haemoglobinemia is rare[74] and its association with renal insufficiency is not frequent in the literature. The first report of a fatal case was made by Wood[100] in 1938. Other cases are described by Ravid and Chesner[89], by Meyers and Rom[74], by Koletsky[61] and by Fox and Ottenberg[47]. Most[47,100,89,61] give necropsy reports.

In most instances the haemoglobin concentration falls quickly 24 to 72 hours after therapy is begun. The patient suffers the symptoms of shock, and passes large amounts of pigment in the urine. The haemoglobin derivatives in the serum are haemoglobin, methaemoglobin and methaemalbumin[47]. The urine volume diminishes, uraemia develops.

Examination of the organs reveals haemosiderosis of the liver and spleen, and the familiar changes in the kidneys previously described. Some of the kidneys show immense numbers of "haemoglobin" casts. Tubular degeneration is not marked. Again, a primary injury, in this instance, shock, is seen along with haemoglobin pigment in the blood and urine, renal failure and a lesion in the kidney strongly reminiscent of the transfusion kidney, the crush kidney or the kidney of blackwater fever[48].

Eclampsia

In 1920 Fahr[40] reported for the first time the presence casts in distal convoluted and collecting tubules

of the kidneys of eclamptic subjects. In 1924 he described tubular casts in 18 out of 33 fatal cases of eclampsia[41]. Acosta-Sison[1] said that convoluted tubular degeneration is part of the pathology of eclampsia, and Baird and Dunn[5] in 1933 wrote of a picture which is remarkably similar to the transfusion kidney and described haemoglobin casts in Henle's loop and in distal convoluted tubules.

Burns

Albuminuria and oliguria are constant findings in severe burns. Haemoglobinuria occurs in such burns as an early complication, but does not always take place. In 1908 Marchand[70] stated that the renal lesion in burn nephritis is similar to that produced in haemoglobinuria from other causes. Shen and Ham[94] found gross haemoglobinuria in nine, and minimal haemoglobinuria in two cases out of forty, second and third degree burns. The maximum excretion was during the first 12 to 24 hours after which it fell quickly. The urine of these patients was acid and was black, red or light brown in color. Spectroscopic examination of the serum showed oxyhaemoglobin and Fairley's[43,44,45] methaemalbumin. The urine was found to contain oxyhaemoglobin and methaemoglobin. Shen and Ham examined the kidneys of nine patients, three of which had not had haemoglobinuria, and these three showed normal kidneys, while in the other six there was a histological picture like that seen in transfusion reactions. Emphasis was placed on the fact that low urinary pH, oliguria and albuminuria, although seen in severe burns, are never associated with azotemia except when haemoglobinuria is also present, and then nitrogen retention develops in four out of five patients who live more than five days.

In severe burns, then, there is shock, haemoconcentration, and some resultant kidney damage of a mild nature. If there is

haemoglobinuria too, it is often followed by progressive renal failure, and the kidneys of those who die in uraemia show morbid changes very much like those seen in fatal transfusion reactions.

The Common Features

The foregoing paragraphs have unmistakably established a common pattern, in all these disorders. To recapitulate, the examination of each ailment discloses some primary injurious factor, be it the shock of a burn, of a crush injury, or an anaphylactoid crisis, and this is followed by a release of "heme" pigments into the circulation. Subsequently, the kidney fails, having undergone an anatomical change which results in a specific morbid picture. It has thus been shown that the search for a single explanation is justifiable. How does this type of lesion come about? What part, if any, is played by the pigments? Has the primary injurious factor any significance? Are various substances produced which cause a non-specific kidney lesion to which haemoglobin casts are added? In the search for a final common path it is well at this time to review the work of others.

THE MECHANISM OF RENAL FAILURE

Precipitation Of Pigment; Blockage

Since circulating and precipitated pigment is a constant feature, it was logical to believe that the pigment casts cause renal failure by simple mechanical blockage of tubules. In 1911 Yorke and Nauss[101] injected haemoglobin into normal rabbits, in amounts varying from 1.5 to 13 grams. At first they could obtain no results but later, after attempting to imitate blackwater fever by a dry diet and by bleeding the animals, they brought about renal failure and demonstrated casts in the distal convoluted and collecting tubules, and in Henle's loop. They concluded that precipitated

haemoglobin blocks the tubules and is the cause of anuria.

Real impetus was given the 'blockage' theory by the work of Baker and Dodds[6] in 1925. They injected carefully prepared haemoglobin into some rabbits on a normal diet, and hence with alkaline urine, and into others on a dry diet, excreting acid urine. The acid animals died showing high blood urea level and pigment casts in the urine and kidneys. The alkaline animals survived. There were no casts. In vitro experiments were performed and it was found that haemoglobin precipitated as methaemoglobin at a pH of 6.4 to 5.81. The intensity of the methaemoglobin bands reached a maximum at pH 5.42 but then diminished. This was explained by conversion of methaemoglobin to acid hematin. It was then observed that a second factor in precipitation was the presence of inorganic salts in certain concentration, and since sodium chloride was the only salt present in the kidney in required amounts (1%), they concluded that it was the important one. These findings led Baker and Dodds to postulate two factors, namely, a pH of 5 to 6 and a minimum concentration of about 1% NaCl, in order for haemoglobin to precipitate in the kidney.

These observations are substantiated by the experiments of De Gowin et al[29] who gave haemoglobin with cell stroma to dogs with acid and alkaline urine, achieving no result in alkaline animals and the typical nephropathy in most of the acid animals. They said "when the urine is acid, transfusions of haemoglobin sooner or later produce renal insufficiency". Although it was believed that tubular blockage can and does account for kidney failure, they also felt that there was still another factor besides urinary acidity which remained to be elucidated.

In 1938 Melhick et al[73] found they could develop incom- ... in dogs by repeated infusions of dog erythrocytes, and

reported pigment casts in renal tubules.

This work, then, leads one to believe that haemoglobin can precipitate in the renal tubules of, otherwise, normal but acidotic animals, and once precipitated, can cause anuria and uraemia by simple mechanical blockage. This concept of the mechanism has stirred up great controversy and most writers feel it worthy of consideration, either to refute or attempt to confirm. The reports of Mandelbaum[69], Bushby et al[19] and Dieckmann and Kramer[34] show how widely the theory was and is accepted. Morrison[77] also supports the blockage idea, offering as evidence a comparison between the kidneys in myelomatosis and in crush injuries. He says that the tremendous casts and great dilatation of tubules cannot take place in the crush kidney because it is too acute, and blockage stops any process - that is, the other tubular changes which are usually expected from blockage, cannot happen in the quickly occurring crush syndrome.

The principal objection to the obstruction theory is that very frequently not nearly enough tubules are cast filled, yet, uraemic death takes place just the same. Again, many who die in uraemia show a number of blocked tubules which is no greater than that seen in many cases that lived to die of something else. Maigraith and Findlay[67] found that in 35 cases of blackwater fever the urinary pH bore no relationship to the oliguria, and Maigraith and Harvard[68] stated that if a kidney is damaged the urine may remain acid even in the presence of an alkalosis, pointing out that when the urine could be rendered alkaline, they still could not prevent the onset of anuria. In contrast to the work of Baker and Dodds, these facts show that anuria comes to alkaline patients, often when there has been little haemolysis, and that there is frequently no anuria when there has been considerable

haemolysis in conjunction with acid urine. They also drew attention to the fact that before urinary suppression sets in, the NaCl concentration in the urine is less than 1%. The literature contains a host of refutations to the blockage theory, reasoning along similar lines. For example, one can refer to the papers of Georgopoulos[51], Bywaters and Beall[20], Dunn et al[35], and Ayer and Gauld[4].

Besides, these reasonable objections, there is a good deal of experimental evidence which casts considerable doubt on the results of Baker and Dodds.

Physiology of haemoglobin excretion - It would be well to pause at this point, and review the physiology of haemoglobin excretion. Monke and Yuile[76] showed that there is a renal threshold for haemoglobin of 100 milligrams per 100 c.c.'s of blood, excretion above this level being proportional to the plasma concentration, up to thirteen times the threshold value. The volume of plasma cleared of haemoglobin in one minute was discovered to be 1.9% of the volume cleared of creatinine. They suggested that in each hundred glomerular pores, three permit the passage of haemoglobin, since of all the plasma filtered, 3% lost its haemoglobin. It was learned that the tubules recover 2 milligrams of haemoglobin per minute and if a greater amount comes through, haemoglobinuria is seen. In another paper, Yuile et al[103] established the possibility of lowering the threshold by repeated injections of haemoglobin, the fall due to a decrease in the maximum rate of tubular re-absorption. The demonstration that above the new threshold the rate of haemoglobin excretion paralleled the initial rate, was proof of this fact. Using radio-active iron these investigators found that in spite of a higher estimated re-absorption rate, normal kidneys re-

tained less iron than did low threshold kidneys, and to account for this phenomenon they suggest that there must be a corresponding drop in the rate of removal of haemoglobin from the tubules in these low threshold kidneys.

Yuile and Clark[104] investigated the renal clearance of myohaemoglobin and found a threshold value of 20 milligrams per 100 c.c.'s of plasma, above which level excretion rate is directly proportional to plasma concentration. The rate of myohaemoglobin excretion was shown to be twenty-five times as great as the speed of haemoglobin elimination. The smaller size of the myohaemoglobin molecule accounts for the difference in rate of glomerular filtration. The whole problem of haemoglobinuria is reviewed by Yuile in a later publication[105].

It is thus clear that haemoglobin and myohaemoglobin are excreted by the normal kidney under certain definite laws which fit the modern concept of renal function.

Sellards and Minot[94] in 1916 and Gilligan et al[52] in 1941, injected haemoglobin into man with no ill effect. Hueper[58] gave haemoglobin to dogs; he obtained no casts. Individuals with paroxysmal haemoglobinuria do not suffer renal impairment. In fact, De Navasquez[32] caused severe haemoglobinuria in such a patient, having first rendered him acid by the administration of 30 grains of ammonium chloride. The urine showed casts and pigments which spectroscopic examination proved to be oxy- and methaemoglobin, yet the patient developed no untoward reaction. Again, "March haemoglobinuria"[53,80] is a benign condition.

What, then, causes haemoglobin to precipitate in renal tubules? Can it really be precipitated in acidotic, but otherwise normal, experimental animals? De Navasquez[32] gave repeated injections of .69 to 1.0 grams of haemoglobin to acid and alkaline

rabbits, and could not produce anuria, or any abnormal histology. Bing[9,10] injected pure haemoglobin, and could find no change in acid or alkaline animals. Myohaemoglobin, too, was innocuous, even in the acid state. Methaemoglobin, however, when given to an acid animal, caused anuria and death, the kidneys showing the changes seen in the crush syndrome, but there were very few pigment casts.

Injury to Tubular Epithelium; The New Factor

Pure haemoglobin or myoglobin cannot be made to precipitate experimentally in a healthy subject. Where casts are seen it is usually very doubtful that they caused serious harm. Since haemoglobin and myoglobin are innocuous, while methaemoglobin gave rise to death with few casts, there must be some other factor in the methaemoglobin which causes a death independent of any tubular blockage. York and Nauss[101] and Melnick et al[73] did not have normal animals. De Gowin[29] injected stroma with his haemoglobin but the findings of Baker and Dodds are hard to reconcile with those described above.

In the light of this knowledge a new factor must be postulated but it is not necessary that this factor should always be associated with the presence of methaemoglobin even though this pigment is often identified spectroscopically in the illnesses under discussion. De Gowin, Warner and Randall[31] state that in the earlier experiments[29] dogs #23 and 24 received the same doses of the same solution of haemoglobin in the same hour. Both died in 7 days. The kidney of dog #23 showed moderate tubular necrosis and practically no obstruction by casts while dog #24 showed minimal necrosis and extensive obstruction. Human cases showed the same variations. They concluded that there is another independent factor which causes tubular necrosis and may be severe enough to cause death. Warner[98] reiterates this concept of two independent

mechanisms working in combination. He believes that this second factor is a nephrotonic substance which produces tubular damage. Ravid and Chesner[89] discussing the kidney of sulphonamide haemolysis, support the idea of these two factors - obstruction and tubular degeneration. It is apparent that the new factor operates by injuring tubular epithelium.

