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PATHOLOGY DEPARTMENT
UNIVERSITY OF EDINBURGH

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George Lyon

INFLAMMATORY CHANGES IN THE KIDNEY

An Experimental Study of the action of some Toxines and Poisons upon the Kidney, and also upon the Spleen.

A THESIS

submitted for the Degree of Doctor of Medicine,

by

GEORGE LYON, M.B., Ch.B.

The work, which forms the basis of this paper, which I present as a Thesis for degree of M.D., has been done in the Pathological Laboratory of the University of Edinburgh. It was undertaken, on the suggestion of Professor Greenfield, and has been conducted entirely as an experimental research. Its primary object was to determine the earliest changes which occurred in the kidney in conditions of acute inflammation, and to ascertain if possible, the relationship between lesions of the blood vessels and those of the secreting structures. As the research advanced, the scope of the work was extended, for in the systematic examination of the other organs in the experimental animals, changes were found, especially in the spleen, which rendered it advisable to include this organ in the research.

The pathogenesis of spontaneously arising kidney inflammation and the changes which it initiates, have long been the subject of much dispute. That this should be the case, is readily apparent when we consider, that in the study of human pathology an opportunity is rarely presented of studying the earliest changes in such inflammatory conditions. Most frequently, we have to study the organ in later/

later and more advanced conditions of the lesions when it is often impossible to say what has been the exact evolution of the process. It was therefore deemed possible that the experimental method might be of some service, as we could thereby produce artificially, acute inflammatory conditions of the kidney by the introduction of certain poisons or toxic substances, and determine the earliest changes, and ascertain if possible, the relationship of these changes to each other. The phenomena of inflammation in the kidney are essentially of the same nature as in other tissues and organs, but we here have to deal with an organ which possesses a more complex structure. Then, now-a-days, in the consideration of this question, due regard must be given to the new ideas of phagocytosis and chemotaxis as developed and extended by Metchnikoff and his school, in addition to the vascular phenomena, which, since Cohnheim enunciated his views on this subject, have been regarded until recent times as the essential characteristics.

HISTORICAL PART:

The literature on kidney inflammation is so voluminous that only a short review will be here attempted. From the time Bright published his observations/

observations on "Renal disease accompanied by the Secretion of Albuminous Urine", in 1827, many classifications of Bright's disease have been advanced, but even at the present time, there is no one which commands universal allegiance among pathologists. We can now regard the term Bright's disease as being synonymous with nephritis or inflammation of the kidney, if we exclude those septic inflammations due to presence of micro-organisms in the organ. Included also in the category of Bright's disease, is that form which is dependent on primary changes in the blood vessels, and is degenerative in character, but to which inflammatory changes are frequently superadded. Among older writers, the true pathogenesis of this latter variety was not recognised. Thus, we find that the pathological processes in Bright's disease were regarded as manifestations of a single disease, and the different naked eye and microscopic appearances which were found, corresponded to different stages. The process, however, was regarded as essentially, inflammatory in nature. Reinhardt(56) in 1850 and Frerichs(21) in 1852 supported the earlier views and regarded the shrunken granular kidney as representing the last stage in the process. However, as early/

early as 1853, Wilks[65] dissented from this view of the unity of the process and although up to 1875 most German authorities, (e.g., Klebs) regarded the acute and chronic forms as stages of one process, yet by this time most English pathologists divided Bright's disease into two groups, those in which the secreting tubules were principally affected and those in which the chief affection was in the interstitial tissue, the latter being regarded as essentially chronic in nature. Tranbe[60] in 1871 was among the first to separate the granular contracted kidney from the other varieties of Bright's disease, both on clinical and pathological grounds. Gull and Sutton[26] supported Tranbe's views and described the condition which they designated, arterio-capillary fibrosis. They regarded the contracted kidney as due to the local expression of this general disease. Johnson,[30] although he made many accurate observations on the lesions in the arteries in Bright's disease, still believed the small granular kidney was due to primary degeneration and disintegration of the renal epithelium. But it was Greenfield[23] in 1879, who clearly recognised the atrophic kidney as due to primary disease of the blood vessels. Ziegler, about the same time, also came to similar conclusions/

conclusions, as a result of his study of the diseases of the blood vessels. Much confusion long existed owing to the inclusion in the category of Bright's disease of those parenchymatous changes which are purely degenerative and occur in such diseases as diabetes and alcoholism and in phosphorous poisoning.

Weigert,(62) in 1879, seperated these degenerative changes from those which were inflammatory in nature. Meantime, the classification of inflammatory lesions must be founded on an anatomical basis, since an aetiological classification is not yet possible, for different substances may give rise to similar lesions, while the same substance in different individuals may give rise to various forms of lesions.

Among recent writers, the lesions are classified according to the tissue of the organ especially affected and these are variously classified as acute, subacute, and chronic. Thus we have an acute glomerulo-nephritis and an acute parenchymatous, or tubular nephritis. Councilman,(13) designates a third type as acute interstitial nephritis, where the chief lesions appear to be in the interstitial tissue. Under Subacute, are especially included those/

those forms, which most frequently arise insidiously, and in which the interstitial and glomerular changes are prominent. Under chronic nephritis, besides the primarily vascular variety, are grouped those types which commonly present induration, and in which the overgrowth of fibrous tissue has progressed so as to form the main characteristic. These cases of chronic diffuse nephritis are for the most part of slow, insidious origin and simply represent a more advanced condition of subacute diffuse nephritis. Subacute and Chronis diffuse nephritis may be sequel of an acute inflammation of the kidney. This is sometimes seen in the nephritis due to Scarlet fever, but the great majority are subacute or chronic from the outset, and are due to the influence of some unknown agency. Waxy disease of the kidneys, is included in most recent classifications of Bright's, for in this condition there is invariably, associated inflammatory lesions.

PATHOLOGICAL CHANGES IN THE CONSTITUENT TISSUES OF THE KIDNEY:

We shall now pass under review, the various pathological changes which the different structures of the kidney undergo, as the result especially of inflammatory/

inflammatory processes. Thus, we have to consider changes in the blood vessels, in the Malpighian bodies, in the glandular structure, and in the interstitial tissue.

BLOOD VESSELS:

The Kidney has an abundant blood supply, and Bowman[7] was the first to accurately determine its circulation in the cortex. He demonstrated the presence of two distinct sets of capillary systems, the one in the glomerular tuft in association with its afferent arteriole, the other between the tubules in association with the efferent vessel of the tuft. The branches of the Renal Artery distributed to the cortex were regarded by Cohnheim as being end arteries: but this conception is not strictly accurate, as there is an anastomosis, but very imperfect, between arteries distributed to neighbouring areas. The afferent arteriole to the glomerular tuft is not the only source of blood supply to its glomerular unit, for there is anastomosis with the capillaries distributed to adjacent tubules. This distribution of blood vessels in renal cortex is responsible for the occurrence of many pathological conditions in the Kidney. As the result of embolic blocking of interlobular artery or its branches/

branches, there is the production of an infarct, and the imperfect anastomosis which exists between capillaries in the obstructed area and those in the capsule and surrounding areas, is responsible for the evanescent engorgement which occurs in the area of infarction. Further, Greenfield[23] has pointed out, that in the atrophic kidney, the terminal parts of the interlobular arteries show the earliest changes and the consequent area of atrophy corresponds in shape and extent to that seen in infarction, the result of embolic obstruction. The ingrowth of new capillaries from the vessels of the capsule into the area which is being insufficiently nourished, and is undergoing atrophic changes, was noted by the same observer.

MALPIGHIAN BODIES:

Under this heading, the changes in the afferent and efferent arterioles and glomerular capillaries, and those in the glomerular epithelium, and in Bowman's capsule with its lining endothelium, come under consideration. Where there is obstruction to the blood flow in the afferent arteriole, or in the glomerular capillaries, important changes result in the glomerulus as a whole, and also in the tubules/

tubules which form constituent parts of the glomerular units. The circulation through the glomerulus may be prevented by the occurrence of changes in the walls of the vessels, by the formation of thrombi within them, and in addition by changes originating outside the vessels. In Scarlet fever, Klein (36) described a hyaline swelling of the intima of the afferent arteriole, and also of the glomerular capillaries, whereby partial or complete occlusion of their lumina was produced. A similar acute hyaline swelling of these vessels has been observed in other acute infectious diseases, particularly in diphtheria, by Oertel and other observers. The occurrence of this hyaline swelling is a phenomenon of considerable interest in relation to the action of toxic diseases and the consequent lesions in the kidney. Does the circulating toxine act primarily upon the vascular structures, and are the changes in the tubules to be regarded as secondary and consecutive?

/Oertel (50)

In Scarlet fever, there can be no doubt that the hindrance to the circulation in consequence of the hyaline swelling of the vessel walls, is in many cases a factor in the evolution of the tubular changes, apart from any direct action of the toxine/

toxine upon them. A more subacute or chronic hyaline degeneration of afferent arterioles and tuft capillaries occurs in association with similar changes in the vessels of the spleen. This is probably due to the action of a toxine of unknown nature, and when many of the glomerular tufts are affected, there is extensive atrophy of the Kidney tubules.

Thrombi of various kinds may be found within the capillaries of the tuft. In acute inflammatory conditions of the kidney occurring as the result of acute infectious diseases, hyaline and fibrinous thrombi have been found by Klein (36) and Councilman (14)

In addition, the lumina of the capillaries may become blocked by a cellular mass derived from the proliferation of the lining endothelium. This has been described by Welch (63) in Acute poisoning by Cantharidin, and by Councilman (14) and Langhans (38) in many of the acute inflammations of the kidney in man.

Compression and obliteration of glomerular vessels, in consequence of changes originating external to them, are seen, especially in the Subacute and Chronic inflammations, where there is proliferation of the endothelium lining Bowman's capsule.

Sometimes/

Sometimes the proliferation and accumulation of cells is especially advanced in the vicinity of the root of the glomerulus, and the larger vessels may be thus early compressed. As regards the presence of connective tissue cells between the capillaries of the tuft and their proliferation in acute inflammatory conditions so that thereby the capillaries become compressed, there is not much conclusive evidence, although Klebs (35) supports this view. Councilman also describes a proliferation of connective tissue cells between the capillaries in acute inflammations. But in the course of the more chronic fibrous atrophy of the glomerular body, connective tissue cells can frequently be demonstrated between the capillaries. Whether they have originated from connective tissue cells normally present, or have extended inwards along the vessels, it is difficult to say.