Given this other factor, haemoglobin or allied pigments can be made to precipitate in the acid state (York and Nauss, De Gowin et al, Bing) but the significance of such precipitation is questionable from examination of human and experimental material. It follows, then, that this factor must be the important feature in the mechanism of the renal failure. There are many ideas in the literature as to just what this factor is - a glimpse has been had of the nephrotoxic theory[9,10,98] and many types of nephrotoxins have been suggested, methaemoglobin being one of these[9,10]

Nephrotoxic substances - A substance can occasion renal tubular injury either by acting on the epithelial cells as a poison, or indirectly, through some mechanism such as vasoconstriction. The noxious agent may be a "heme" compound, or a new, or newly released substance which is formed by the primary disease process. The possibilities then are,

(a) haemoglobin derivative acting directly on tubular cells,

(b) haemoglobin derivative effecting vasoconstriction which, in turn, causes damage,

(c) nephrotoxic substance injuring tubular cells, and

(d) nephrotoxic substance acting indirectly.

Pigment toxins - direct and indirect action: Having investigated kidney changes in experimental haemoglobinuria, Levy [63] came to the conclusion that haemoglobin is a nephrotoxic substance and

damages tubular epithelium directly. This was in 1904 and it has since been proven that haemoglobin is harmless. In his excellent treatise on transfusion reactions Bordley[12] was drawn by the similarity between the renal changes in this disorder and those in mercuric chloride poisoning, and was, hence, of the opinion that some haemoglobin derivative exerted a direct toxic action on tubular cells. Brown[13,14,15,16,17] caused marked renal injury by the injection of hematin, and in this vein Anderson et al[2] injected di-sodium ferrihemate of pH 7.6 into dogs via the subcutaneous, intraperitoneal, and intravenous routes. Severe renal alterations were observed only when ferrihemate was administered intravenously. The glomeruli showed engorgement, hyaline thrombi, proliferation and leukocytic infiltration. Tubular changes were more constant in the cortex, and consisted of massive necrosis with calcification. Pigment casts were present, but were not prominent. The storage of this substance in the reticulo-endothelial system for a long period of time suggested that ferrihemate is not a normal product of bile pigment metabolism, and these workers suggest that it is the nephrotoxic substance. Bywaters and Popjak[23] succeeded in reproducing the crush syndrome in rabbits by the application of a rubber band to a limb for 4 to 5 hours. Upon release the rabbits showed haemoconcentration, azotemia (largely due to increased tissue breakdown), creatinuria, a fall in carbon dioxide combining power and a rise in urinary acidity. There was neither myohaemoglobinuria nor renal failure and they thought that these two negative factors may be causally related, so in another publication Bywaters and Stead[24] reported the injection of human myohaemoglobin into acid rabbits with the production of renal failure. They felt that myohaemoglobin had a direct effect on renal tubules. Bing[9,10]

found no deleterious effect of myohaemoglobin and suggested species differences to explain the discrepancy. But it is likely that a heterologous reaction supplied the added factor in the experiments of Bywaters and Stead. As previously mentioned, Bing achieved renal failure in his dogs by the injection of methaemoglobin in the presence of low urinary pH. He showed that in acidosis produced by lactic acid, clearances fell and methaemoglobin appeared in the urine, indicating that renal failure is caused by a combination of acidosis and methaemoglobin. He thought it possible that these are toxic through inhibition of respiratory enzymes in tubular cells. The finding of casts in only two instances led him to conclude that pigment cast formation is the sequel and not the cause of renal failure.

Mason and Mann[71] were interested in determining if any portion of the red blood cell could exert a vasoconstrictor action on the vessels of the kidney. They performed plethysmographic studies and concluded that haemoglobin exerts a specific vasoconstriction. This contraction of renal vessels was produced whether haemoglobin itself was introduced or whether it was liberated as the result of in vivo laking of erythrocytes. In another study large amounts of distilled water and laked blood were injected. An increase in the speed of injection resulted in a secondary transient decrease in kidney volume, indicating that renal vessels tolerate a certain amount of haemoglobin but when this is raised above a certain threshold they respond by contracting. Hesse and Filatov[56] showed that haemoglobin has a specific vasoconstrictor effect on the kidney which they said could be relieved by transfusion of compatible blood. Peters[82] quotes Mason and Mann, Hesse and Filatov, and as further evidence of renal spasm he cites the condition of

reflex anuria where the kidney is swollen and congested like the transfusion kidney, and believes that the congestion in both must be due to spasm of efferent vessels, trapping blood in the kidney. Since experimental irritation of splanchnic nerves has been shown to cause oliguria, and section of these nerves prolonged polyuria, and since good results have been obtained in reflex anurias by splanchnic block, Peters suggests splanchnic block as an added therapy for transfusion reactions. To support his contention, he records a case of a haemolytic reaction with anuria in which recovery followed splanchnic block. Following his earlier work[2], Anderson[3] argues for the nephrotoxic theory, pointing out that the constant finding of tubular degeneration is strong supporting evidence and he believes that since haemoglobin is the common factor, it or its derivative is toxic. He mentions his own work with hematin and recalls the work of Fairley[43,44,45] who found a new pigment in the blood in association with intravascular haemolysis. This pigment, methaemalbumin is the heme or hematin combined with the crystalbumin fraction of serum albumin and Fairley said that hematin free in the blood rapidly combines to form this pigment. These facts, then, suggest to Anderson that this new pigment is really the nephrotoxic substance which, he thinks, may bring about the injury by constriction of vessels.

Non-pigment toxins - direct and indirect action: Some writers considered a newly released nephrotoxin to act directly on the kidney. In 1933 Georgopoulas[51] writing about the azotemia in blackwater fever did not believe that haemoglobin possessed poisonous properties, but felt that the malarial parasite was culpable either directly or through increased protein breakdown. The effect on the kidney, he thought, may be either direct or through the liver. To

add weight to his contention he recalled the work of others who explained oliguria postoperatively and in other conditions by the toxic action of breakdown products of protein metabolism and pointed out that increased protein breakdown in blackwater fever is evidenced by an excess rise in blood urea which is not essentially related to the phenomenon of haemolysis, for in other haemolytic diseases one encounters no such urea increase. Hall(54) thought protein metabolism in blackwater fever to be halted at proteases, which act chemically like snake venom. Investigations into the mechanism of renal failure in the crush syndrome were conducted by Eggleton (37) and reported in the "Lancet" in 1944. It was found in cats that complete prolonged ischaemia of the limb appeared to be the essential factor, suggesting the formation of a toxic product. If this "poison" reached the kidney in concentration, it reduced its capacity to excrete creatinine. If liberated slowly by gradual release of the limb, it had no effect when the cats' livers were intact, but had the same effect as if it were rapidly released, if the cats were eviscerated. The liver seemed able to detoxify. Differences in human symptoms, she said, can then be explained by differences in the degree of ischaemia and in the functional capacity of the liver. Since extract of fresh muscle had no effect and that of ischaemic muscle considerable effect, she concluded that, both showing considerable urinary pigment, this could not be held responsible. Extract of dead muscle produced renal damage only if the muscle had been taken from 4 to 10 hours after death. She suggested that the toxin is an early intermediate breakdown product of a large mdecule, formed under anaerobic conditions and probably never seen during normal protein catabolism in an aerobic environment.

The final possible nephrotoxic mechanism obtains when a newly released substance other than pigment acts indirectly on kidney tubules by causing vasoconstriction. Iljin[59] injecting human and autohaemolyzed blood into dogs reported that repeated small doses of small amounts (7 cc. per kg. of body weight every 24 hours) of human, rather than autohaemolyzed blood, brought about death of the dogs after the 3rd day. He found that the early vascular phenomena of shock are not due solely to kidney disturbance, and thought that the late severe renal insufficiency is probably due to a nephrotoxic substance. Although Iljin does not say so, the reader obtains the impression that he believes this substance operates by some vascular mechanism. Petrov and Bogomalova[83] in 1936 and again in 1937[84] reported a series of experimental studies on the nature of haemolytic shock in blood transfusions. Plasma, red blood cells and especially haemolyzed red blood cells of heterogeneous blood caused a drop in blood pressure and in kidney volume, believed due to the foreign proteins; the higher toxicity of erythrocytes explained by their greater concentration of protein. The greater toxicity of plasma over serum was thought due to fibrinogen. Albumin and globulin had marked toxic depressor action while boiled blood and dialysate did not. They said that if there is incompatibility between proteins of donor and recipient, structural protein changes may occur giving rise to toxic substances. Although they were interested in death due to circulatory collapse they showed that the part of autolyzed incompatible blood which caused the fall in blood pressure and kidney volume, could get through a colloidal membrane and reminded them of histamine in its action. Corcoran and Page[25] showed that repeated lengthy and severe hypotension impairs the ability of the kidney to recover towards normal clearance and plasma flow, and they thought this phenomenon due to

release of a humorally circulating vasoconstrictor substance.

Anaemia, low blood pressure, circulatory collapse - It has been seen that one of the important lines of thought in the nephrotoxic theory is the concept of vasoconstriction with resultant anoxaemia of tubules. Is it not also possible that such anoxaemia may be considered due to some other factor without postulating any such toxic substance? Fox and Ottenberg[47] dealing with sulphonamide haemolytic anaemias considered the anuria and oliguria due to the shock associated with the loss of blood volume and severe anaemia. Smith and Evans[95] suggest that the first step in the mechanism of renal failure is the degeneration in the tubules from severe anaemia. Corcoran and Page[25] showed that renal blood flow is decreased by hypotension due to bleeding. Maigraith and Findlay[67] blamed peripheral circulatory collapse. The possibility that intravascular haemolysis is itself responsible for the lesions is considered by Wakeman and Morrell[97] although this is easily ruled out by the examples of other disorders in which haemolysis is unaccompanied by renal failure.

"Functional" Theory. (Anaphylactoid, Hypochloremia, Inadequate Glomerular Flow)

Finally, it might be held that neither pigment precipitation nor tubular injury is the essential factor. Robinson[90] held that acute uraemias seen after transfusion, surgical operation and in gastro-intestinal intoxication are of a similar nature. He believed that the tubular degenerations are insufficient and too varied, indicating that the kidney findings are secondary, and that the uraemia is of a functional nature, capable of existing in the presence of a normal kidney. From observations on human "transfusion" kidneys, Warner[98] concluded, "We are forced to the

assumption that the physiologic disturbance is greater than the anatomic change would indicate". Ayer and Gauld[4] said that the clinical renal failure in transfusion reactions is probably not due to the morphologic kidney changes, and the same conclusion was reached in regard to the renal insufficiency in burns, by Shen and Ham[94].

Perhaps anaphylactoid shock[66] causes renal failure in many instances. Such a cause must operate either functionally or by ischaemic tubular injury. The work of Brown et al[18] on the nephritis of gastro-intestinal intoxication, and Root and Henson[91] on postoperative urinary suppression, brings into focus a metabolic factor, namely a fall in chlorides. This "azotemie par manque du sel" is refuted by the absence of hypochloremia before the onset of renal insufficiency in transfusion reactions[12,31]. De Navasquez[82] thought it reasonable to suppose that anuria and pigment retention are the result of inadequate glomerular flow. He suggested that low blood pressure resulting from "shock" may be the cause.

The Path For Investigation

Review of the literature has indicated that pigment precipitation is certainly not of prime importance in the mechanism of renal failure in these conditions. The theory of a nephrotoxic substance has been examined from many angles and it has been seen that some consider purely functional disturbance of prime significance.

Having studied the various disorders and assembled the many proposals, what stands out prominently is that if the lesion is responsible for the renal failure, the salient feature is tubular degeneration and necrosis. One may then ask - since these conditions are so varied in their fundamental nature - is it at all necessary to accept one single cause for the tubular degeneration? It seems

likely that different causes or combinations of causes act through the "final common path" of tubular injury. The conflicting evidence as to the importance of pigment precipitation commands further investigation. In fact in 1944 Maigraith and Findlay[67], recognizing that the kidney lesions are not the result of haemoglobin passage, said, "the effects of the passage of similar amounts of haemoglobin upon tissues damaged by circulatory failure or by some chemical substance, have yet to be determined". In 1943, Dunn, Sheehan and McLetchie[36] discovered alloxan diabetes while seeking to bring about selective tubular damage before injecting haemoglobin. They did cause tubular cast formation, but could not continue their work because the animals died of a metabolic disorder.

The significance of urinary pH has also been the subject of much controversy. While some advocated alkalinization on the basis of their experimental work, others found no differences between acidotic and alkaline animals. Yet the finding of most of the pigment casts in distal convoluted tubules must have some relation to the observation that urine in the frog and rat is acidified exclusively in distal convoluted tubules in moderate acidification[38]. The discrepancy found in the literature seems to hinge on differences in experimental procedure - De Gowin[29] using impure haemoglobin, Yorke and Nauss dealing with injured animals[101], Bing[9,10] with a nephrotoxic substance. This question requires clarification.

PART 2: EXPERIMENTAL INVESTIGATIONS

INTRODUCTION

In the following experiments an attempt is made to determine, definitely, whether or not pigment precipitation plays a part in the mechanism of renal failure, what factors influence precipitation, and what importance is held by urinary pH. As has been seen, previous difficulty in estimating these factors has partly been due to the difficulty in reproducing the lesion, and since it is known that tubular injury is most probably the underlying disturbance in all the relevant conditions, the present work was first concerned with an attempt to reproduce the morbid anatomy by some method of tubular injury followed by the infusion of haemoglobin. Having achieved this structural picture one could then try to assay the relative importance of renal tubular degeneration and pigment cast formation.

Based on the reviewed theories, tubular damage was accomplished in two ways. First, ischaemia was produced by the temporary application of a clamp to the renal pedicle of the experimental animals, and second, a selective tubular poison, sodium tartrate, was injected subcutaneously. The use of sodium tartrate was indicated by the work of Friedman and Kaplan(50). In the definitive experiments, the injury was followed, after an interval, by the injection of purified haemoglobin. Acid and alkaline animals were used. The functional disturbances resulting from this procedure were studied and an attempt made to correlate them with structural changes observed.

METHODS

The work on experimental methods occupied a large part of the first year of these investigations, and was carried out by Dr. Yuile

and Dr. Hinds. No part of it was performed by the author, and, therefore, the methods will not be described in great detail.

White domestic rabbits were used. Their average weight was 2.75 kgms., with a range of from 2 to 3.2 kgms. They were all four to six months old. Metabolism cages were employed.

The animals were divided into two groups according to diet. The first group was given rabbit chow (purina) and vegetable scraps, resulting in the excretion of normal, alkaline urine, while the others were fed oats and stale bread, and their urine thus rendered acid. The water containers were kept full.

The urine was collected and measured every twenty-four hours. Male animals were catheterized to determine the degree of acidity, and this was measured using Nitrazine papers (Squibb). Levels of pH5. and 5.5 were found in the group receiving oats, and pH8. and 8.5 in the alkaline rabbits.

Rabbit haemoglobin was prepared in the following manner. The blood was obtained by cardiac puncture, immediately citrated, using 1 cc. of saturated sodium citrate per 100 ccs. of blood, then centrifuged at 2800 R.P.M. for 25 minutes. Withdrawing the plasma and leukocytes with a pipette, physiologic saline in an equal amount was added, the red cells and saline gently mixed, and the whole centrifuged again for 20 minutes. The supernatent saline was then withdrawn and the washing and centrifuging repeated. The packed erythrocytes were then laked by the addition of one-third their volume of ether and one-half their volume of distilled water. The whole was then vigorously shaken. After further centrifuging the stroma was removed, the haemoglobin solution was filtered first through coarse and then through fine (Whatman #50) filter paper in the presence of a vacuum which removed the ether. Thus, a pure solution of haemoglobin was prepared, free from cell stroma. This

method was described by Monke and Yuile[76]. The concentration of the solutions was determined photo-electrically according to the method of Evelyn and Malloy[39]. The standard curve was obtained by testing various dilutions of a known amount of haemoglobin, determined by the Department of Biochemistry of McGill University. The haemoglobin was given in doses ranging from 1.32 gms. to 2.35 gms., the corresponding volumes ranging from 8 to 16 ccs. Injection was made into marginal ear veins of the rabbits. It was calculated that the amounts injected corresponded to the amount released when complete intravascular haemolysis of 200 to 400 ccs. of blood takes place in a human being weighing 150 pounds. In most instances the solutions were not rendered isotonic. It was not considered necessary to do so in view of the small volumes actually injected.

In one group of experiments selective tubular damage was accomplished by the use of a small clamp which was applied to the renal pedicle. Some animals were clamped for 15 minutes and then released, others for 25 minutes. In this way, the renal artery, renal vein and ureter were completely occluded. This method of injuring tubules was suggested by the work of Scarff and Keele[92]. Haemoglobin was injected 5 minutes prior to removal of the clamp. In an early series, the clamp was applied to the left renal pedicle and haemoglobin injected, the right kidney being left in place as a control, and this series was used for histological study only. In all the other clamping experiments, the right kidney was removed 7 to 14 days before the experiments and the animal allowed to recover with a stabilized blood non-protein nitrogen level. The operations were performed with aseptic technique and great care was taken to avoid unnecessary manipulation. The rabbits were anaesthetized with intravenous sodium nembutal and inhalation ether.

The second method of causing tubular damage was by the injection of sodium tartrate in 20 per cent solution. Doses of 0.8 to 0.95 gm. per kgm. of body weight were given to non-anaesthetized animals. Haemoglobin was injected when the animals were recovering as indicated by falling blood non-protein nitrogen levels.

The selection of an accurate and consistently reliable method of measuring the blood non-protein nitrogen consumed considerable time, and finally a modification was used, of the method described by Hoffman[57].

This is the procedure that was followed:
3/4 cc. samples of blood were taken from the artery of the ear and put into test tubes containing one small drop of saturated sodium citrate. Volumes of 1/2 cc. were then added to 4.5 ccs. of 5 per cent trichloracetic acid to remove the proteins. The glassware was kept scrupulously clean. After allowing fifteen minutes for the proteins to precipitate, the whole was then filtered through Whatman No. 40 paper, the first drop being re-filtered. 3 cc. amounts of the filtrate were poured into Kjeldahl tubes. The pipettes were rinsed with distilled water and this was added to the tubes. This was found to make the results more constant by eliminating the factor of "sticking" in the pipettes. 1 cc. volumes of 50 per cent sulphuric acid were added as a digestion mixture and the tubes were then slowly boiled with micro-burners until white fumes appeared. Funnels were kept in the tops of the tubes. After cooling 3 to 4 drops of 30 per cent hydrogen peroxide decolorized the charred fluid, and then boiling was repeated until white fumes reappeared. After a second period of cooling amounts of distilled water were added to 50 ccs., part of the same water being poured through and around the funnels to remove and measure any nitrogen

deposited there. The tubes were shaken vigorously, then 10 ccs. aliquots were taken into colorimeter tubes. Carefully prepared gum ghatti was added in amounts of two drops per tube. This was found to stabilize the final color. The tubes were brought to a steady temperature of 25°C. in a water bath. Nesslers reagent, which had been prepared according to the method described by Wicks (99) was quickly added in 3 ccs. quantities. Blanks were prepared by boiling 3 ccs. of trichloracetic acid with 1 cc. of digestion mixture. A standard Nessler curve was obtained by dissolving ammonium sulphate in distilled water to give 1 mgm. of nitrogen in 10 ccs., re-dissolving to give 0.1 mgm. in 10 ccs., and using varying amounts of this solution treated in the same manner as above, and measured after the addition of 3 ccs. of Nessler's reagent. The curve was a straight line, giving concentration of nitrogen against colorimeter readings.

A Luxtrol colorimeter was used for all photo-electric determinations.

Tissues to be stained with haematoxylin and eosin were fixed in 10 per cent formol-saline. For a special haemoglobin stain the method described by Lison (65) was used. The tissue was fixed in a 3 per cent solution of potassium ferricyanide in 10 per cent formol-saline for 4 to 6 hours, and then washed in distilled water. Frozen sections were then cut, stained for 2 to 3 minutes in a special reagent. This was prepared by adding 10 gms. of powdered zinc and 2 ccs. of glacial acetic acid to 100 ccs. of a 1 per cent solution of Patent Blue, heating until colorless, filtering and pouring 2 ccs. of glacial acetic acid and 1 cc. of 30 per cent hydrogen peroxide into 10 ccs. of the filtrate. The sections were washed, counterstained with Lithium Carmine, then treated with absolute alcohol, xylol, and then mounted.

OBSERVATIONS

Two acid and two alkaline animals were given haemoglobin intravenously, without any previous treatment, in order to eliminate the possibility of haemoglobin precipitation in the renal tubules of normal kidneys. No casts were seen.

Histological Studies

+The first experiments were designed to reproduce and to study only the morbid anatomy of the "transfusion" kidney. In the first group a clamp was applied to the left renal pedicle of non-nephrectomized acid and alkaline rabbits. The duration of clamping was set at 15 minutes in an effort to produce only minimal to moderate tubular injury. Clamping for 1 hour results in severe tubular necrosis. Animals killed 24 hours after the 15 minute clamping showed slight swelling, granularity and vacuolization of proximal convoluted tubular epithelium of the clamped kidney. The picture was independent of the urinary pH. Unclamped kidneys were normal. Alkaline animals clamped and given haemoglobin showed, in addition to the slight tubular damage, a moderate number of haemoglobin casts in the clamped kidney 24 hours later, while the unclamped kidney contained no casts. The kidneys of similarly treated acid rabbits contained many haemoglobin casts on the clamped side, and occasional casts on the unclamped side. Thus, in all animals given haemoglobin after injury to tubules, casts were found, but these were notably more numerous in acid than in alkaline rabbits. These haemoglobin casts were seen chiefly in the distal convoluted and collecting tubules, but also in the ascending limb of Henle's loop. In haematoxylin-eosin preparations they stained

+ The greater part of this work was performed by Dr. Yuile and Dr. Hinds.

brownish-red, they were negative to Prussian Blue, and were strongly positive for haemoglobin with the special stain.

In another group of purely histological investigations, acid and alkaline animals were subjected to the damage of a specific tubular poison, sodium tartrate. Controls were killed 48 hours after the subcutaneous injection of this substance, while others were given haemoglobin at this time and allowed to survive for another 15 to 18 hour period.

Again, it was desired to produce only moderate disturbance for it was obvious that severe injury would of itself kill the animal. Several different doses of sodium tartrate were used, and, finally, this trial and error method led to the determination of an average optimum dose of 0.85 gms. per kgm. of body weight. There was considerable variation in the response of different animals, both acid and alkaline, the greatest irregularities being noted in the latter group while acid animals showed consistently greater damage so that it was frequently necessary to administer up to 0.95 gms. per kgm. to the alkaline rabbits in order to obtain comparable results.

Control animals in both acid and alkaline groups given tartrate alone showed the following picture: Sometimes the convoluted tubules had undergone only mild degenerative change consisting of epithelial swelling and granularity. In other cases the damage was very severe with considerable necrosis of tubular epithelium, reducing some tubules to masses of coagulated protein, others to clear spaces lined by basement membrane. Hyaline casts were found in collecting tubules, their number varying with the degree of injury. In some instances calcium granules were seen in necrotic tubules. Haemoglobin stains in both acid and alkaline rabbits showed no pigment precipitation.

In animals given haemoglobin after tubular injury by tartrate, typical coarsely granular haemoglobin casts were found in distal convoluted and collecting tubules and in the ascending limb of Henle's loop. These were superimposed on the changes described as due to the sodium tartrate alone. Casts were seen both in acid and alkaline animals. They were, however, much more numerous in those with acid urine. It is interesting to note that casts were plentiful only in those cases where the original tartrate damage was mild and the necrosis minimal. When the initial insult was severe, haemoglobin casts were scarce.

†Functional Experiments

All the other observations were concerned with an evaluation of the functional disturbances encountered when the described morphological alterations are taking place. The experiments were divided into three main groups, the first and second being fifteen and twenty-five minute clamping experiments, and the third sodium tartrate studies. In the first two groups animals were used whose right kidney had been previously removed. The experiments consisted of clamping the left renal pedicle for periods of fifteen minutes in the first and twenty-five minutes in the second group. Some animals were kept as controls, others received haemoglobin intravenously five minutes before removal of the clamp. Blood non-protein nitrogen levels were studied in all animals and in many, urinary output was measured. The effect of haemoglobin injection was evaluated in the third, or tartrate group by withholding the administration of the pigment until retention of nitrogen had begun to diminish. In all the experiments rabbits with low and high urinary pH were employed.

† This is the part of the original investigative work in which the author actively participated with Dr. C. L. Yuile.

Renal pedicle clamp - 15 minutes: A summary of the results obtained when the renal pedicle clamp was applied for 15 minutes, is seen in Table I.