GLOMERULAR EPITHELIUM:

Herring (29) has shown, in his work on the development of the Malpighian bodies, that the epithelium which covers the tuft is to be regarded as essentially different in origin and function from that lining Bowman's capsule. In the foetus, the capillaries/

illaries of the tuft are covered by a single layer of cells almost cylindrical in form, and the division of the glomerulus into lobes results from the penetration of the epithelial cells between the capillary loops. When glomerulus is fully formed, these cells become smaller and appear flattened. Bowman's capsule, on the other hand, is lined by cells which are long and flat from an early stage, and are developed from cells closely allied to those which form the lining of the general peritoneal cavity. The cells covering the glomerular tuft are more highly organised, and their reaction in disease is comparable to that occurring in the secreting cells.

In acute inflammations, the glomerulus is affected/ed to a greater or less extent, according to the nature of the exciting cause. Klebs[35] first drew attention to these changes in the glomeruli, which he found especially in Scarlet fever and called the condition "glomerular nephritis". Swelling of tuft with dilatation of its capillaries occurs, and sometimes is very considerable. Mention has already been made of the occurrence of hyaline swelling of vessel walls and of the presence of thrombi within the lumina. These swollen tufts always/

always show a richness in nuclei, and much discussion has arisen concerning their origin. There are several possible sources. They may arise by proliferation of the glomerular epithelium, of the endothelium lining the capillaries, or by proliferation of connective tissue cells, if such normally are present. In addition, there may be, as elsewhere, an accumulation of leucocytes within the vessels, and these may emigrate from the capillaries and be found lying between them or free in the capsular space. This accumulation of leucocytes and their emigration into the capsular space has been described by Cornil and Brault,(12) as occurring in an extreme degree in acute cantharidin poisoning. In acute inflammations occurring during the course of acute diseases, Cornil and Brault regarded the increase of nuclei in the tuft as derived from a proliferation of the glomerular epithelium. These cells, according to them, are not epithelial in nature, but exhibit the characters of connective tissue cells. Fibrillary intercellular substance was formed between them and the subsequent contraction produced compression of the capillaries. Kelsch and Kiener,(34) also regarded the increase in cells, as epithelial in origin. Whilst in all acute inflammations of the glomeruli, these cells/

cells are found to show swelling and frequent desquamation, no one has supported Cornil and Brault's contention that they form fibrous tissue. This view is in entire opposition to all that is known of the nature and character of these cells. While most observers admit a detachment and a certain degree of proliferation of these epithelial cells, many regard the majority of the nuclei to be derived from a proliferation of the capillary endothelium. Nauwerk,(48) in a case of diphtheria, observed what he believed to be a proliferation of these cells. Langhans,(38) has made a careful investigation into the changes occurring in the glomeruli in acute inflammations, especially in Scarlet fever. Besides a detachment and proliferation of glomerular epithelium in association with the emigration of leucocytes, he found the capillaries filled with a protoplasmic mass rich in nuclei. The nuclei, he believed to arise by a proliferation of the endothelium, although there was the absence of mitotic figures. This accumulation of nuclei lying in a protoplasmic reticulum, leads to an occlusion of the lumina. Welch has described the glomerular changes as the result of poisoning white rats with Cantharidin, and has used the term "intra-capillary glomerulitis"/

glomerulitis" to describe these cases in which accumulation of proliferated endothelial cells within the capillaries, was the chief phenomenon. Councilman,[14] as the result of numerous observations, found proliferation of cells in and among the vessels of the glomeruli in practically all cases of Acute and Subacute inflammatory conditions of the glomerulus. Those within the vessels, he regarded as endothelial, and some of those between the vessels as connective tissue cells. Klebs, who was the first to study the glomerular changes in Scarlet fever, describes proliferation and desquamation of the cells covering the glomerulus, and also an increase in nuclei in the glomerular tuft, which he regarded as arising by the proliferation of connective tissue cells between the capillaries. Hansemann[27] came to the same conclusion as Klebs. Ribbert,[57] regarded the accumulation of cells within the capillaries as a thrombus of white blood corpuscles.

BOWMAN'S CAPSULE AND ITS LINING ENDOTHELIUM.

There is nothing definitely known concerning its origin and intimate structure. There is much evidence in favour of the view that it consists of a mosaic of flattened cells, which have undergone

a/

a fibrous transformation with the presence of potential nuclei imbedded in its substance. In the development of the kidney, Bowman's Capsule, as seen in adult kidney, is not present, but is represented by several layers of flattened cells. In many pathological processes, specially in Subacute and Chronic Nephritis, it often seems to become separated into several layers with intervening nuclei. Bowman's Capsule is not reflected over glomerular tuft. The cells lining Bowman's capsule are elongated flattened cells, and Greenfield (23) had long ago pointed out the similarity in reaction of these cells to endothelial cells. Herring's work supports this view, and he regards the cavity of Bowman's Capsule as a body cavity specially differentiated for urinary secretion. When we trace these lining cells towards the point of origin of the secreting tubule, we find that the flattened cells are replaced by more cubical cells as we reach the neck. In the cat's kidney, these cubical cells, with granular protoplasm, extend inwards, lining Bowman's Capsule to a varying distance beyond the neck of the tubule. In the rabbit, this is not so prominent. These cells resemble the secreting cells in structure and show the same reactions, and in many acute conditions, they become detached and accumulate in the capsular space. Greenfield (23×24) first drew/

drew the attention of pathologists to the importance of the glomerular changes, especially those occurring in Bowman's Capsule and its lining endothelium, at a time when the tubular changes were considered of paramount importance. He pointed out that distinct and important lesions were to be found in the glomeruli constantly in subacute and chronic nephritis and in many acute cases, especially in Scarlatinal nephritis. Proliferation of the endothelium covering Bowman's Capsule had been noted by previous observers, but its true significance was not appreciated. Klebs and Klein,(36) in their studies on the Kidney in Scarlet Fever, had observed the presence of nuclei imbedded in granular masses in the capsular space, and concluded they were derived from a proliferation of the cells of Bowman's Capsule. Kelsch,(33) in Scarlatinal nephritis, recognising that the morbid process starts frequently around the glomeruli, considered the changes which occurred in Bowman's Capsule as secondary to interstitial changes, which were found around the glomeruli. Greenfield showed that proliferation of the capsular endothelium was of constant occurrence in the subacute and chronic nephritis, and also in many acute cases. Rapid pro-

liferation/

liferation of the capsular endothelium accompanied by a swelling of the Capsule itself, is found in many acute diseases. It occurs most constantly in Scarlet fever. In other acute infectious diseases, while there may be swelling of the capsule itself accompanied by a granular swelling of the endothelial cells, actual proliferation has not been so commonly observed. Councilman,(14) however, found proliferation in the great majority of his acute cases, where glomeruli were especially affected. Welch(63) found in white rats poisoned with Cantharidin, the condition which he designates "desquamative glomerulitis." There was proliferation and desquamation into capsular space of the endothelium of Bowman's Capsule. Cornil and Brault described similar appearances in their Cantharidin experiments. When there is rapid proliferation, masses of flattened cells arranged in a crescentic fashion, lie between the capsule and the tuft. Proliferation once initiated, leads frequently to progressive changes, which finally end in the obliteration of the glomerular tuft. In those cases in which the proliferative changes may have originated during the course of an acute disease like Scarlet fever, or, as is much more frequently the case, have started as a slower/

slower and more insidious process in consequence, probably of an intoxication of unknown nature, the progressive changes which occur in relation to the proliferated capsular endothelium are the direct causative factors in the glomerular obliteration, and the consecutive changes which thereafter occur.

But in those cases where there is primary lesion in the blood vessels, the proliferative changes of capsular endothelium are secondary and consecutive to occlusion of glomerular capillaries. The proliferated cells often form attachment to the glomerular tuft and extend inwards between the capillary loops. There is the projection of capillaries into the mass of newly formed cells, through Bowman's capsule, and there is the formation of organised connective tissue, a process which is analagous to endarteritis obliterans. The glomerular tuft becomes compressed and shrinks, and its capillaries become occluded, most frequently by a simple hyaline thickening of their basement membrane, but there may be also an increase in the connective tissue cells between the capillary loops. Some observers have described the occasional obliteration of the capillaries in another way. This occurs where there is a marked proliferation of capillary endothelium and a hyaline change in these cells/

cells will lead to complete occlusion. Bowman's Capsule also shows changes. It may appear single and thickened, but more commonly it is represented by several hyaline layers separated by nuclei. There is an associated periglomerular proliferation of fibrous tissue.

CHANGES IN THE TUBULES:

All the glandular elements, with the exception of the collecting tubes, are mesoblastic in origin. The collecting tubes are probably of epiblastic origin. When the Kidney is subjected to the action of injurious agents, the collecting tubes enjoy a much greater immunity than the secreting tubules. The convoluted tubules of the cortex and the aseending limb of Henle's loop have a similar structure and function, and their reaction in disease is closely parallel. For long, the lesions occurring in the tubular epithelium were regarded as the essential and primary lesions in all varieties of Bright's disease. In all acute diseases, in which there is the presence of a toxic substance in the blood, the secreting epithelium is always more or less affected according to the nature of the circulating toxine. While some of the soluble toxines and poisons act especially upon the secreting epithelium/

epithelium, others may produce more marked effects upon the glomeruli or interstitial tissue. While all the structural elements of the Kidney are interdependent as regards their reaction in acute or chronic inflammatory processes, it is difficult to estimate the true relationship of the lesions occurring in its different constituents. The atrophic and degenerative changes in the tubules, when there is slow obliteration of afferent arterioles or glomerular capillaries, and the necrotic lesions in embolism of renal artery or its branches, are readily correlated to their cause: but in those cases in which the toxic agent produces acute lesions in the glomeruli and there is the occurrence of rapid hyaline swelling of glomerular vessels, or it may be, the presence in their lumina of hyaline thrombi or swollen and proliferated endothelial cells, so that partial or complete occlusion of many glomerular capillaries results, it is then difficult to exactly gauge how much of the tubular change is due to the direct action of the toxine and how much is secondary to the vascular lesions. Some of those toxic substances appear to be excreted by the glomeruli, producing effects in these parts, and the toxic urine, by its presence in the capsular space and in the lumina of the tubules, will thus affect these structures/

structures later. On the other hand, the soluble toxine may pass in a more concentrated form through the glomerular tuft to the intertubular capillaries and tubules, the glomeruli not being gravely affected. Some poisons, as will be shown later, will affect the secreting tubules very irregularly and this distribution of the lesions must be referred to one of several causes. It is quite probable that all the glomeruli do not functionate simultaneously, so that the toxine or poison will only produce its effects in relation to the functioning glomerular unit. The possibility that different glomerular units possess different vulnerabilities is not to be gainsaid. We have evidence of this in certain cases of subacute diffuse nephritis, where, as pointed out by Greenfield, the glomeruli in the deep part of superficial cortex always show the earliest affection. There is no conclusive evidence in support of the view that the site of some acute lesions is determined by physical condition of the circulation. The tubules also may be affected secondarily to infiltration of cells in the interstitial tissue. A necrosis of epithelium may be thus produced in consequence of compression of tubules and vessels. The changes which occur in relation to the tubules in inflammatory conditions/

ions correspond to those seen on any secreting surface, but the cells of the secreting tubules represent a very highly specialized tissue. There is transudation of varying quantity of fluid from the subjacent vessels into the lumen of the tubules and into the cells.

Associated with the occurrence of this transudation into the lumen, is the question of the origin and formation of tube casts, which will be considered later. Leucocyte emigration is here, as elsewhere, not wanting. The emigration of leucocytes is always more abundant into the lumen of the tubules than into the capsular space. The form of leucocyte which emigrates, is the polymorphic leucocyte and the number present seems to be dependent on the degree of destruction of tubular epithelium. The mononucleated leucocytes do not appear to be attracted by the degenerating or necrotic epithelium. While the changes in the cells of the collecting tubes are comparatively simple in character, those occurring in the secreting cells, present a much greater variety. In the secreting tubules, the lesions of the cells are either necrotic or degenerative in character, but the consideration of these cellular changes as they affect the protoplasm and nuclei will be taken up later./

later. In nephritis of any duration, there is always evidence of regeneration of tubular cells, and the phagocytic properties of collecting tubule cells will be demonstrated afterwards.

INTERSTITIAL TISSUE:

In the fully developed Kidney, this is small in amount, while in foetal Kidney, it is abundant. In the adult Kidney, it is in most abundance between the larger ducts in the medulla. In the cortex, the amount between the tubules is insignificant and in normal kidneys, the tubules appear separated merely by the intertubular capillaries. It is still, however, present in the form of embryonic connective tissue cells, which possess developmental potentialities. It is more abundant around the larger blood vessels, beneath the capsule, and around the bases of the glomeruli. There is much diversity of opinion as regards the presence of connective tissue cells between the capillaries of the glomerular tufts. The probability is, that there are some connective tissue cells within the tuft, and these may proliferate as described by Klebs(35) and Hansemann.(27) In all acute inflammations of the Kidney, changes which vary greatly in degree are to be found in relation to the/

the interstitial tissue. Every writer on acute Scarlatinal nephritis has described an accumulation of round cells in the interstitial tissue of the cortex, especially around the glomeruli and the larger vessels, and this infiltration of cortex with round cells seemed to be chief lesion in some cases. Kelsch(33) regarded it as the typical lesion in Scarlatinal nephritis. This condition was variously described as a lymphoid or round cell infiltration, and whilst many regarded it as a primary interstitial change, others believed it to be secondary to a primary degeneration in the tubules. The cells, by most observers, were considered to be emigrated leucocytes, though by some, their origin was thought to be from the connective tissue cells.

With the advance in histological methods, their true nature and origin has been determined. Councilman(13) has made an exhaustive study of these cells. He found an accumulation of these cells in the interstitial tissue of the cortex and medulla, not only in Scarlet fever, but in diphtheria and other acute infectious diseases, especially in children. This condition, he designates acute interstitial nephritis, and considers it to be a primary interstitial lesion accompanied by, but independent of any degeneration of epithelium. Councilman believes these/

these cells to be derived from the lymphoid cells of the blood and regards them as identical with the plasma cells of Unna. They may emigrate as plasma cells, or may be formed from emigrated lymphoid cells. Local accumulation of similar cells may also frequently be found in Subacute and Chronic diffuse Nephritis.

TOXINES AND POISONS USED IN THIS RESEARCH:

To study the acute inflammatory changes in the Kidney, it was necessary to use toxines and poisons which produced these constantly and in a sufficiently marked degree. For this purpose, such poisons as phosphorus, which only produce degenerative changes, were of course useless. In the experimental study of the early inflammatory changes in the Kidney, a considerable number of substances have been used. The chief have been Sulphuric Acid, Chromic Acid and its salts, Cantharidin, various salts of Mercury, Oxalic Acid and Aloin. The action of various organisms and their toxines upon the kidneys have been investigated, either as special studies or incidentally in the course of other observations. The action of snake venom, serum of eel, ricin and abrin has also been studied.

Munk/

Munk and Leyden[47] experimented with sulphuric acid, and succeeded in producing an inflammatory condition of the Kidney, and observed especially a nuclear increase in the interstitial tissue. This substance was also used by Litten,[41] who found that inflammatory changes were generally absent during the first few days, but frequently found in the second week, some evidences of inflammatory reaction. In the hands of other observers (e.g., Burmeister) no inflammatory reaction could be induced by this substance, and the changes which were found were purely degenerative changes in the epithelium. Chromic Acid and its salts have been extensively used, especially neutral Ammonium Chromate, and the lesions produced, are necrosis of the secreting cells and a considerable emigration of leucocytes around the larger vessels in the early days of intoxication. In intoxications of longer duration, there was considerable increase of interstitial connective tissue. This substance has been most fully investigated by Burmeister.[9] Cantharidin has not been used by many investigators and the changes induced have been described as a glomerulo-nephritis. The salts of Mercury have been much used, and this poison produces a necrosis of/

of the secreting cells in which there is subsequent calcification. As both these substances have been used in this research, the literature will be referred to later. Novak[49] has studied the histological changes produced in the organs by snake venom. Necrotic changes were found in the secreting tubules of the Kidney. Almost identical lesions were produced in the kidney by the use of serum of eels (Petit)[54]. Lindemann[40] in a research upon the mode of action of renal poisons, studied the effects of the substance vinylamine. The Kidneys lesions closely corresponded to those seen as the result of the action of snake venom and serum of eels, but the glomerular changes were more intense. Flexner[20] in acute ricin and abrin poisoning, found marked degenerative changes and actual necrosis of the secreting cells. The glomerular, but chiefly the capsular epithelium, suffered even more severely often, than that of the tubules. Within certain glomerular capillaries, leucocytes accumulated and at times fused to form imperfect thrombi. The presence of fibrin, in a fibrillar form, in larger vessels and glomerular capillaries was occasionally observed. The changes in the epithelial cells, which are provoked by metallic salts, such as those of/

of Mercury and by Chromic acid and its salts are of the type called coagulative necrosis. These animal and vegetable poisons produce changes in the cells, which resemble those produced by many bacterial toxines. There is destruction of the epithelial cells, characterised by vacuolation and disintegration.

Pernici and Scagliosi,(53) studied the action of various micro-organisms upon the Kidney. They injected animals with Anthrax bacillus, Staphylococcus pyogenes aureus, Bacillus pyocyaneus, and Micrococcus prodigiosus and found the constant occurrence of a glomerulo-nephritis. In one animal, inoculated with Bacillus pyocyaneus, there was very considerable proliferation of the capsular endothelium. Filtrates of cultures of Anthrax bacillus and Bacillus pyocyaneus produced similar, but less intense lesions. The streptococcus toxin and cholera vibrio produce diffuse parenchymatous lesions, but necrotic changes in the cells are not common.

In this investigation, I have used a considerable number of different toxines and poisons, but most numerous experiments have been with Diphtheria toxine, Corrosive Sublimate, Cantharidin, and with the filtrates of cultures of Staphylococcus Pyogenes/

genes Aureus whose virulence had been intensified by passage through animals. Ricin, abrin, tetanus toxine and snake venom were also used in a few cases. The use of diphtheria toxine was adopted, because of its potency to produce hyaline changes in the vessels, and it was deemed the one most likely to throw light on the question of the interdependence of vascular and tubular changes. Cantharidin had been shown by several investigators to produce glomerular changes, and this substance was therefore used to study the origin and evolution of these changes. Corrosive sublimate and other poisonous mercurial salts, cause in lethal doses, a widespread necrosis of the secreting tubules, but in sublethal doses, necrotic changes are much less diffuse. Blocking of many tubules arises in consequence of the calcification of necrosed cells, and one was naturally lead to expect consecutive and consequent changes in the glomeruli, which were in relation to the occluded tubules. So also did chronic mercury poisoning present an opportunity for the study of the changes, if any, which occurred in the interstitial tissue around calcified tubules.

Of late years, special attention has been directed/

directed to the condition of acute interstitial inflammations in the Kidney, to which reference has been already made. The causative factor in this condition is to be referred to the presence of some soluble toxic substance circulating in the blood. Morse (45) succeeded in producing an interstitial change in the kidneys of three rabbits subjected to injections over several months, of a toxine prepared from a virulent culture of staphylococcus pyogenes aureus. These results seemed to offer grounds for the further study of this question, and moreover, in human pathology, the prolonged action of some circulating toxine is the only feasible explanation of the origin of many subacute and chronic inflammatory processes in the interstitial tissue and glomeruli. To study this question, pure cultures of Staphylococcus pyogenes aureus from two cases of acute osteomyelitis, were grown in broth after their virulence had been intensified by passage. The broth cultures were incubated for ten days, and were then killed by the addition of toluol. The fluid, which was then injected, contained the dead organisms and whatever toxines had been produced by their growth. In one of the two samples, 6 c.c. of the fluid was fatal to a rabbit

of 1600 grams in three days. Six rabbits were subjected to the injection of gradually increasing quantities of this fluid every second or third day for periods varying from one to three months. In all these cases, although there were degenerative changes in the tubules, in only one was there increase in number of cells in the interstitial tissue, and that only in a very small degree. The most noteworthy feature in this series of experiments, was the constant evidence of destruction of red blood corpuscles in the spleen, and the accumulation of masses of iron-containing pigment in the pulp. The organs were examined for the presence of waxy disease in their vessels, but this was entirely absent. Waxy disease of vessels or changes closely allied, have only been successfully produced by the use of living cultures of staphylococcus pyogenes aureus (Davidsohn,[17] Lubarsch,[42] Green.[22])

Finally, were the effects of Chronic lead poisoning investigated. In man, as a result of Chronic lead poisoning, it is common to find a granular contracted kidney. Charcot and Gombault[11] have described, as the result of feeding guinea-pigs with white lead for several months, areas of atrophied tubules, in which later, there was formation of fibrous tissue. The vessels and glomeruli were only slightly/

ly affected, and they regarded the alterations as secondary to those occurring primarily in the tubules. I subjected several rabbits for two to three months to subcutaneous injections of lead acetate, and although the animals gradually wasted and finally died, the effects on the kidney were negative. In this present work, the results obtained by the use of diphtheria toxine, corrosive sublimate, and cantharidin will alone be described.