Both acid and alkaline rabbits who were clamped but not given haemoglobin suffered no renal functional disturbance, the blood non-protein nitrogen levels remaining unchanged. One alkaline rabbit, No. 31, was given 1.6 gms. of haemoglobin intravenously, five minutes before removal of the clamp; no change was noted in the blood non-protein nitrogen level. Two acid rabbits, numbers 32 and 43, developed azotemia following clamping with haemoglobin injection. No. 32 received 1.32 gms. of haemoglobin, the non-protein nitrogen reaching a peak level of 170 mgms. per cent on the second day, and No. 43 received 1.5 gms. of haemoglobin reaching a peak level of 163 mgms. per cent on the third day. The non-protein nitrogen reached a normal level on the 8th day in rabbit No. 32, and had falled to 120 mgms. per cent on the 6th day in rabbit No. 43, who was accidentally killed at this time. Urine volumes were measured for rabbit No. 43, revealing an oliguria (5 cc. output) on the 2nd day, gradually returning to normal. The kidney of this animal showed many haemoglobin casts and moderate tubular degeneration, with some tubules showing tubular dilatation and epithelial compression (Fig. 4a). In Table Ia, there are presented some of the actual protocols, and Fig. 1 graphically illustrates this series.

Renal pedicle clamp - 25 minutes: Although 15 minutes of clamping caused slight structural abnormalities, only if haemoglobin was given to an acid animal could functional disturbance be elicited. In order to obtain greater tubular injury, of a degree sufficient to cause an elevation of non-protein nitrogen in control animals, the clamp was applied for a period of 25 minutes to each rabbit in the 2nd group.

TABLE I

Summary of 15 minute Clamping Experiments

Rabbit No.	Weight Kg.	Reaction of Urine.	Renal Pelicle Clamped. Minutes	Haemoglobin Injected. Gm.	Effect on Blood N.P.N.	Effect on Urine Volume	Remarks
30	2.02	Alk.	15	0	Unchanged	Not determined	Recovered
31	3.18	Alk.	15	1.60	Unchanged	Not determined	Recovered
33	3.25	Acid	15	0	Unchanged	Not determined	Recovered
32	2.55	Acid	15	1.72	Initial level 58 mg.% Maximum-day 2-170 mg.% Return to normal 8th day	Not determined	Recovered
43	2.30	Acid	15	1.50	Initial level 47.5 mg.% Max. 1st day 3-162 mg.% Day 6-falling-120 mg.%	Average normal vol. 50 cc. Accidentally Oliguria 2nd day - 5 cc. Killed 4th day Gradual return to normal. Hgb. casts +++	

TABLE Ia

Representative Protocols - 15 Minute Clamping Experiments

Alkaline-Control

Rabbit #30 - Wt. 2.02 kg.

Left Renal Pedicle Clamped 15 mins.

Day of Exp.	N.P.N.*
Pre-Clamp Level	36
1	45
2	35.6
3	31

Alkaline-Hgb.

Rabbit #31 - Wt. 3.10 kg.

Left Renal Pedicle (1.6 gm. Hgb. I.V.*) Clamped 15 mins.

Day of Exp.	N.P.N.*
Average Pre-Clamp Level	37.3
1	36
2	39
3	46
4	37.6
5	-
6	37.6

Acid-Control

Rabbit #32 - Wt. 3.25 kg.

Left Renal Pedicle Clamped 15 mins.

Day of Exp.	N.P.N.*
Pre-Clamp Level	46.6
1	35.6
2	35.6
3	30
4	36.6
5	-
6	37.3

Acid-Hgb.

Rabbit #17 - Wt. 2.6 kg.

Left Renal Pedicle (1.5 gm. Hgb.* I.V.*) Clamped 15 mins.

Day of Exp.	N.P.N.*	Urine Vol.*
Average Pre-Clamp Level	47.5	71
1	104	60
2	145	5
3	163	42
4	-	-
5	153	42
6	120	-
Died of Tamponade		

*Hgb. - Haemoglobin

*I.V. - Intravenously

*N.P.N. - Blood non-protein nitrogen in milligrams per 100 cc.

*Urine volume in cc.

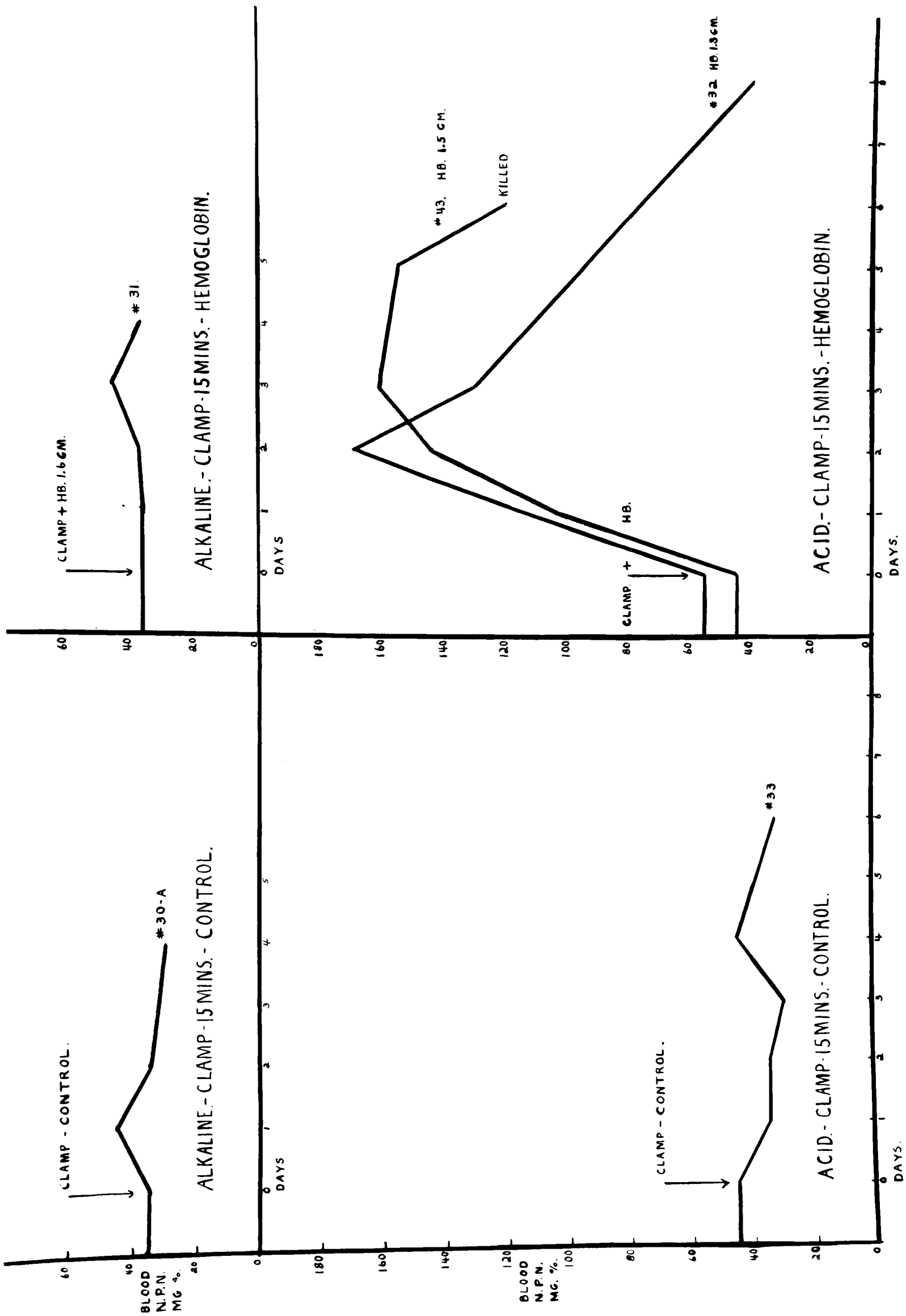

FIG.1. Non-protein nitrogen curves in experiments in which the left renal pedicle of previously nephrectomized rabbits was clamped for fifteen minutes. Details of the various experiments are to be seen on the chart.

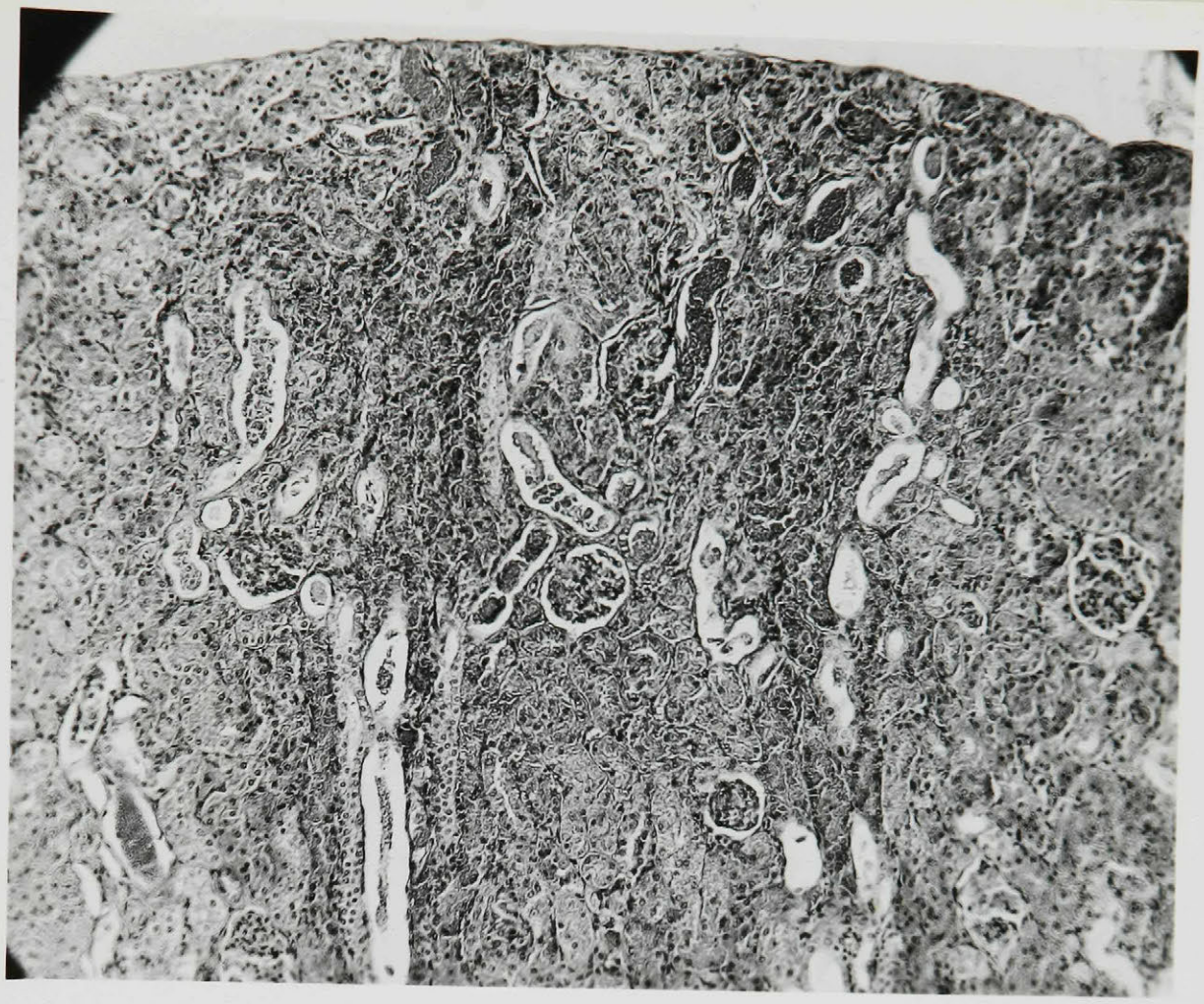

FIG.4a. Kidney, rabbit No.43 (hematoxylin and eosin X 110)
Acid urine. Previous right nephrectomy. Left renal pedicle clamped for fifteen minutes and hemoglobin injected. There are many hemoglobin casts in the distal convoluted and collecting tubules. These tubules also show dilatation and compression of the epithelium. Mild degenerative changes are seen in proximal convoluted tubules.

The effects of haemoglobin injection superimposed on such an injury, were studied.

Table II and Fig. 2 record the results of these experiments. Rabbits with alkaline urine who did not receive haemoglobin developed a transient rise in blood non-protein nitrogen reaching a peak on the first to the third day and steadily falling to normal within seven days after clamping. In the alkaline rabbits given haemoglobin along with the clamping a notably different course was observed; the rise in non-protein nitrogen, while not exceeding the level seen in the control animals, was more persistent, and did not fall to normal until the ninth to the eleventh day. All these animals suffered a temporary suppression of urine on the first or second day. They all recovered showing no obvious after effects. Rabbit No. 58, having received 1.6 gms. of haemoglobin was sacrificed on the twelfth day and his kidney examined. There were only very occasional haemoglobin casts.