The rabbit has been almost exclusively used as experimental animal. In several preliminary investigations, the cat was used, but as a variable quantity of fat is always present in the secreting tubules of its kidney, its use had to be discontinued. It was necessary to use an animal in whose kidney epithelium, fat was normally absent so as to determine the occurrence of fatty metamorphosis in the course of the various degenerative or necrotic cellular changes. In normal rabbits and guinea-pigs, fat is not found in the tubular epithelium. The animals were all full grown. In young animals, the frequency with which we find evidences of proliferation of cells leads to confusion in the results obtained. The autopsies were made as soon as possible, rarely beyond three or four hours after/

after death. The tissues were thus fixed in perfectly fresh condition, before they had undergone any post mortem change. As fixing agents, saturated solution of corrosive sublimate, alcohol, Zenker's fluid and formalin have been used. As a cell fixative, corrosive sublimate was found to give the best and most constant results. The tissues were imbedded in paraffin. As staining agents, alum haematein and eosine have been most commonly employed. For the demonstration of hyaline changes, in the vessels, von Gieson's stain was most satisfactory. Methylene blue and eosine combination was of special service in differential staining of certain cells. Benda's stain and Weigert's fibrin stain have been used, as occasion demanded. Fresh frozen sections of the Kidney were made in all cases, and stained with sudan or osmic acid, for the demonstration of fat.

EXPERIMENTAL DIPHTHERIA INTOXICATION.

METHOD OF INVESTIGATION:

The toxine employed, was obtained from the Jenner Institute, where it was used in the production of antitoxine. It was made according to Martin's method. Its lethal activity was determined on rabbits and .007 c.c. was usually fatal to an animal of 1000 gram weight on the 4th or 5th day. This was the only sample of toxine employed and during the three or four months of its use, its toxic power was found to deteriorate only very slightly. It was preserved under toluol and kept in the dark. It was injected intravenously into the ear vein and also subcutaneously beneath the skin of the abdomen, but the method of introduction did not produce any variation in the results obtained. Before each inoculation, the toxine was diluted ~~in~~ with normal saline solution. Thirty animals were used for the purpose of this investigation and were subjected to acute and subacute intoxication, and duration varied from 24 hours to 16 days. By the injection of one or several lethal doses, the animals died usually within 5 days, but it was found that no constant results as regards duration of life of animal could be obtained by the injection of a single/

single graduated sublethal dose. In some cases, the animal survived as late as the 12th day, but many more recovered and when these were killed at a subsequent period, I have been unable to discover any lesions of a chronic nature in their kidneys. Enriquez and Hallim (19) have described the presence of granular kidneys in an animal dead 10 months after single injection with diphtheria toxine. I have not found any evidences of such chronic processes in animals killed several weeks or months after a single inoculation. In this work, there was recourse to the method of successive sublethal injections. These were repeated every 3rd or 4th day. For our standard lethal dose, we take the amount of toxine killing 1000 gram rabbit on the 4th to the 5th day. This amount was ascertained to be .007 c.c. If a dose of .004 - .006 c.c. per 1000 gram were introduced, the animal might die within twelve days, or death might not occur even though albumin was found in the urine in the days succeeding the injection. On the other hand, when .003 c.c. per 1000 gram weight was injected, animal almost invariably survived and showed no symptoms, but if the same dose was repeated on 3rd day subsequently, death occurred 7-10 days after the date of the first injection. It was rare to find the presence/

/ Hallion

presence of albumin in the urine, after a single injection of .003 c.c., but it almost invariably appeared accompanied by casts, on the days succeeding the second injection of similar amount. All animals dead later than the fifth day were subjected to several sublethal doses. Attempts were made to obtain a more chronic intoxication by the injection of very small doses over longer periods, but in the animals which ultimately died, some after four weeks' treatment, the changes in the kidney did not differ from those found in animals dead within 16 days. If to animals, in which definite inflammatory changes had been induced by the inoculation of several sublethal doses, as evidenced by the presence of albumin and casts in the urine, no further injection was given and if animal ultimately recovered and was killed several weeks or months later, I have not succeeded in finding the development of chronic progressive lesions in the Kidney. All attempts, therefore to study the changes in the Kidney consequent on and consecutive to acute inflammatory lesions have failed. The Kidneys, in the cases studied, appeared to be restored to its normal integrity after the subsidence of the acute inflammatory phenomena. It would have been of considerable interest, in relation to human pathology, if one had succeeded in the production/

duction of chronic processes subsequent to the acute lesions. The following series of observations refer to acute and more subacute processes, as result of acute or subacute intoxication. I shall not give the protocols of all the experiments: a few examples will serve the purpose.

Rabbit No. 2, Female, Wt. 1870 grams. Single dose .1 cc., D. Toxine; lived 30 hours. No urine obtained.

Rabbit No. 5, Male, Wt. 1528 grams. Single dose of .01 cc. D. Toxine; lived 4½ days, Wt. 1274 grams. Albumin in urine on the day after inoculation and persisted till death. Hyaline and granular casts.

Rabbit No. 10, Male, Wt. 1755 grams.

1st day .003 cc. per 1000 gram wt.
4th " .003 cc. " 1000 " "
10th " dead, wt., 1275 grams.

Albumin and casts in urine after last injection.

Rabbit No. 15, Female, Wt. 1760 grams.

1st day .003 cc. per 1000 gram wt.
4th " .002 cc. " 1000 " "
8th " .002 cc. " 1000 " "
14th " dead, Wt. 1410 grams.

Slight trace of albumin in urine after 2nd injection/

tion which disappeared. After 3rd injection, albumin on succeeding day, present as a trace, but increased till it amounted to 2½ grams per litre on day before death. Hyaline and granular casts. Numerous red blood corpuscles on the 3 days preceding death.

Rabbit No. 17, Male, wt. 2020 grams.

1st day	.0015 cc.	per	1000	gram	wt.	
3rd "	.0015 cc.	"	1000	"	"	
6th "	.002 cc.	"	1000	"	"	
9th "	.002 cc.	"	1000	"	"	
12th "	.002 cc.	"	1000	"	"	
15th "	dead, wt. 1415 grams.					

Trace of albumin after 2nd dose, which disappeared. After the next two injections, trace of albumin only on day succeeding. After the last injection, on 12th day, trace again appeared, which increased till it reached ½ gram per litre on day before death. Hyaline casts and a few red blood corpuscles.

Rabbit No. 20, Male, wt. 1800 grams.

1st day	.0017 cc.	per	1000	gram	wt.	
3rd "	.0017 cc.	"	1000	"	"	
4th "	.0025 cc.	"	1000	"	"	

6th day .003 cc. per 1000 gram wt.
9th " .003 cc. " 1000 " "
13th " .003 cc. " 1000 " "
17th " dead, wt. 1190 grams.

No albumin till day succeeding 4th injection. It did not persist. After 5th injection, on the 9th day, albumin along with a few red blood corpuscles and hyaline casts appeared in urine, but these disappeared in two days. After the last injection on 13th day, albumin, red blood corpuscles and casts in urine, which persisted till death.

In acute and subacute intoxications, the lesions were of the same nature, although they varied in intensity. No detailed account will be given of the general symptoms which were observed in the inoculated animals. In animals, which did not die within two days, there was always progressive loss of body weight. In those which lived beyond ten days, this was extreme, the loss varying from 30-35% of body weight. After each injection, there was slight rise of temperature, but this did not persist beyond a day or two. After several injections had been made in the same area, the hair over the part frequently fell out, but there was never any other marked local reaction such/

such as necrosis of skin and subcutaneous tissues, seen after subcutaneous injections of ricin and abrin. Paresis or paralysis was of rare occurrence, and was only observed in those animals which died later than the 9th day. The hind limbs were the only parts affected. This occurred in 3, of 15 animals, which died after this period.

EXAMINATION OF URINE:

In all animals dying within 4 or 5 days, as result of a single lethal dose, albumin was present in the urine, as well as casts, but no red blood corpuscles were ever found. In these acute cases, the amount present on day succeeding inoculation was usually small, but in the later days it increased, and homogeneous hyaline casts feebly refracting, were invariably to be found. Attached to these, were a few granular cells, some of which could be identified as polymorph leucocytes. Definitely granular casts also occurred, hut in smaller number and at a later period than the hyaline variety. The reaction of the urine was neutral or feebly alkaline.

Amount of urea was increased per ounce of urine, but as all the urine could not be collected, the daily output of urea was not determined. Sugar was never found in the urine. In animals, which received/

received the successive sublethal doses, the appearance of albumin in the urine was taken as the index of definite damage to the kidney. On the day succeeding the earlier injections, a trace of albumin usually appeared, but did not persist beyond one or two days, but after the final injection, it progressively increased, reaching in some cases, 3 grams per litre. In the majority of animals which lived beyond ten days, red blood corpuscles were present on the days immediately preceding death. Sometimes the urine was blood-red in colour, in other cases only a few were found. The red blood corpuscles were found discrete, and were never fused to form definite casts. Within recent times, much importance has been laid upon the freezing point of blood, of patients suffering from Bright's disease. This is especially in relation to surgical practice. The freezing point of the blood of several of the experimental animals was determined by means of the cryoscope. Normal blood or blood serum freezes .56°+-.58° C. below the freezing point of distilled water. The blood of these animals was found to freeze at a slightly lower temperature. In the results which I obtained, freezing point varied from .59° - .61°C. below that/

that of distilled water, but I have not sufficient data to draw any definite conclusions.