Acid animals subjected to clamping alone exhibited elevations which corresponded to those seen in the alkaline controls. There were, as before, variations in the heights reached, but all came to a peak and were normal by the sixth day. The most striking changes took place in those acid rabbits which received haemoglobin in addition to the clamping. Rabbits No. 46 and 57 showed a continuous sharp rise to levels of 256 and 227.5 mgm. per cent respectively, and both died on the fifth day while the blood non-protein nitrogen concentration was still ascending. Both rabbits were completely anuric from the first day onward. The kidney in both showed only moderate tubular degeneration with some hydropic change. There was no necrosis of tubules. Both showed a multitude of haemoglobin casts. The kidney of No. 57 is illustrated in Fig. 4b.

Paradoxically, acid controls neither rose as high nor suffered as much urinary suppression as did alkaline controls.

The results in No. 49b appear to be out of line with No. 46 and No. 57. It must be pointed out that this animal was the subject in two separate experiments. In the first (No. 49a), a 25 minute clamping control experiment was performed. An interval of one month elapsed and then a second clamping (49b) was carried out, at which time haemoglobin was injected. At operation considerable scar tissue was found about the kidney, and while this did not involve the renal vessels, it did prevent their complete closure by the clamp. This experiment, then, illustrates the result obtained with haemoglobin and a 25 minute period of incomplete occlusion as compared with complete interruption of the renal circulation to which injection of haemoglobin is added. It will be noted that the outcome in No. 49b is almost exactly the same as that seen in acidotic subjects given haemoglobin after 15 minutes of complete renal ischaemia. This animal was killed on the 8th day and his kidney showed minimal degeneration and moderate numbers of haemoglobin casts. The complete protocols of some of the rabbits in this group are given in Table IIa.

Sodium tartrate injection - Experiments in which sodium tartrate was used to produce tubular injury, were not as consistent as clamping experiments. There were notable individual variations in response to the chemical, and while some animals died of sodium tartrate poisoning before further investigation could be carried out, others did not respond at all. Thus many rabbits had to be discarded, the desired effect being the infliction of some damage, not severe enough to cause death, and permitting a study of the effects of haemoglobin per se. It was found that animals with

TABLE II

Summary of 25 Minute Clamping Experiments

Rabbit No.	Weight Kg.	Reaction of Urine	Renal Pedicle Clamped Minutes	Haemoglobin Injected Gm.	Effect on Blood N.P.N.	Effect on Urine Volume	Remarks
41a	2.70	Alk.	25	0	Initial level-43.6 mg.% Maximum-day 1-110 mg.% Returned to normal 6th day	Anuria-1st day Immediate return to normal level 100-130 cc. per day	Recovered
52	3.10	Alk.	25	0	Initial level-57.8 mg.% Maximum-day 3-151.6 mg.% Normal-7th day	1st day - 10 cc. 2nd day - 0 cc. 3rd and subsequent days average 85 ccs.	Recovered
42	3.20	Alk.	25	2.35	Initial level-48 mg.% Irregular elevation to 105 mg.% 7 days Approximately normal by 11th day	Slight oliguria on 1st and 2nd days only	Recovered
58	2.15	Alk.	25	1.60	Initial level-53 mg.% Irregular elevation to 98 mg.% 6 days Normal by 9th day	Oliguria 3 ccs. 2nd day only	Recovered Killed 12th day Very occasional Hgb. casts.

TABLE II (continued)

Summary of 25 minute Clamping Experiments

Rabbit No.	Weight Kg.	Reaction of Urine	Renal Pedicle Clamped Minutes	Haemoglobin Injected Gm.	Effect on Blood N.P.N.	Effect on Urine Volume	Remarks
41b	2.70	Acid	25	0	Initial level-53 mg.% Maximum-day 1-81.6 mg.% Normal on 6th day	Moderate oliguria 1st-6th day	Recovered
49a	2.70	Acid	25	0	Initial level-52 mg.% Elevated to 80 mg.% 2nd to 5th days Normal 6th day	Unchanged Average 200 cc. per day	Recovered
46	3.00	Acid	25	1.75	Initial level-71.6 mg.% Continuous rise to 256 mg.% on 4th day	Initial average 100 cc. per day Complete anuria 1st to 5th day	Died 5th day Hgb. casts ++++
57	2.77	Acid	25	1.70	Initial level-57 mg.% Continuous rise to 227.5 mg.% on 5th day	Initial average 130 cc. per day Complete anuria 1st to 5th day	Died 5th day Hgb. casts ++++ (massive)

TABLE II (continued)

Summary of 25 Minute Clamping Experiments

Rabbit	Weight	Reaction of Urine	Renal Pedicle Clamped	Haemoglobin Injected	Effect on Blood N.P.N.	Effect on Urine Volume	Remarks
No.	Kg.		Minutes	Gm.			
49b	2.70	Acid	25 (Partial)	1.60	Initial level-56 mg.% Maximum-day 3-156 mg.% Decline by day 8 to 74 mg.%	Average over 100 ccs. No appreciable change during experiment	Killed 8th day HGL. casts ++

TABLE IIa

Representative Protocols - 25 Minute Clamping Experiments

Alkaline-Control

Rabbit #41a - Wt. 2.7 kg.

Left Renal Pedicle Clamped 25 mins.

Day of Exp.	N.P.N.	Urine Vol.
Average Pre-Clamp Level	43.6	66
1	110	0
2	-	-
3	76	230
4	62	170
5	57	190
6	-	145
7	33	130

Alkaline-Hgb.

Rabbit #42 - Wt. 3.2 kg.

Left Renal Pedicle (2.35 gm.* Hgb.* I.V.) Clamped 25 mins.

Day of Exp.	*N.P.N.	*Urine Vol.
Average Pre-Clamp Level	48	125
1	85	53
2	93	23
3	72.5	120
4	80	102
5	91	95
6	105	36
7	90	160
10	70	-
23	52.6	-

Acid-Control

Rabbit #41b - Wt. 2.7 kg.

Left Renal Pedicle Clamped 25 mins.

Day of Exp.	N.P.N.	Urine Vol.
Average Pre-Clamp Level	53	46
1	81.6	15
2	78.3	10
3	74	6
4	-	-
5	-	-
6	46.6	-

Acid-Hgb.

Rabbit #[illegible] - Wt. 2.7 kg.

Left Renal Pedicle ((1.7 gm. I.V.) Clamped 25 mins.

Day of Exp.	N.P.N.	Urine Vol.
Average Pre-Clamp Level	57	130
1	83	10
2	141	0
3	151	0
4	187	0
5	227.5	0

*Hgb. - Haemoglobin

*I.V. - Intravenously

*N.P.N. - Blood non-protein nitrogen in milligrams per 100 cc.

*Urine volume in cc.

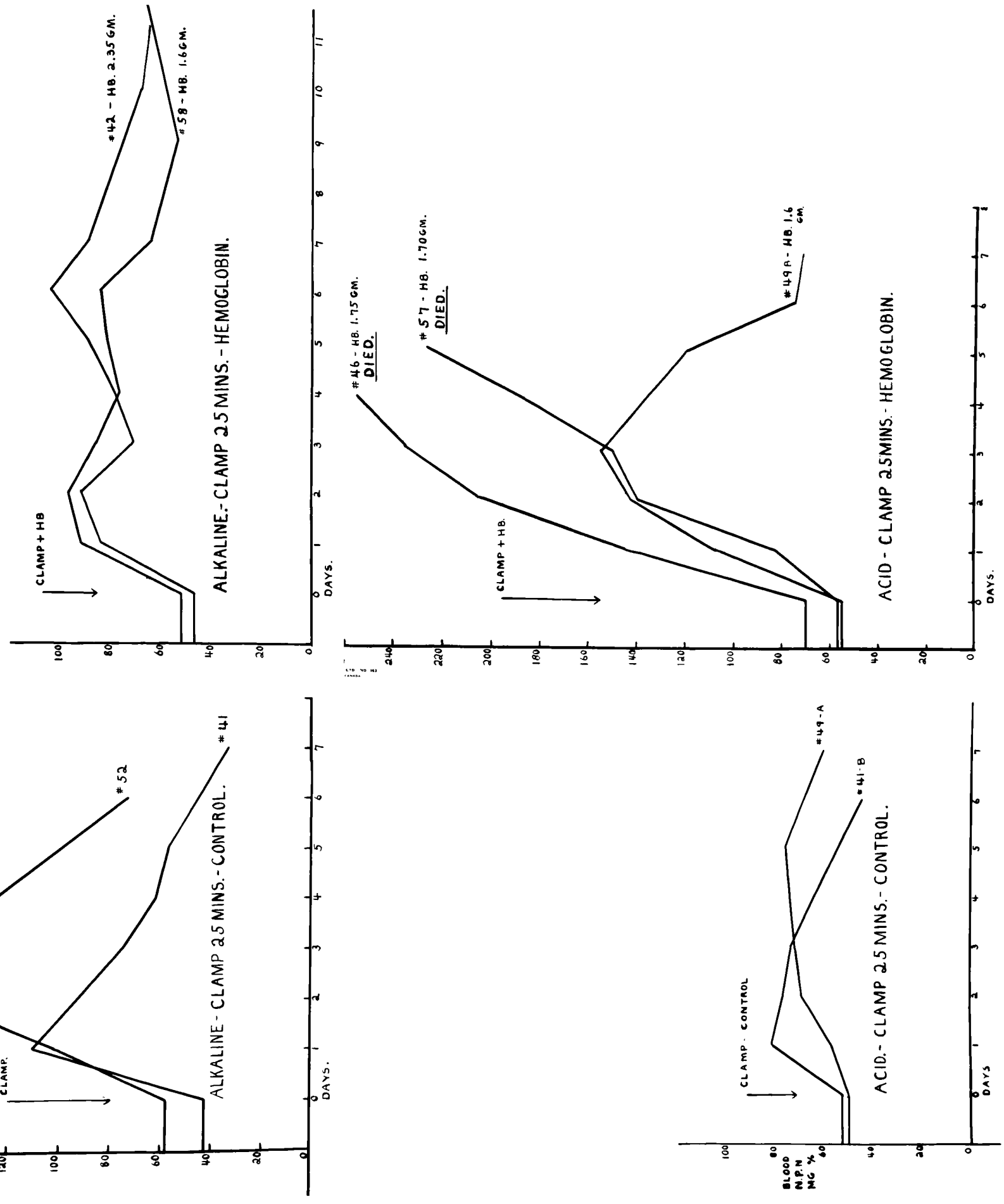

FIG.2. Non-protein nitrogen curves in experiments using a left renal pedicle clamp for twenty-five minutes in order to bring about tubular damage in rabbits which had previously been subjected to right nephrectomy. Details of the various experiments are to be seen on the chart.

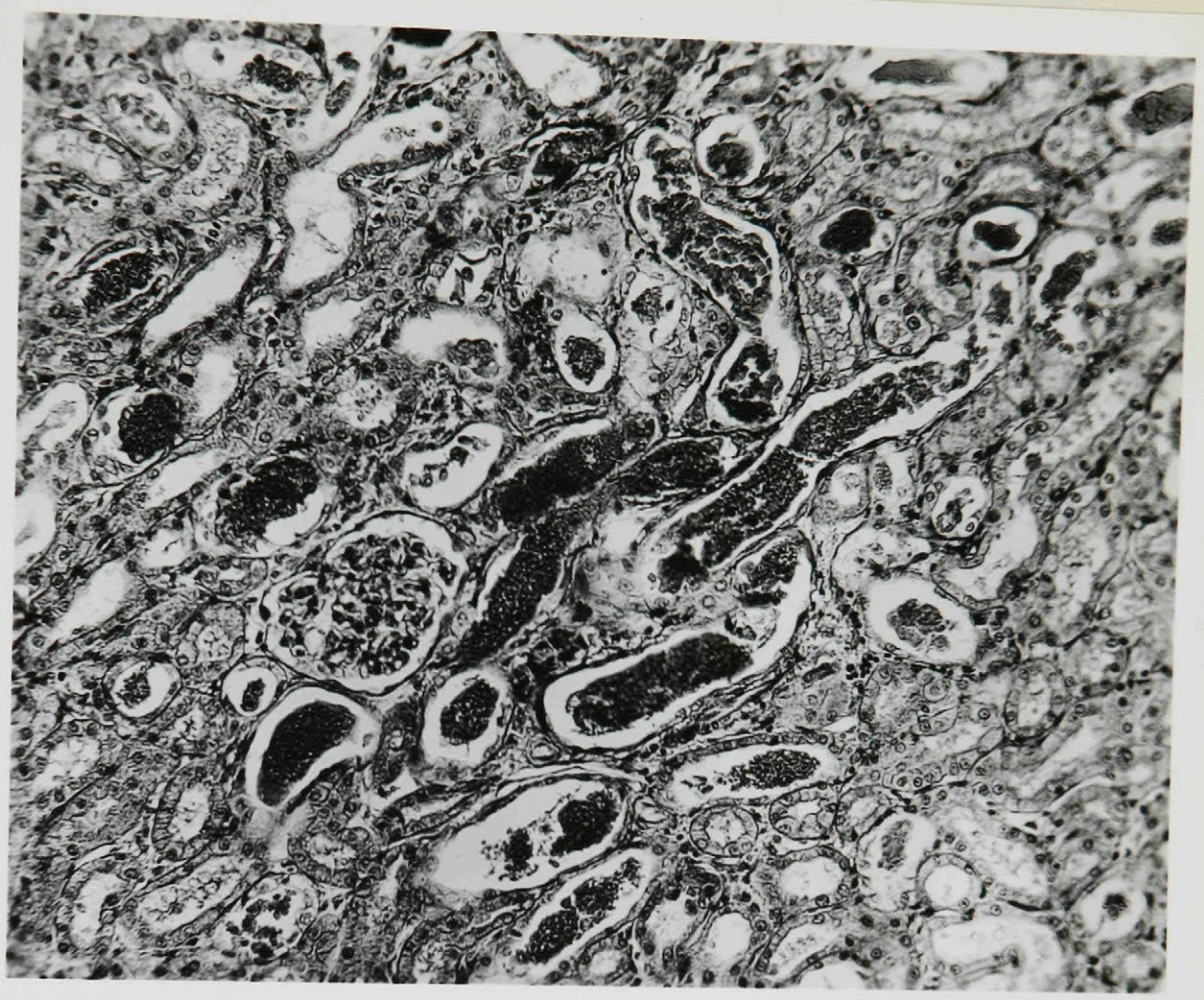

Fig.4b. Kidney, rabbit No.57 (hematoxylin and eosin X160)
Acid urine. Previous right nephrectomy. Left renal pedicle clamped for twenty-five minutes and hemoglobin injected. Died on fifth day. There are great numbers of hemoglobin casts in the distal convoluted and collecting tubules. The granular nature of these casts is demonstrated, and the tubular dilatation evident. Proximal convoluted tubules show hydropic changes, but no necrosis.

alkaline urine were more erratic and less susceptible than acid animals, and later therefore, they received larger doses. It appears that body weight is not an accurate index of dose, but lack of time prevented development and use of a more careful criterion. When sodium tartrate injection resulted in a mild to moderate, seemingly reversible nitrogen retention, the animal was used. After the first few experiments it became relatively simple to decide which animals were only moderately injured and would recover.

Table III summarizes all the experiments in which functional studies were carried out, and Fig. 3 presents a graphical picture of the blood non-protein nitrogen levels plotted against days of the experiments. Alkaline control animals, that is, those receiving sodium tartrate but no haemoglobin, showed an abrupt elevation in non-protein nitrogen following the injection, and this promptly returned to normal. Concentrations between 93 and 117 mgms. per cent were reached, on the first to the third day, and the rapidity of the return to normal appeared to depend on the degree of nitrogen retention, the rabbit reaching a level of 117 mgms. per cent requiring 5 days to recover. The output of urine was affected in only one instance, and only very slightly.

In order that any effect of haemoglobin might be detected and evaluated, haemoglobin injections were given in most instances, when the animals were well on the path to recovery from the initial insult, as indicated by a steadily falling non-protein nitrogen concentration. In this manner, No. 27,38 and 45 were given 1.25, 1.81, and 2.32 gms. of haemoglobin respectively. Non-protein nitrogen levels, which were falling before the injections continued to fall after administration of haemoglobin, and promptly returned to normal. The normal levels were reached in 6 to 7 days, recovery

occurring 1 to 4 days later than in the controls, and this is attributable to the fact that the initial levels after sodium tartrate were higher in this group. Notwithstanding the fact that, in general, the initial injury in the definitive group was more severe than that seen in control animals, haemoglobin injection made no appreciable difference in the rate of recovery. Rabbit No. 38 was sacrificed on the 8th day. The kidney showed occasional haemoglobin casts (Fig. 4c). The data for Rabbit No. 56 appears inconsistent with the other results. In this animal sodium tartrate injection resulted in a blood non-protein nitrogen concentration of 216 mgms. per cent on the 3rd day. At the time of haemoglobin injection on the 6th day, the level was 132 mgms. per cent. There was a secondary rise in non-protein nitrogen after the haemoglobin injection persisting until the animal's death on the 15th day. Autopsy revealed a marked degree of tubular necrosis with calcification. <u>There were no haemoglobin casts</u>.

Control animals in the acid series suffered temporary nitrogen retention of a moderate degree, a course no different from that seen in alkaline controls. Of the acid rabbits receiving haemoglobin No. 29 and No. 62 showed no appreciable haemoglobin effects. In contrast to the alkaline animals given haemoglobin, these rabbits received relatively small doses, No. 29 getting 1.25 gms. of haemoglobin, and No. 62, 1.35 gms. The pigment was given to No. 29 at the peak of the tartrate effect, while No. 62 was injected well along in the recovery period. No. 62 was killed on the 12th day, and examination of the kidneys disclosed only occasional haemoglobin casts in relatively normal renal architecture.

Other acid rabbits, No. 36 and No. 37, were given larger doses of haemoglobin and showed striking results. No. 36 received

only mild tartrate injury, and was recovering well, with a non-protein nitrogen level of 79 mgms. per 100 ccs., when the haemoglobin was injected on the 3rd day of the experiment. There followed a progressive increase of nitrogen retention and a persistent oliguria, and the animal died on the 9th day, while the non-protein nitrogen level was still rising. Examination of the kidneys revealed great numbers of haemoglobin casts with tubular dilatation and moderate degenerative changes in the tubular epithelium (Fig. 4d). Rabbit No. 37, was given haemoglobin while recovering from moderate tartrate injury, and this was followed by a secondary rise in blood non-protein nitrogen, which slowly declined after 4 days. The animal died 9 days after the haemoglobin injection, and many pigment casts were found in the renal tubules along with the moderate degree of degenerative changes due to sodium tartrate. In Table IIIa are shown some of the protocols of this series.

Further histological observations - The functional studies revealed added histological information, for many of these rabbits died, or were killed, 5 days or more after the injection of haemoglobin, thus permitting a study of the late changes that occur.

If an animal whose tubules had been injured by sodium tartrate survived longer than 4 days, a new tartrate change was seen, and this consisted of oedema and fibrosis of interstitial tissue of the kidney. Often, too, there was evidence of tubular regeneration.

Again, when a comparison was made between these "late" kidneys and those of the purely histopathological experiments, certain significant differences were noted. In the morphological studies, alkaline animals given haemoglobin after tubular injury had been inflicted, showed occasional to moderate numbers of haemoglobin casts. In the functional studies, where the animals survived more than the short interval allowed in the histological experiments,

Summary of Sodium Tartrate Experiments

Rabbit	Weight	Reaction of Urine	Sodium Tartrate Injection	Haemoglobin Injection	Blood N.P.N. Initial Level	Following Sodium Tartrate Max. conc.	Day of Exp.	At Haemoglobin Injection Max. conc.	Day of Exp.	Following Haemoglobin Injection Max. conc.	Day of Exp.	Final Determination conc.	Day of Exp.	Effect on Urine Volume	Remarks
No.	Kg.		Gm/kg.	Gm.	mg.%	mg.%		mg.%		mg.%		mg.%			
35	3.15	Alk.	.95	0	63.0	93	1	-	-	-	-	63.0	3	Not determined	Recovered
44	3.20	Alk.	.95	0	38.0	94	3	-	-	-	-	38.0	4	Slight oliguria 3-4 days	Recovered
60	2.23	Alk.	.90	0	37.0	117	3	-	-	-	-	40.0	5	No change	Recovered
27	2.85	Alk.	.85	1.25	49.3	160	2	160	2	163	4	48.5	7	Not determined	Recovered
38	2.70	Alk.	.85	1.81	49.1	93	2	70	4	57	5	43.0	6	No change	Killed on 8th day. Occasional hgb. casts
45	2.96	Alk.	.85	2.32	54.5	142	2	93	3	80.6	5	66.0	7	Oliguria marked 3rd-7th day	Recovered
56	2.80	Alk.	.95	1.75	49.0	216	3	132	6	201	9	150	15	Anuria Day 2 and 3; and day 7 and 10	Died 15th day

[redacted] III (continued)

Summary of Sodium Tartrate Experiments

Rabbit	Weight	Reaction of Urine	Sodium Tartrate Injection	Haemoglobin Injection	Blood N.P.N.								Effect on Urine Volume	Remarks
					Initial Level	Following Sodium Tartrate		At Haemoglobin Injection		Following Haemoglobin Injection		Final Determination		
						Max. conc.	Day of Exp.	conc.	Day of Exp.	Max. conc.	Day of Exp.	conc.	Day of Exp.	
No.	Kg.		Gm/kg.	Gm.	mg.%	mg.%		mg.%		mg.%		mg.%		
23	2.07	Acid	.80	0	47.8	110	1	-	-	-	-	36.0	4	Not Determined	Recovered
28	2.42	Acid	.85	0	43.3	99	2	-	-	-	-	30.0	5	Not Determined	Recovered
55	2.71	Acid	.90	0	57.0	92	1	-	-	-	-	61.0	6	No change	Recovered
29	2.75	Acid	.85	1.25	38.0	140	2	140	2	140	3	55.0	9	Not determined	Recovered
36	2.77	Acid	.85	2.00	52.0	97.2		79	3	180	8	180	8	50% oliguria after hgb.	Died 9th day Hgb. casts ++++
37	2.65	Acid	.85	2.08	34.0	170	4	107	6	138	10	61	15	Not Determined	Died 15th day; Hgb. casts ++++
62	2.83	Acid	.85	1.35	38.0	210	4	119	7	117	8	60	12	Oliguria Days 1 and 3	Recovered. 12th day Occasional hgb. casts.

TABLE IIIa

Representative Protocols - Sodium Tartrate Experiments

Alkaline-Control

Rabbit #14 - wt. 3.2 kg.

Sodium Tartrate 0.85 gm./kg.

Day of Exp.	N.P.N.	Urine Vol.
Average Pre-Clamp Level	36	110
1	48	53
2	84	130
3	94	30
4	38	5
5	-	-
6	43	30

Alkaline-Hgb.*

Rabbit #[illegible] - wt. 2.7 kg.

Sodium Tartrate 0.85 gm./kg.

Day of Exp.	N.P.N.*	Urine Vol.*
Average Pre-Clamp Level	49.1	127
1	[illegible]	140
2	93	215
3	86	100
4	70	77
Haemoglobin 1.81 gm. I.V.		
5	57	127
6	43	120
7	-	-
8	48	170
KILLED		

Acid-Control

Rabbit #28 - wt. 2.42 kg.

Sodium Tartrate 0.85 gm./kg.

Day of Exp.	N.P.N.	Urine Vol.
Average Pre-Clamp Level	43.3	Not Determined
1	85	" "
2	99	" "
3	85	" "
4	82.5	" "
5	30	" "

Acid-Hgb.

Rabbit #36 - Wt. 2.77 kg.

Sodium Tartrate 0.85 gm./kg.

Day of Exp.	N.P.N.	Urine Vol.
Average Pre-Clamp Level	52	125
1	92	125
2	97	135
3	79	75
Haemoglobin 2 gm. I.V.		
4	111	65
5	-	50
6	123	70
7	153	12
8	180	70
DIED		

*Hgb. - Haemoglobin
*I.V. - Intravenously
*N.P.N. - Blood non-protein nitrogen in milligrams per 100 cc.
*Urine volume in cc.