POST MORTEM APPEARANCES:

The kidneys were enlarged, and in some cases, there were haemorrhages in the perinephric tissues. In those animals, whose urine contained large numbers of red blood corpuscles, the pelvis of one kidney, more rarely of both kidneys, was distended and completely occupied by a red blood clot. The ureter, in these cases, was also greatly dilated and filled with a loose red clot. The bladder, in some cases, was found to contain a large quantity of similar clot. In one case, the pelvis of both kidneys, both ureters and the bladder were completely filled with clot. There were submucous haemorrhages in the pelvis of the kidney and this seemed to be the main source of the blood. No haemorrhages were found in the mucous membrane of the ureter or bladder. The capsule stripped easily and the surface presented a fine mottling, produced by the alternation of small congested and pale areas. On section, the cortex was always increased in width, and in the acute cases, which showed the most intense changes, it was dark red in colour. In other less acute cases, the cortex was pale, and medullary/

medullary rays were swollen. When the pelvis was distended with a blood clot, the papillae were flattened.

The liver was usually blood-rich and more friable than normal, and in some cases, there were evidences of fatty changes. The spleen, in animals dead within two or three days, was enlarged, soft and congested; in those dying later, congestion was absent and Malpighian bodies were distinctly visible. The suprarenals, as a rule, showed congestion of ~~its~~ the medulla, and at times, actual haemorrhages. The lymphatic glands were larger than usual and the mesenteric and retroperitoneal glands were frequently congested. Some increase of fluid in the serous cavities was common, the pleural sacs being the most frequent site of the effusion. In a few, the hydrothorax was so considerable, as to cause almost complete collapse of one or both lungs. The lungs did not show any lesions, beyond the occasional presence of some small subpleural ecchymoses. Similar subserous haemorrhages were also found on the surface of the heart, whose muscle was usually pale and soft. The right cavities, and less frequently the left, were full of dark red blood clot. In male rabbits, effusion into the tunica/

tunica vaginalis was almost a constant phenomenon. In one rabbit, there was an extensive subdural haemorrhage over the cerebral cortex.

In animals in which the duration of intoxication exceeded ten days, it was common to find the presence of a great number of minute submucous haemorrhages scattered diffusely over the surface of the stomach, and in many, there were small superficial ulcers.

LITERATURE ON THE KIDNEY LESIONS IN DIPHTHERIA:

Since albuminuria was observed to be frequently present in diphtheria, much attention has been given to the lesions in the Kidney. Oertel,[50] in his studies on diphtheria, has found the Kidneys to be the seat of marked lesions in the majority of his cases, and showed that these lesions could occur in the kidneys, without the actual local presence of the organism. Oertel most commonly found a focal nephritis, and there were haemorrhages in the interstitial tissue and into the tubules.

In the glomeruli, there was proliferation of the capillary endothelium, and a degeneration and desquamation of glomerular and capsular epithelium. In the capillaries of the tuft, leucocytes, showing degenerative changes in their nuclei, accumulated.

In/

In the capsular space, an exudation of albuminous nature was occasionally present. The tubules were also markedly affected, the secreting always to a greater degree than the collecting. The cell lesions were of the nature of granular disintegration, accompanied by loss of their nuclei. Fatty changes were less frequently observed. In the cortex, around Bowman's Capsule, between the tubules, and in relation to the larger blood vessels, were focal accumulations of round cells, which varied in size and showed degenerative changes. These were sometimes observed between the tubules in the medulla, and Oertel states that the focal accumulations of round cells did not seem to have any relation to the damage in the tubules. The small blood vessels in the cortex showed hyaline change in their walls associated with proliferation of their endothelium. Other observers have not found such constant and extensive changes in the kidney. Langhans,(38) in his cases, found a granular disintegration of the convoluted tubule cells, with hyaline masses in their lumina. Fatty changes also occurred, especially in the cells of the ascending limbs of Henle in the boundary layer and in the medullary rays. The interstitial tissue was rarely affected,/

affected, and Langhans regarded the affection of the stroma, as secondary to lesions in the glomeruli and tubules. In the glomeruli, there was slight swelling of the capillary endothelium. Cornil and Brault[12] found in diphtheria, that the tubules were specially affected, the glomeruli and interstitial tissue showing no marked changes beyond congestion of their blood vessels. Bernhard and Felsenthal[4] found focal accumulations of round cells around the glomeruli, only in a few of their cases. The glomerular and capsular epithelium showed degeneration, and in the capillaries of the tuft were hyaline changes, along with evidence of proliferation of their endothelium. Rosenstein,[58] in his cases, found no marked or constant changes. These consisted chiefly of swelling of the convoluted tubules, with the presence of some fatty degeneration. Changes in the interstitial tissue were rarely observed. Councilman[16] has more recently made important additions to this subject. In many cases, there was an acute glomerulo-nephritis, with proliferation of capillary endothelium. Hyaline thrombi were often present. Proliferation and desquamation of capsular and glomerular epithelium were also noted, and the capsular space sometimes contained/

contained a haemorrhagic or fibrinous exudation. The secreting tubules showed various necrotic and degenerative changes in their cells. In 24 of 103 cases of diphtheria, Councilman found the lesion was an acute interstitial nephritis in which there was a focal or more diffuse accumulation of large round cells in the cortex and medulla. These were most abundant at the bases of the pyramids in the boundary zone, beneath the capsule, and around the glomeruli. In addition to these cells, which he regards as identical with Unna's plasma cells, polymorph leucocytes were found in small numbers, together with a few cells, epithelioid in character, which were probably derived from connective tissue cells of the part. The glomeruli, in these cases, showed no changes, except where acute glomerular changes occurred in addition to the interstitial lesions. Simple parenchymatous or necrotic lesions occurred in the cells of the secreting tubules. In the Kidneys in diphtheria, the lesions as described by various observers, present variations as regards their nature and intensity. The secreting cells constantly suffer, undergoing changes varying from simple degenerative to more necrotic lesions. The affection of the glomeruli and interstitial tissue varies. In some they may be/

be so severely affected so that the lesion partakes of the nature of an acute glomerular or interstitial nephritis.

EXPERIMENTAL DIPHTHERIA:

The lesions in the Kidneys in experimental diphtheria, have been studied along with those in other organs, especially in relation to the investigation of the question as to whether the lesions produced by the diphtheria toxine were identical with those produced by living cultures of the bacillus. Babes[2] first called attention to the occurrence of lesions in the organs of experimental animals, and did not consider that the effects upon the organs of the diphtheria bacillus and its soluble toxine, were identical in nature. In the kidneys of rabbits, inoculated with diphtheria bacillus, he found, in addition to tubular changes, accumulation of leucocytes with fragmented nuclei in the glomerular capillaries, and he gives drawings showing Karyokinetic figures in the endothelium of the glomerular capillaries, in the capsular epithelium, and in the cells of the labyrinth. There was hyaline swelling of vessel walls. In animals, inoculated with the soluble toxine, there was extreme parenchymatous degeneration of the cells, with disappearance/

disappearance.of their nuclei, but the proliferation of the endothelial cells, and the fragmentation of the nuclei of the leucocytes were usually absent. Welch and Flexner[64] have made a series of investigations regarding the action of living cultures of diphtheria bacillus and its soluble toxine. They found the lesions were indistinguishable from each other. In the Kidney, there was usually some fatty change in the cells of the tubules, but in some cases, there was none. Hyaline swelling of the glomerular capillaries and smaller arteries, and the occasional presence of a hyaline mass within the capillaries were particularly noticed in kittens. Flexner,[20] in a more recent work, has described the lesions more fully. There was sometimes necrosis, chiefly by fragmentation of the endothelial cells, and mitotic figures also occurred in the vessels. The white blood corpuscles occasionally showed fragmentation of their nuclei. The alterations in the epithelial structures were most profound, when there was hyaline degeneration of vessel walls. The capsular epithelium was also implicated, undergoing disintegration. In rare instances, a deposition of lime salts was noted in the straight tubules. There was complete absence of intertubular round cell infiltration.

PERSONAL OBSERVATIONS:

In fresh frozen specimens, any extreme degree of fatty degeneration has never been found. In the animals, which survived as long as a fortnight, evidence of any fatty metamorphosis of cell protoplasm was indeed frequently wanting. The fat, when present, was in the form of fine granules or minute globules, and it was most common to find it in the cells of ascending limb of Henle. At other times, this change was irregularly distributed throughout the convoluted tubules of the cortex, and in some cases it was limited to the collecting tubules. In staining, with osmic acid, the protoplasmic granules of the secreting cells frequently assumed a darker tint than normal. Fatty metamorphosis did not thus seem to be an accompaniment of constant frequency in the various degenerative and necrotic changes, which the cells undergo. It occurs in acute poisoning as well as in animals which survive for some time. Thus, in rabbit No. 2 in which the intoxication lasted 30 hours, there is quite marked fatty degeneration especially in ascending limbs of Henle. As a rule, the more intense and rapid the necrotic changes are, the less is the degree of fatty change.

CHANGES IN THE BLOOD VESSELS:

The congestion of the blood vessels is always a striking feature in acute cases, but is less noteworthy when intoxication has been of longer duration. The vessels of the cortex and medulla equally suffer, and in some cases there may be fibrinous thrombi in the large veins at the junction of the cortex and medulla, as seen in specimen No. 12, but the blocking of the vessel is never complete. The extreme distension of the interlobular veins, sometimes leads to a post-mortem occlusion of the accompanying artery. The intertubular capillaries are also greatly congested, where these have not been compressed by a swelling of the tubules, but no actual haemorrhages in the interstitial tissues have been found, except in the submucous tissue of the pelvis. Haemorrhage into the tubules is of rare occurrence. In some cases, a localized distension of the collecting tubules in the cortex seems to have been produced by compression exerted by distended veins.