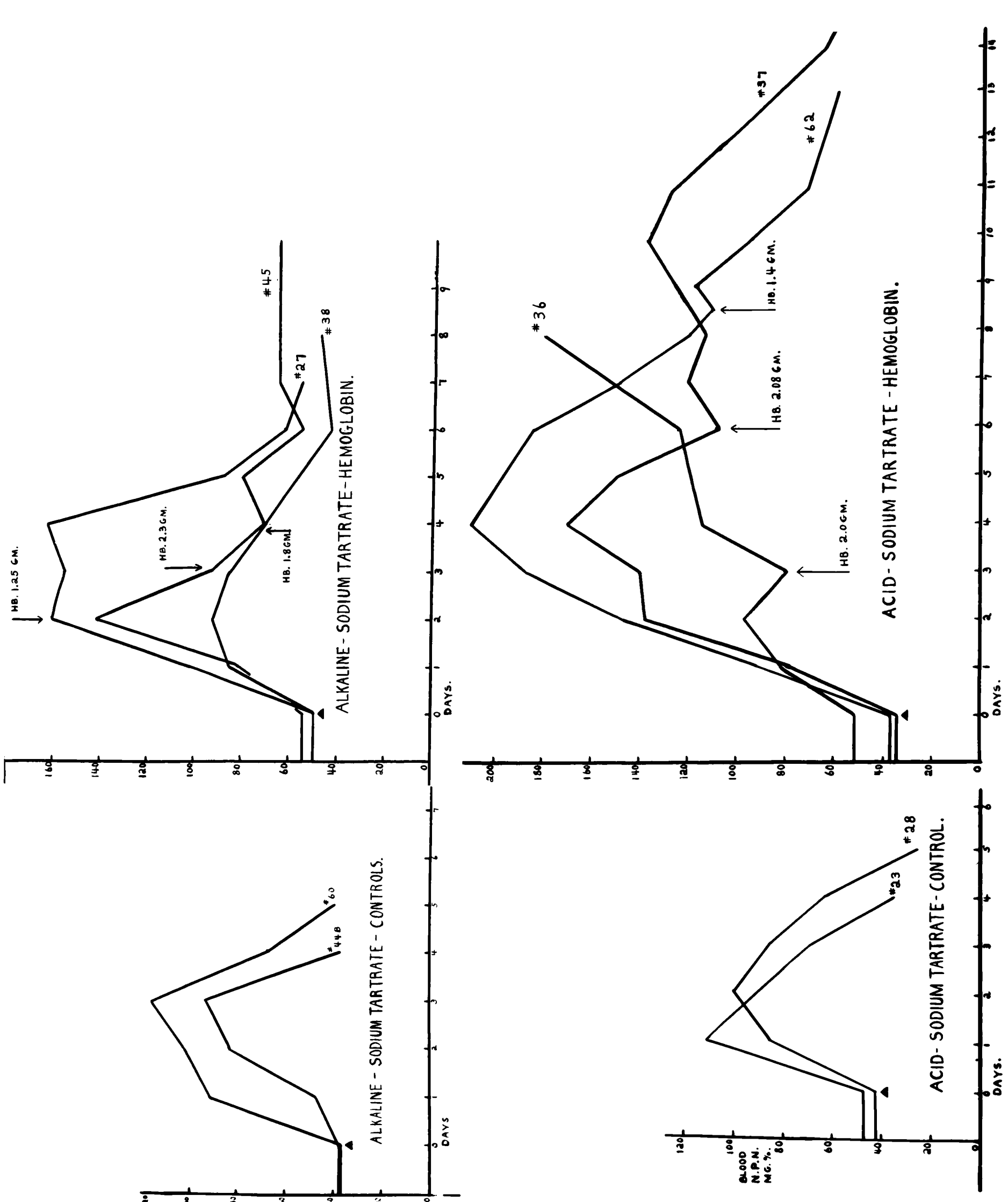

Fig.3. Non-protein nitrogen curves when sodium tartrate produced renal injury. The black arrow-heads indicate injection of the chemical on day 0. Other details are to be seen on the chart.

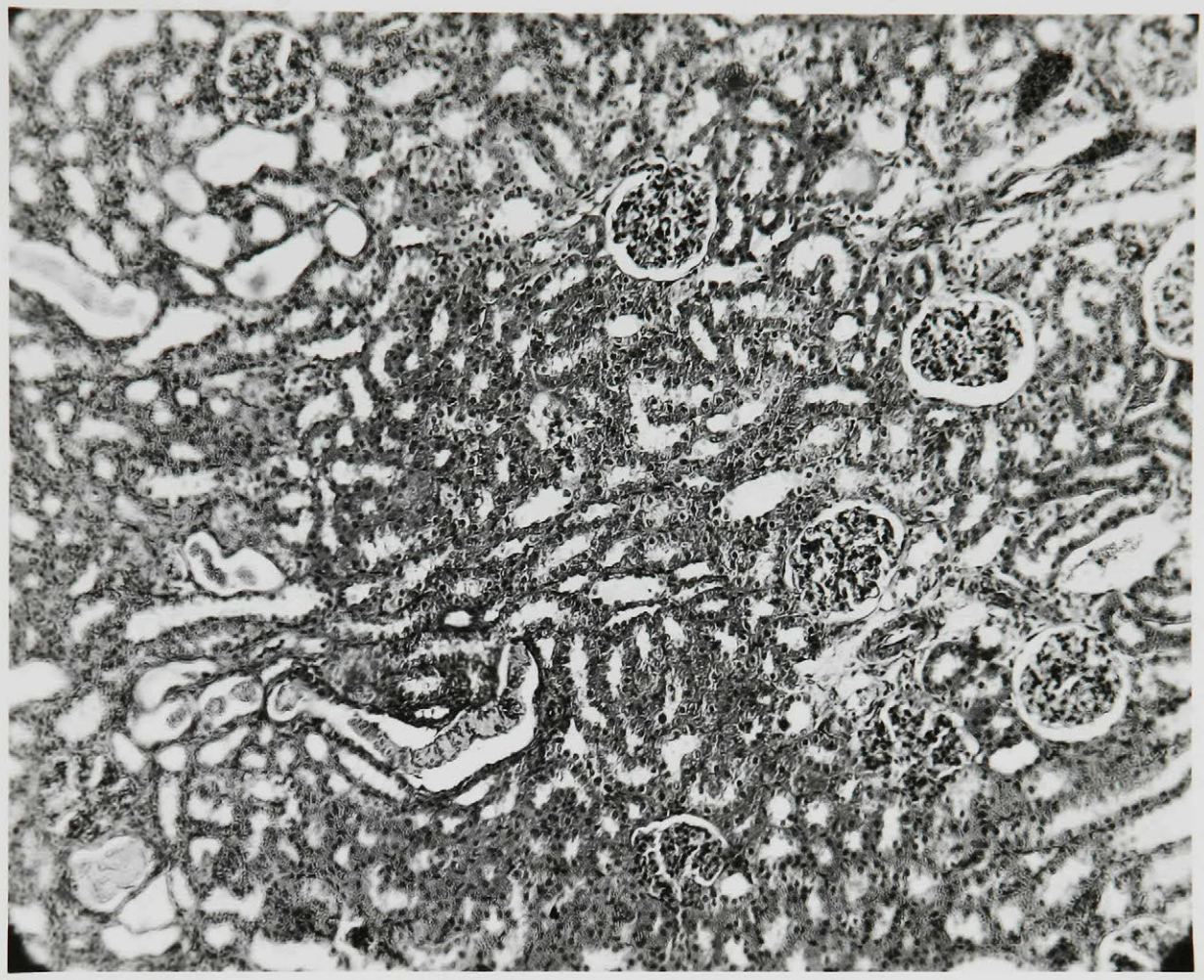

Fig.4c. Kidney, rabbit No.38 (Hematoxylin and eosin X 110)
Alkaline urine. Treated with sodium tartrate subcutaneously and with hemoglobin intravenously when the N.P.N. was falling. Animal recovered. Occassional hemoglobin casts are seen in distal convoluted and collecting tubules. T he rest of the kidney is normal.

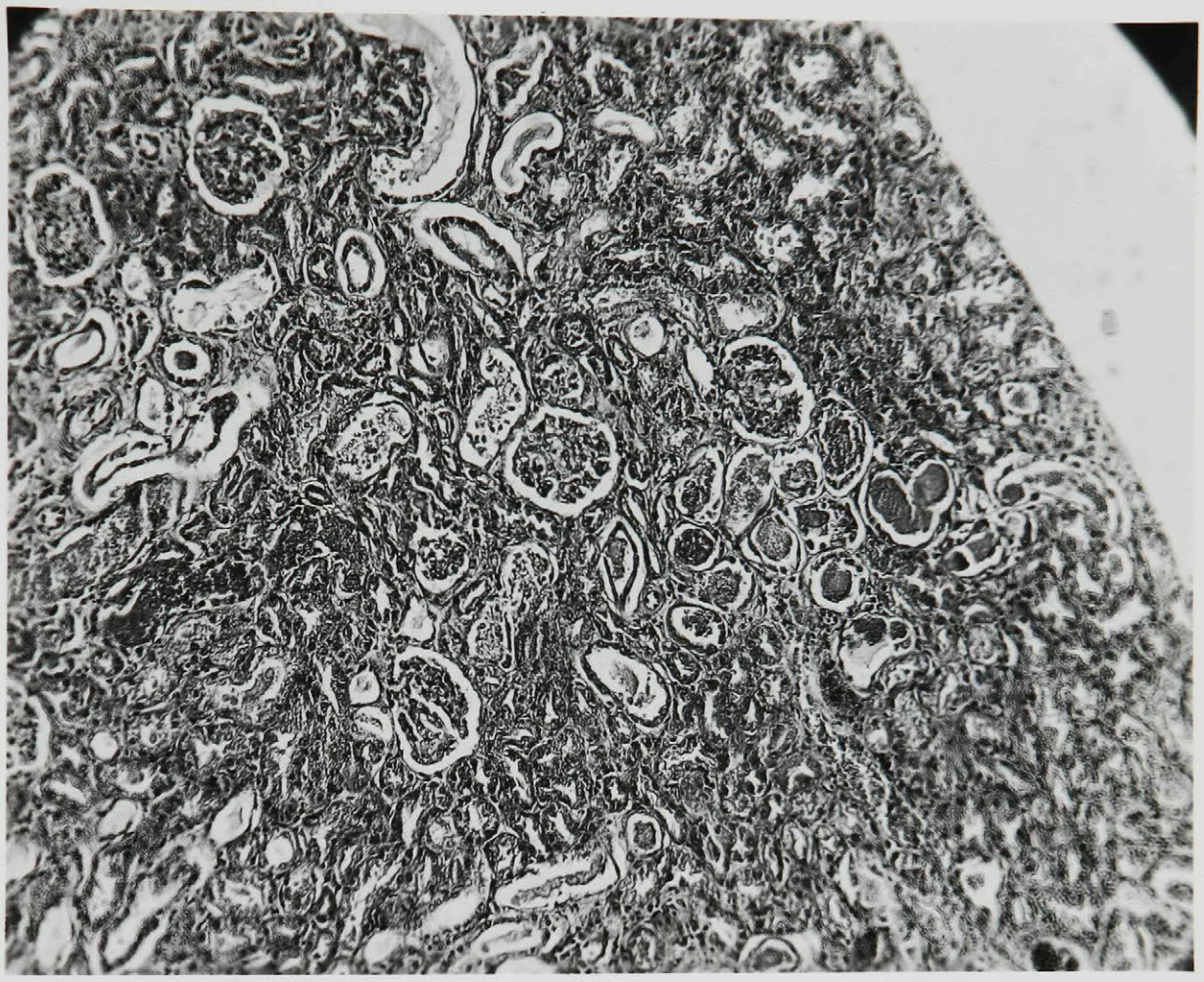

Fig 4d. Kidney, rabbit No.36 (hemotoxylin and eosin X 110) Acid urine. Treated with sodium tartrate subcutaneously and hemoglobin intravenously when the N.P.N. was falling. Died six days after hemoglobin injections. T here are many hemoglobin casts in dilated tubules. Degenerative changes are mild.

haemoglobin casts were occasional or rare. In other words, irrespective of the method of tubular injury, the alkaline kidneys lost many of their casts within a few days after haemoglobin injection. On the other hand, in the histological studies on acid animals many haemoglobin casts were seen, and no fewer casts were noted in the group used for functional studies; so that haemoglobin casts persisted in the kidneys of acid animals, and did not remain in those of alkaline animals. The persistent casts were denser than the initially precipitated haemoglobin casts, and were associated with greater degrees of tubular dilatation and epithelial compression.

PART 3: DISCUSSION

Perusal of the literature and a survey of the foregoing experimental findings, brings to light certain factors which play an integral part in the mechanism of the renal failure seen in transfusion reactions, Blackwater fever, the crush syndrome, and many other conditions in which anuria is associated with the release of haemoglobin or related pigments into the blood plasma. In discussing these factors it would be well first to examine the data observed in these investigations, and then to relate this with the work of others.