MALPIGHIAN BODIES:

The glomerular lesions differ according as the intoxication has been acute or subacute. When the intoxication has been fatal within 3 days, as seen in specimens Nos. 1, 2 and 3, the glomerular tuft occupies almost completely the whole space. Although/

though many of the capillaries are much dilated, the lumina of others are partially or completely occluded by hyaline masses. Similar hyaline thrombi occur in the afferent arterioles. In some capillaries, the lumen is much reduced in size by the regular deposition of this material around its whole circumference, but on the other hand, the hyaline mass may lie free in the lumen, surrounded by red blood corpuscles, or form a spindle shaped deposit at one side of the vessel, projecting into its interior. These masses stain red with Picro-fuchsin and usually present a homogeneous hyaline, or it may be a more fibrillated appearance. These hyaline thrombi in glomerular capillaries and afferent arteriole are represented in Plate 1. These hyaline masses must be regarded as thrombi. This is quite evident, when they lie free in the lumen, but when the deposition occurs at one side of the vessel or circularly around its lumen, it might be mistaken for a hyaline swelling of the basement membrane of the capillary. This, however, is not the case. The capillary basement here and there may show slight swelling, but at this period it is neither marked nor extensive. These peripheral masses lie internal to the endothelium of the capillaries/

illaries and arterioles, and must in consequence, be a derivative of some of the albuminous constituents of the blood serum. Similar thrombi are not found in the intertubular capillaries, but are common in the arterioles between the tubules. The larger arteries and veins contain no thrombi. A considerable part of these hyaline masses gives the characteristic fibrin reaction with Weigert's fibrin stain, as shown in the specimens and also in Plate II. Definite fibrin threads may be seen imbedded in a homogeneous mass which stains more faintly, or red blood corpuscles may be included in their meshes. Many of these hyaline thrombi are probably albuminous coagula, or they may represent a hyaline transformation of the more definitely fibrinous masses. These thrombi are not to be regarded as corresponding to masses of fused blood platelets. At this period, there is no increase in the nuclei of the glomerular tuft. Normally, the glomerular epithelium can be easily distinguished from the capillary endothelium, by its larger and more vesicular nuclei. The cells of the glomerular epithelium possess a very small amount of protoplasm, and there is no definite line of division between adjacent cells. In these acute cases, the greater part/

part of surface of tuft is devoid of its epithelial covering. The epithelial cells between the capillary loops, and those which remain attached to the surface are swollen. Their protoplasm is increased and has become more granular, and many of the nuclei show deficient chromatin staining. The endothelial cells of the capillaries are not swollen, and their nuclei stain deeply. Some capillaries are bereft of their endothelium, but there is no accumulation of desquamated cells in the capillaries. There is an increase in the number of polymorph leucocytes in the tuft and many show fragmentation of their nuclei, as shown in specimen No. 2. In a few tufts, some of the dilated capillaries have evidently ruptured with the formation of larger blood containing spaces. The blocking of some capillaries by hyaline thrombi seems to be the direct causative factor. The capsular spaces are devoid of contents, except a small minority which contain some granular debris. This granular debris lies in the vicinity of the point of origin of the tubule, and although some of it may represent an albuminous transudation from the vessels of the tuft, most of it appears to be derived from the granular disintegration of the cells in the neck of the capsule, which correspond in/

in structure and reaction to secreting cells. Neither red blood corpuscles nor leucocytes are found in the capsular space. Bowman's Capsule and its lining endothelium, as yet, do not suffer. Here and there, there is swelling of the Capsule, but the endothelium is intact and shows no proliferation and desquamation. We now pass to the consideration of the glomerular changes in the less acute and in the subacute cases. These are found in specimens No. 4 -20. We now find the congestion of the capillaries is less extreme, but there is the development of other changes. One of the most noteworthy of these, is the occurrence of hyaline swelling of the walls of the glomerular capillaries and afferent arterioles. In the capillaries, it is rare to find any marked diminution in their calibre; on the contrary, the capillaries are usually dilated. In the arterioles at the bases of the glomeruli and between the tubules, the lumen in a few cases, as shown in Specimen No. 10 and Plate V., is occluded; in the majority, however, it remains patent, though diminished in size. In the arterioles, the hyaline swelling affects chiefly the muscular coat, the affection of the elastic lamina and inner coat being less prominent, although at times, a spindle shaped mass projects/

jects into and diminishes the lumen. The swelling of the middle coat is homogeneous, and the muscle cells appear to undergo hyaline transformation. Their nuclei are diminished in number, but those which remain are large and swollen, showing deficiency of chromatin. By a comparative study of the specimens, we can trace what appears to be the stages in the development of this change. In animal No. 4 in which the intoxication lasted 4 days, the capillary loops are separated by a homogeneous substance, which resembles an albuminous transudate. The contour of the capillaries appears double. This material does not give a characteristic hyaline reaction, but is stained faintly pink by picro-fuchsin and eosine. It does not give fibrin reaction. The early hyaline swelling of walls of small arterioles reacts similarly to staining reagents, as shown in specimen No. 4. The swelling of the vessel wall appears to be produced by a soaking into it of some albuminous material from the blood. Specimens Nos. 4, 5 and 6 show these early changes. At a later period the hyaline substance stains more deeply, being coloured characteristically red by picro-fuchsin. While in the earlier periods, the appearances which have been described, are seen throughout the greater part of/

of the tuft, in intoxication of longer duration, the thickening of the capillary walls is much less diffuse and occurs in patches, especially over the superficial capillaries. This is shown in Plate IV. a drawing from specimen No. 7. Within the capillaries, hyaline thrombi are now rarely found. In specimen No. 4, a few are still present. The glomerular epithelium is constantly affected. The cells are greatly swollen and are increased in number, although I have never found mitotic figures. The protoplasm is more abundant than normal, and is granular, but in many cases the protoplasm becomes hyaline, and when many cells undergo this change, they fuse and produce hyaline masses which are found on the surface of the tuft and between the capillary loops. The capillary walls then appear thickened. The cell nuclei also become swollen, and vary in form. Commonly, they are ovoid and somewhat vesicular; at other times, they are much elongated and may become constricted, as if undergoing direct division. They possess a well defined nuclear membrane, but do not stain so deeply as usual. The cells covering the surface of the tuft project towards the capsular space, a few become detached, others still remain attached by their stalk of protoplasm/ thin

plasm. I have never observed any marked desquamation and accumulation of these cells in the capsular spaces. In the body of the tuft, these cells can be readily differentiated from the capillary endothelium. They lie between the capillary loops, and possess a larger and less deeply staining vesicular nucleus, and a greater quantity of protoplasm. When these cells undergo hyaline transformation, their nuclei remain imbedded in the hyaline mass. In animals surviving beyond the fifth day, and subjected to several sublethal doses, there is the development of cyst like spaces in the body of the glomerular tuft. These can be studied in various stages of development in specimens Nos. 7-20 and are shown in Plates VI., VII. and VIII. They vary greatly in size. Some tufts are represented by one large rounded cavity, lined with endothelium, and filled with blood corpuscles and a varying quantity of hyaline or fibrinous material. More commonly, the tuft contains several dilated spaces, unequal in size, which correspond to the individual lobules of the glomerular body. The capillaries, which lie between adjacent spaces, are usually compressed. These cyst-like spaces are round or ovoid in form, and are situated superficially, though some are seen to/

to be situated within the substance of the tuft, and surrounded by compressed capillaries. These blood containing spaces must be regarded as a result of the rupture and confluence of several capillaries. This can be frequently observed, and the lumina of adjacent capillaries communicate through the rupture of the intervening basement membranes. They possess a distinct limiting membrane, which often shows diffuse or patchy swelling, and are lined by endothelial cells. No evidence of rupture into the capsular space has been seen, and it is rare to find any red blood corpuscles free in the spaces. In the acute and intense cases, similar dilated blood spaces are occasionally seen, and the presence of hyaline thrombi completely blocking many capillaries, seems to be a sufficient explanation of their origin. But in subacute intoxication, hyaline thrombi are no longer found. Here, the blocking of the capillaries with consequent dilatation and rupture of others, must be produced in some other way. The hyaline swelling of the basement membrane may be one of the factors, as the vessel wall may thereby be weakened, and lead to its easier distension and ultimate rupture. For evidence of a more direct causative factor, the changes which occur in the lining endothelium/

elium of the capillaries, must be studied. The endothelial cells become swollen, their nuclei increase in size, stain less deeply and are more vesicular. They are attached to capillary wall, but many have desquamated and are free in the lumen as large cells with granular protoplasm and pale nuclei. Mitotic figures in the endothelial cells are of rare occurrence. The lumen of some capillaries is found to be partially or almost entirely occluded by the swollen endothelial cells, either attached or free in the lumen. If blocking be complete, the consequent stasis of blood in other capillaries will readily explain their dilatation and rupture. The endothelial cells are occasionally seen to contain yellow granular pigment and chromatin granules. There is a great increase in the number of the polymorph leucocytes in the tuft. They accumulate even in acute cases, but are always most numerous when intoxication has been of longer duration, and many of the large spaces may show their presence in large numbers, as shown especially in Plate VI. These leucocytes always show many degenerated forms, and sometimes, not a single normal one is to be found in the tuft. The nuclei assume a great variety of forms, and the most bizarre figures are produced/

duced. They stain deeply and the nuclear fragments are often arranged in a radiate fashion, so as to simulate the appearance of a mitotic figure. Finally, the fragmentation of the chromatin masses ends in the production of smaller chromatin granules which are scattered irregularly through the body of the cell, or lie free in the capillary lumen. Much of the nuclear debris is taken up by the endothelial cells, which act as phagocytes. There is no accumulation of mononucleated leucocytes in the tufts. There is no emigration of leucocytes into the capsular space. At most, a few mononucleated leucocytes occasionally are seen lying between the capillary loops. This may be seen in specimens Nos. 4, 5 and 6. The contents of the large spaces vary somewhat in nature. Plates VI. and VII. show them filled with red blood corpuscles and polymorph leucocytes with fragmented nuclei. Many of the red blood corpuscles are sometimes broken down, giving rise to granular debris. In Plate VIII. the spaces contain hyaline and fibrinous masses. These masses may completely fill the spaces, but more frequently, they are deposited around their peripheries, forming hyaline layers, which may have attached to them, fibrinous threads.

By/

By referring to the specimens stained according to Weigert's method, the hyaline masses do not give the same characteristic reaction as the fibrinous threads. Desquamated endothelial cells also occur in these spaces, and some contain pigment and nuclear fragments. Bowman's Capsule and its endothelium are not specially affected. The Capsule may be swollen and homogeneous in appearance, but occasionally it presents a more fibrillated consistence. In some glomeruli, the endothelium is swollen and granular; in others, it is little altered. In the most marked cases, the endothelial cells become more cubical; their increased quantity of protoplasm is granular, and their nuclei are larger, more ovoid, and stain less deeply. Sometimes there is increase in number of nuclei, but mitotic figures are rare. There is never the formation of laminated layers of cells; at most, a double layer of cells is seen over localized areas, the cells of the more superficial layer becoming desquamated. The cells at the neck are swollen and granular, and frequently undergo disintegration with loss of nuclei. The granular debris accumulates in this vicinity. The capsular space contains few contents besides this granular material. A few red blood corpuscles may occur/

occur, and in one case they were derived from the rupture of a capillary through Bowman's Capsule. Cells which have desquamated from the capsular or glomerular epithelium are few in number.

TUBULAR CHANGES:

The changes in the tubules vary in intensity, and are chiefly found in the cells of the secreting tubules. The cellular lesions may be simple degenerative or necrotic. They are always most intense in the acute cases, but the lesions in the subacute cases are similar in nature, but the definitely necrotic are less predominant. In those cases in which many of the afferent arterioles and glomerular capillaries become blocked by hyaline thrombi, it is difficult to estimate the part played by the vascular obstruction in the production of the cell lesions, but although this influence cannot be excluded, I have never been able to satisfy myself that it accounts for the more intense changes which are found. Here the intoxication is rapid and intense, and this seems quite sufficient to explain the difference. In areas, where the glomerular vessels are patent, the tubules in association with them, show as marked changes as those in relation to the blocked/

blocked glomerular vessels. We will first describe the cell lesions as they are seen in these intense and acute cases (Specimens 1, 2 and 3.)

The secreting cells are swollen and coarsely granular and the lumen may be entirely occluded. The granules are large, round or angular and stain deeply with eosine. In many tubules, the changes have progressed to more or less complete disintegration of the cell protoplasm. The disintegration may affect the part of the cell next the lumen, the basal portion, or be localized around the nucleus. The division between individual cells is lost in many tubules. Vacuolation of the cells is frequent, and commonly results from the granular disintegration of localized areas of protoplasm; in some cases it may be due to a fluid transudation into the cells.

All the convoluted tubules are not equally affected. Some merely show granular swelling with well preserved nuclei. The basement membrane of the tubules is swollen. In acute intoxication, and also in subacute cases, the ascending limbs of Henle suffer most severely. They are the least resistent parts, and definitely disintegrative and necrotic changes are more common here, than elsewhere. In the rapidly acute cases (Specimens 1, 2 and 3.) these tubules are dilated, the cells are detached

from/

from the basement membrane, and have generally fused to form a finely granular mass around a lumen, which is almost entirely obliterated. (Plate X.) The nuclear changes are particularly noteworthy in the most intense cases. The nuclei are not so early affected as the cell protoplasm, and in many cells in which disintegration of protoplasm is advanced, the nuclei may be little altered. Disappearance of the nuclei occurs in several ways. A rapid solution of the chromatic substance, so that the nucleus remains for some time longer as an achromatic body, is not common. This achromatic body possesses a distinct limiting membrane and reticulum and stains feebly with acid dyes and can be differentiated from the protoplasm in which it lies. Finally, it entirely disappears. Fragmentation of the chromatin, however, is the common result of nuclear death, in these intense cases of intoxication. This process is well seen in specimen No. 3, where it is more extensive and advanced than in Nos. 1 and 2. For the purpose of description, we recognise two chief types of nuclear fragmentation, or Karyorhexis as it is called. In the one type, the nucleus becomes condensed and stains solidly and homogeneously. The form is round, or it may be irregular with peri-

pheral/

pheral projections. All trace of nuclear reticulum is lost. The term, pyknosis, is applied to this altered nucleus. These pyknotic nuclei are seen in the convoluted tubules, but particularly in ascending limb (Plate X). In longitudinal section of these tubules, every one of their cells may show nucleus in this condition. When necrotic cells are free in the lumen of any of the tubules, their nuclei also show this transformation and its further stages. If the subsequent fate of these altered nuclei be traced, the condensed chromatin mass is found to break up into smaller masses, varying from 2-6 in number. These lie free in the cell, or are discharged into tubular lumen. These chromatin masses may undergo further fragmentation, but finally the chromatic substance is lost and they are no longer visible. The other type of nuclear fragmentation is most commonly seen in the convoluted tubules. The nucleus becomes slightly decreased in size, but some are large and swollen. The nuclear membrane stains deeply and is well defined, and at first contains deep-staining granules scattered throughout its body. Later, the chromatin granules become peripherally placed in relation to the nuclear membrane, and the body of the nucleus is then pale/

pale and homogeneous or may contain a few granules of varying size, either discrete or joined by chromatic threads. The granules may appear to be imbedded in the nuclear membrane, or larger semispherical masses of chromatin project inwards from the nuclear membrane. Plate XI , shows these changes. In some cases, the peripheral part of nucleus is pale, and the chromatin is condensed into a centrally placed mass. Some of the chromatin masses may project outwards, and become free in the protoplasm of the cell. These nuclear changes are advanced at the end of 24 hours, not only in secreting tubules, but also in the smaller collecting tubules. Further changes occur, resulting in the complete disintegration of the nucleus. There is first of all, the disappearance of the chromatic substance of the nuclear membrane in localized parts of the circumference or more diffusely. The achromatic persists till later, and then the chromatin granules become free in the cell protoplasm. These fragments are smaller and more numerous than those resulting from the fragmentation of the pyknotic nuclei. They are scattered diffusely throughout the cell, and also among the granular debris in the lumen. The chromatic substance of these granules finally becomes dissolved or altered in its staining/

staining properties. The cells of the descending loop are swollen and granular, but do not suffer disintegration. The collecting tubules, particularly the smaller in the medullary rays, suffer more now than in longer durations of intoxication. Protoplasm is slightly swollen and granularity is increased, so that they simulate, in staining reaction, the secreting cells. They are frequently detached en masse from their basement membrane, or the lumen may be filled with discrete desquamated cells. (Plate IX) There is no extensive disintegration of protoplasm; at most, this change is localized around the nucleus. The nuclei of the collecting tubules stain deeply, but many undergo changes similar to those described in the nuclei of the secreting cells. In some collecting tubules, as well as in secreting, a mitotic like figure may result in consequence of nuclear fragmentation.

In the lumina of the secreting tubules, there is an accumulation of granular protoplasmic debris, which may be diffusely scattered or aggregated into round granular masses. Frequently, the lumen appears filled by a conglomeration of large vacuoles, separated by a network-like arrangement. This appearance is produced by the close apposition of cells, whose/

whose cell membrane is still intact, but whose protoplasm has almost entirely been disintegrated. In some tubules, there is a felted fibrinous mass, apparently derived from the protoplasmic network with which it is continuous. This is seen in Plate IX . In addition, discrete necrotic cells with deeply stained hyaline protoplasm and condensed or fragmented nuclei, lie free in the lumen. Homogeneous or more definitely fibrillated casts, which stain faintly, are present in the collecting tubules of the medullary rays and in the ascending limbs. Many of these, at this period, give fibrin reaction and on the surface of some, is a layer of granular debris or some adherent polymorph leucocytes. A few red blood corpuscles are found in the larger collecting tubules in the medulla, and polymorph leucocytes may be seen emigrating between two adjacent cells. Necrotic cells are found in the lumen, and some of these have been ingested by the collecting tubule cells and lie within vacuoles in their protoplasm as shown in Plate XII .

We now pass on to the study of the tubular changes in subacute intoxication. The cellular lesions are similar in nature, but less intense in degree. Necrotic changes in the cells are now less diffuse/

diffuse, though never entirely wanting. The secreting tubules are not uniformly affected. The convoluted tubules in superficial part of cortex are usually swollen, and lumen may be occluded. Other tubules show cells which are comparatively normal beyond an increased granularity of their protoplasm, and the lumen of many is dilated. Complete granular disintegration only occurs in single cells, here and there, while other cells may show vacuolation and disintegration around the nucleus or in the basal portion. The cell divisions are distinct, and ciliated border is intact. Many secreting cells contain round colloid masses in their protoplasm. These stain deeply and uniformly with eosine, and are found free in the lumen. As in the rapid cases of intoxication, the ascending limbs are the most severely affected. These always show more marked and diffuse disintegration of their cells. The nuclei of the secreting cells are well preserved, and many are unaltered. Some are swollen with deficient chromatin staining. In the more severely damaged cells, the various forms of necrotic nuclear changes already described are seen, but disappearance of nuclei by simple solution of their chromatin without a preliminary fragmentation is more common. Pyknotic/

Pyknotic nuclei in cells of ascending limb are, however, more numerous than elsewhere. Beyond the fourth day, mitotic figures occur in the convoluted tubules and ascending limbs of Henle, but this process of regeneration is never extensive (Plate XIV) In the cases where the great majority of the glomerular tufts were transformed into haemorrhagic cysts, the lesions of the tubules did not differ from those where the glomeruli were less extensively affected. The descending limbs of Henle suffer: at most, there is slight granular swelling of their protoplasm. Phagocytosis of cellular debris by these cells, has not been observed. The collecting tubules, likewise, enjoy a considerable degree of immunity. There is increased granularity of their protoplasm, and their free extremities may form semispherical projections into the lumen. The protoplasm around their nuclei is sometimes disintegrated. The nuclei are well preserved, but it is not infrequent to find condensation of their chromatin with the formation of irregular figures and fragmentation. In some cells, enormously swollen pale nuclei are seen. The large tubules in the papillae possess healthy epithelium. Mitotic figures occur in the collecting tubules of the medullary rays. It is not/

not uncommon to find considerable dilatation of the collecting tubules, either in localized areas or diffusely throughout the organ. In specimens Nos. 12 and 15, the diffuse dilatation has occurred in consequence of complete blocking of the pelvis and ureter by a blood clot. In specimen No. 13, though pelvis was completely occupied by a clot post-mortem, this feature is absent. The dilatation of the smaller tubules in the cortex is always more marked than that of the larger tubules in the medulla, and their cells become flattened. In such cases, some of the ascending limbs may show a slight degree of dilatation, but convoluted tubules are unaffected. Even with absence of obstruction in pelvis and ureter, localised dilatation of groups of collecting tubules in the cortex is often a noteworthy feature. In specimens Nos. 7 and 16, a very general dilatation of these tubules in the cortex has occurred. The explanation of this phenomenon must be referred to a blocking of their lumen at some part by the presence of casts. In one case, Specimen 19 and Plate XVI, localized dilations have arisen in consequence of the obstruction produced by calcified masses within the lumen. Further, in some cases, the compression of collecting tubes by the large dilated veins at the junction of the cortex and medulla seems to be/