The preliminary histopathological studies proved conclusively that haemoglobin will not precipitate in the tubules of a normal kidney. This was also shown by many investigators (vide supra).

Other morphological experiments did make it clear, however, that haemoglobin casts would form in a damaged kidney, irrespective of the urinary pH and the quantity of circulating haemoglobin. As long as the kidney was injured, some casts were always found. Yorke and Nauss[101] did not have normal rabbits, De Gowin[29] gave impure haemoglobin, Melnick et al[73] were dealing with abnormal conditions resembling transfusion reactions, and Bywaters and Stead[24] probably caused renal damage by giving human myoglobin. Only the results of Baker and Dodds[6] cannot be explained, and it is felt that lack of confirmation is ample refutation of their beliefs.

Having established the factor necessary for haemoglobin precipitation, its role in the production of renal failure must be determined. Are the haemoglobin casts merely an incidental finding? Are they simply the result of the renal failure, playing no part in its mechanism?

This work has shown that they do play a part, and that their effect is dependent on the pH of the urine. Other workers, whether

consciously or unwittingly producing kidney damage, had no way of knowing exactly what caused the functional disturbances they observed, some saying that haemoglobin casts blocked the tubules, others that the pigments were toxic to the tubules, and others, that the pigments were of no consequences. In the experiments described above, it was seen that in the acid state, the injection of haemoglobin after tubular injury results in the formation of persistent pigment casts and, in itself, leads to an elevation of blood non-protein nitrogen which may progress until death ensues. In alkaline animals, however, although the damage allows haemoglobin to precipitate, not nearly so many casts are formed, those that do form do not persist, and no elevation in non-protein nitrogen accompanies their formation. In the 25 minute clamping experiments, the rise in non-protein nitrogen seen in alkaline controls, was prolonged but not increased by the injection of haemoglobin. It is thus evident, that since the greatest nitrogen retention is accompanied by the most extensive cast formation, and no nitrogen retention is seen with little cast formation, that the casts themselves can cause nitrogen retention. Since they can conceivably do this only by blocking the tubules, it follows that tubular blockage does play a part in the mechanism of the renal failure under discussion. The dilatation and compression of tubular epithelium accompanying the haemoglobin casts, lends added support. The production of casts is dependent on the pH of the urine, since the acid rabbits suffered the greatest cast formation The finding of some casts in alkaline animals may possibly depend on the pH of individual nephrons. The one fatality in an alkaline rabbit was not associated with haemoglobin casts in the renal tubules, and was shown by post mortem examination to have resulted from a very severe initial tartrate injury.

Admitting the significance of urinary pH, the fact remains

that precipitation of haemoglobin depends primarily on tubular damage. The observations made in these experiments clarify the relationship between the precipitation and the primary injury. There was found to be an optimum degree of tubular damage in conjunction with which haemoglobin cast formation is at a maximum, and this holds true at any given level of plasma haemoglobin concentration and of urinary pH. From these experiments, the "optimum" damage can only be characterized as moderate, lacking any finer means of distinction. That haemoglobin precipitation may follow very minimal kidney damage was proved by the discovery of casts in unclamped kidneys of acid rabbits subjected merely to anaesthesia and the temporary clamping of the opposite renal pedicle. If one compares the results of the 15 minute and the 25 minute clamping experiments, it will be seen that these conclusions are borne out, that is, there is a relationship between the degree of damage and the degree of cast formation and functional disturbance. Acid animals clamped for 15 minutes and given haemoglobin, formed moderate numbers of casts, and showed transient nitrogen retention, while those clamped for 25 minutes developed rapidly rising non-protein nitrogen levels, complete suppression of urine, and died in uraemia. Cast formation was massive. Again, in the sodium tartrate series, it was seen that the greatest numbers of haemoglobin casts and the greatest nitrogen retention occurred in the acid animals in whom tartrate injury was moderate. Therefore, it can be said, that up to the point of moderate injury, a direct relationship exists between the degree of tubular insult and the quantity of haemoglobin precipitated in the kidney. The manner in which the damage permits the precipitation is open to speculation, but it is known that pH in the tubules is of singular importance. It is of interest to note at this point that the location of the casts co-incides to a great extent with the site

of acidification of urine[38].

Beyond the point of moderate injury the relationship breaks down. When the damage is severe enough to result in widespread tubular necrosis, haemoglobin does not precipitate even though the urine is strongly acid, and a large amount of haemoglobin is injected. This observation was made from the histological and functional experiments with sodium tartrate. No clamping experiments were performed where severe injury was produced by prolonged ischaemia and followed by haemoglobin injection. Nevertheless, the striking similarity in appearance of the kidneys after large doses of sodium tartrate and after lengthy clamping (Scarff and Keele, 92) makes it feasible to assume that haemoglobin would not precipitate when clamping is the cause of great damage to the renal tubules. If increased damage permits correspondingly increased haemoglobin cast formation, why should there be no cast formation when damage is most severe? The most likely answer is that the excretory mechanism is interfered with, preventing the entrance of haemoglobin into tubular lumina.

The level of haemoglobinemia has been casually mentioned above, but its importance must not be overlooked, and requires re-emphasizing. In two acid rabbits given tartrate it was found that haemoglobin injection caused little or no change in nitrogen retention. Since they were given small amounts of haemoglobin, while two others who received larger amounts succumbed with cast filled kidneys, it can be safely said that the degree of haemoglobinemia is a factor in the amount of cast formation and associated functional disturbance. It has been stated earlier in this discussion that the level of haemoglobinemia has no influence on the precipitation of haemoglobin. This seems confusing in the light of what is shown here. Nevertheless, both statements are true. Whether or not haemoglobin will

precipitate is absolutely independent of the quantity of circulating pigment, but, once the stage is set for cast formation, the number of casts formed, depends, among other things, on the level of haemoglobinemia.

There is, then, a complicated mechanism by which the kidney fails in these disorders which are characterized by haemoglobinemia and haemoglobinuria. The first and most important factor is damage to the renal tubules. This is doubly important, because not only can it result in renal failure of itself, but because it is also the pre-requisite for pigment precipitation. The next factor is this pigment precipitation and it has been shown that it, too, can cause death. It has been found that it depends on the pH of the urine and on the degree of tubular damage.

In other words, if a patient suffers a transfusion reaction, he may die of severe injury to the renal tubules. If the tubules are not damaged badly enough to result in renal failure, then the circulating pigment may precipitate in the damaged renal tubules and cause nitrogen retention by mechanical blockage. If the transfusion was small, and/or if the urinary pH is high, only a few tubules may be plugged and the patient may survive. On the other hand, if there is a large amount of circulating haemoglobin and a low urinary pH, blockage may be massive. Again, if death is due to severe tubular injury, extremely few haemoglobin casts will be found. The ultimate anatomical and functional result in any given instance depends upon a fine balance being struck between the degree of renal injury, the level of haemoglobinemia, and the pH of the urine. The degree of renal injury is the most important, for when it is marked, the other factors do not operate at all. This concept offers an explanation for the extreme variations seen clinically. It explains why blockage is so frequently not a prominent feature.

In this work the greatest stress has been laid on the renal precipitation of haemoglobin, the factors which influence it, and its significance, once it has occurred. It is felt that this portion of the problem has been solved, although it remains to be pointed out how tubular damage permits precipitation and how a low pH influences this. It is likely that acid hematin or perhaps methaemalbumin is deposited in the acid state, but why this should occur in damaged tubules and not in normal ones is difficult to say, unless the damage results in a further pH drop, and perhaps also puts a halt to normal tubular re-absorption. Perhaps the low pH in otherwise normal subjects is not low enough to result in the chemical reaction of haemoglobin precipitation, and if lowered still further by tubular damage, the chemical reaction can take place. Or it may even be that the reverse holds true, the damage resulting in an elevation of pH to an optimum level. But this is all conjecture, and one could cover many pages with such speculation.

The mechanism of the production of renal tubular injury has not been investigated in these studies, but deserves some attention here. The literature has been seen to contain a multitude of theories To recapitulate, some laid stress on a pigment derivative directly affecting tubular epithelium(9, 10, 2, 12, 24), others thought pigment to act indirectly(71, 56, 82, 3). Theories of a newly liberated, directly acting, nephrotoxin other than a pigment were offered, (51, 54, 37) the most popular suggestion being an intermediate product of protein metabolism. Others,(59, 83, 84) thought such a substance acts indirectly through a vascular mechanism. Besides these nephrotoxic theories there are others which lay the tubular injury to severe anaemia, loss of blood volume, circulatory collapse (47, 95, 25, 67). It is by no means necessary or wise to attempt to choose one cause and to rule out all others. Rather is it likely

that different factors operate in different conditions. Perhaps toxic action of a protein break-down product is important in black-water fever, and perhaps methaemoglobin and/or methaemalbumin is the factor in the sulphonamide haemolytic crises. It is also likely that combinations of factors operate to produce renal tubular injury. The mode of production of tubular injury in all the disorders spoken of in the introduction, is probably not the same, and, therefore, this part of the problem requires special investigation for each case. All the causes, however, lead to a final common path - the alterations seen in the renal tubules. That such alterations may be etiologically non-specific is also indicated by the similarity of the morphological pictures obtained with a specific chemical poison, sodium tartrate, and by transient complete renal ischaemia.

In reply to those who hold that the renal failure is insufficiently accounted for by the morphological alterations in the kidney[72, 94, 52, 98, 90], one can only draw attention once more to the experimental results obtained in this work. While these experiments do not rule out the possibility of physiologic or functional uraemia, they certainly indicate that the other is much the more frequent occurrence, and this is supported by the fact that human material more often than not, shows adequate anatomical change.

The findings of those who thought they had produced cast formation and azotemia in normal animals, have been explained. The "added" factor has been found to be tubular degeneration, and it has been shown that this is not merely a complementary factor, as De Gowin and others believed, but rather the primary essential feature, which could act singly, or as a basis for the second feature — pigment precipitation, the two then acting together. In considering the essential factor it was argued that it could originate in different ways. The experimental work lent support to the non-

specificity of this factor, but, being chiefly concerned with the second feature, pigment precipitation, proved the importance of urinary pH in this regard, and emphasized the significance of the level of haemoglobinemia. The influence of quantity of haemoglobin was stressed also by Bordley[12] who said that the amount of blood transfused is of definite importance. He found that of those patients who received less than 350 ccs. of blood, none succumbed, while all patients suffering a reaction after more than 540 ccs. of blood, died.

The main discrepancies found in the literature have been explained. No new mechanisms were offered, but a proper evaluation of the old ones was made and some which had been discarded were re-established in their proper light.

PART 4: SUMMARY AND CONCLUSIONS

1. Review of the literature (a) proved that the renal failure in transfusion reactions, crush syndrome, blackwater fever, sulphonamide haemolytic crises, eclampsia with haemolysis, and burns, is of the same nature and must have a common mechanism. (b) It showed the many conflicting theories about the mechanism, part of the mystery being due to the difficulty encountered in reproducing the typical lesion.

2. A readily reproducible anatomical picture, like that seen in the "transfusion" kidney, was obtained by injecting haemoglobin into rabbits whose renal tubules had been previously injured (a) by temporary complete ischaemia and (b) by administration of a specific chemical poison, sodium tartrate.

3. It was found that if the renal tubules are damaged, haemoglobin will precipitate, in contrast to the absence of precipitation in normal kidneys. Damage is the determining factor, and not urinary pH or quantity of circulating haemoglobin.

4. The extent of haemoglobin cast formation does depend on the pH of the urine and on the level of haemoglobinemia - within the limits of moderate tubular insult. In the acid state haemoglobin casts are more numerous and more persistent than in the alkaline state.

5. No functional disturbance results when few casts are formed, while marked nitrogen retention accompanies great numbers of casts. Thus, blockage of renal tubules can have a marked effect on the final outcome.

6. Up to a point the number of casts varies directly with the degree of tubular damage, other factors being constant.

7. When the initial injury is very severe, few to no haemoglobin casts are formed, no matter how high the level of haemoglobinemia, or how low the urinary pH. Animals given haemoglobin after such an injury die of the initial insult, with no functional or histological evidence of haemoglobin effect.

8. Therefore, the result in any one case depends on a balance between tubular damage, level of haemoglobinemia, and urinary pH. If damage is severe, death results from it alone. If damage is mild to moderate, death may be caused by tubular blockage, depending on the amount of haemoglobin present, and the degree of acidity.

9. These experimental truths have been discussed, and the discrepancies in the literature have been dealt with.

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