be the causative factor. We have now to examine the contents of the various tubules, and consider the question of cast formation. In the secreting tubules, the quantity of granular debris is never so abundant as in the acute cases. In many specimens, large round granular masses or shadow cells with a definite cell membrane containing some granules, are found. Other necrotic cells, possessing finely granular or homogeneous protoplasm and condensed or fragmented nuclei, lie free or attached to granular masses. Within the protoplasm of these cells, colloid globules are frequently seen and are sometimes lying free in the lumen. Definite cast formation is rare in the secreting tubules, although pale homogeneous hyaline casts with a surface layer of granular material adherent may be present, or there may be the fusion of colloid globules to form deeper staining, more refracting colloid like casts. It is especially in the collecting tubules of the medullary rays, that cast formation can be studied. In these tubules are numerous necrotic secreting cells with characters similar to those found in convoluted tubules (Plate XIII .) These cells are frequently adherent to the surface of pale hyaline casts which are constantly present in the collecting tubules. They are also found in the descending and ascending/

ascending limbs of Henle's loop. They are homogeneous in consistence, but sometimes are definitely fibrillated, and may be fragmented into isolated masses. They correspond to the hyaline casts which are passed in the urine. In the Kidney, they stain faintly, and often form the basis of the granular casts. These granular casts, with or without a central hyaline mass, constitute the chief type of cast. They stain deeply with eosine and other protoplasmic stains, and are formed by the fusion of necrotic secreting cells or granular detritus. Degenerated nuclei and chromatin granules are found imbedded in them (Plates XIII. XIV.) These casts then, are cellular in origin, but the pale hyaline casts are not to be regarded as the result of cell secretion or disintegration, but as an albuminous coagulable transudate, which has occurred into the tubules when their epithelium has been damaged. In addition to these, another form of cast is found. These casts are characterised by the vacuolated appearance of their surface. The vacuoles are round or ovoid, vary in size, and are devoid of contents. (Plates XIV and XV .) They stain deeply with eosine and saffranin, and are finely granular or homogeneous in consistence. Their borders are irregular in outline and frequently scalloped.

These/

These casts must also be regarded as cellular in origin, and are a variety of the more definitely colloid like casts which are found in the tubules. The formation of these colloid casts can be easily followed. The protoplasm of the desquamated necrotic secreting cells may undergo a colloid transformation en masse, or colloid globules are formed within them. These globules are found free in the lumen, and their subsequent fusion leads to the production of a colloid mass which frequently presents the vacuolated aspect. These cases are found in tubules, whose epithelium is regular in outline, so that the vacuolated condition is not to be entirely explained as an appearance produced by their retraction from the walls of the tubule. Longitudinal sections of casts may be seen, which show transition stages between these various forms of casts. At one part, they are definitely granular, with, it may be, colloid masses adherent to or imbedded in them; in other parts they are colloid and present a more or less vacuolated surface. The larger collecting tubes of the medulla very rarely contain properly constituted casts. In them are found some granular debris, desquamated necrotic cells, and occasionally a few red blood corpuscles. Various views/

views are held concerning the origin and nature of these tubular casts. While some regard them as altered albuminous or fibrinous transudates from the blood vessels, others believe them to be derived from the tubular cells, either as a result of a process of secretion, or of disintegration. In this series of experiments, the capsular spaces have been invariably free from any albuminous transudation. If it has occurred, it has not remained in situ. But once the tubular epithelium has become damaged, transudation occurs into the tubules, the non coagulating part being passed with the urine, while the part which coagulates, leads to the formation of a hyaline or fibrillated cast. It has been maintained that these casts are all fibrinous in constitution, the coagulation being determined by white blood corpuscles or the disintegrated cells within the lumen. That such a hyaline transformation can occur in fibrinous material, is elsewhere observed, especially in acute inflammations of serous and mucous membranes. To determine whether these casts were composed of fibrin, Weigert's fibrin stain has been used, but the results have been most inconstant. While some gave the characteristic reaction, and this was particularly noticed in the acute cases, the/

the great majority stain of a faint blue colour. Weigert's fibrin stain, however, does not give uniform results, even with a definitely fibrin containing substance. While some of the casts may be a simple albuminous coagulum, others may be altered fibrin casts. The colloid or granular casts never gave a positive reaction to Weigert's stain. Colloid casts were never found in the rapidly fatal cases. The only form of leucocyte which emigrates into the tubules is the pseudo-eosinophile polymorphic variety. They are usually attached to the surface of the casts, and they never exhibit phagocytic properties within the tubules. On the other hand, they become degenerated. Emigration of these leucocytes between the cells of the collecting tubes has been frequently noticed. Reference has been already made to phagocytosis of necrotic cells, by the tubular epithelium of collecting tubules. This is quite a common feature in subacute intoxication, and is especially seen in the collecting tubules of the cortex (Plate XIII .) The ingested granular masses, which lie in vacuoles, vary in size and generally contain a condensed nucleus or chromatin granules. Granules of yellow pigment may also be found in the cells of the larger collecting tubes, and/

and more rarely in ascending limbs. These granules are probably derived from blood pigment, but they do not give iron reaction.

Finally, mention must be made of calcification of some tubules and casts. In only one case was this process diffuse (Specimen 19.) Minor degrees are seen in other cases (Specimens, Nos. 8, 10, 14, 16 and 17.) These calcified masses are chiefly localized in the medullary rays, where single necrotic cells of ascending limb are affected, or the whole tubule may be calcified. In the collecting tubules, are calcified casts, which often produce complete blocking. This process of calcification will be discussed later, in the consideration of Mercury poisoning.

CHANGES IN THE INTERSTITIAL TISSUE:

Reference has been already made to the congestion of the blood vessels, and the occurrence of hyaline changes in the walls of the small arterioles. Any distinct separation of the tubules by inflammatory oedema of interstitial tissue is only seen in intense and rapid intoxication. Within the vessels, both of the cortex and medulla, there is an accumulation of leucocytes, which is most abundant in slow intoxication. The great majority of these are pseudo-/

pseudo-eosinophile polymorphs, and like those in the glomerular vessels, degeneration and fragmentation of their nuclei are common. In the great majority of animals which survive beyond the 4th day, there is an accumulation of round cells around some of the cortical vessels. Similar accumulations do not occur in the medulla. This infiltration is always focal in character, and degree is very variable. Normally, there is no layer of lymphoid cells, around the larger cortical vessels of rabbits' kidney. These round cells are found around the large vessels, along the lines of the interlobular vessels, around the afferent arterioles, and less commonly in relation to the intertubular capillaries. They are frequently found localized around a hyaline arteriole. In relation to the interlobular vessels, they accumulate especially in the tissue intervening between the artery and vein. The most extensive infiltration is seen in specimen No. 17 (Plate XVII.) The characters of these cells are best studied in eosine and methylene blue preparations. The nucleus varies in size and is round. It stains deeply, has a well defined nuclear membrane and chromatin in the form of closely aggregated granules. A small nucleolus, staining red with eosine, can be demonstrated in some. Frequently/

quently these nuclei stain almost solidly. The amount of protoplasm varies considerably. They possess no distinct cell membrane, and their outline is rarely circular, when protoplasm is at all abundant. Frequently the nucleus is surrounded by a scarcely perceptible rim of protoplasm. In the larger cells, the nucleus is usually eccentric in position. The protoplasm stains blue in eosine and methylene blue preparations and is homogeneous in consistence. Mitotic figures in these cells are rarely observed. In addition to these cells, a few polymorph leucocytes are present, and there is also a small number of larger cells with pale ovoid nuclei and granular protoplasm, which resemble connective tissue cells. The characters of these cells are represented in Plate XVIII. What is the source and function of these round cells? They exactly resemble the lymphocytes of the blood and are to be regarded as identical with them. They are found within the vessels, around which accumulation has occurred, and can be seen in the process of emigration through the walls of some veins. The irregular outline of many of these cells resembles the appearance of recently emigrated leucocytes. They can emigrate from the blood vessels, by virtue of/

of their amoeboid movement. If the blood of rabbit be examined on a warm stage of 41° - 42° C., the lymphoid corpuscles are found to be actively amoeboid. After emigration from the blood vessels, further increase in number may take place, but I have never found evidence of any active mitosis. These cells do not accumulate in areas where the tubular changes are more advanced than elsewhere, so that we cannot regard the degenerative or necrotic changes in the epithelium as the direct causative factor in producing their emigration. To no physical condition of the circulation in the kidney can their emigration be ascribed. The focal character of distribution resembles that seen in some bacterial infections, but here the agency of bacteria can be excluded. These cells correspond in character to those which Councilman has described in his cases of acute interstitial nephritis. Similar accumulations of round cells are common in the interstitial tissue in chronic nephritis and Ribbert (57) ascribes the focal distribution as due to the action of some toxic substance in the urine, in areas where it becomes more concentrated, or where it escapes into the tissues. It is evident that the emigration of these leucocytes must be dominated by some substance exerting a chemotactic influence, but what/

what the nature of this is, it is difficult to say. Do these emigrated leucocytes act as phagocytes, or do they become transformed into fibroblasts and form fibrous tissue? Although these cells in the splenic pulp are actively phagocytic towards red blood corpuscles, I have never found them endowed with similar properties in the interstitial tissue of the kidney. They do not emigrate into the tubules, and their protoplasm never contains any ingested substances. Transformation of these cells into fibroblasts with the subsequent formation of fibrous tissue has been described by Karvonen,(31) in experimental Mercury poisoning, in the rabbits' kidney. I have been unable to observe such a transformation in my series of experiments. Of some interest in this connection is the recent work of Maximon on the inflammatory new formation of connective tissue. He used rabbits in a number of his experiments, and induced the inflammatory reaction by subcutaneous introduction of a foreign body under aseptic conditions. He found a very important rôle was played by the migrated mononucleated leucocytes, and he believes, in opposition to Ehrlich's views, that the various forms of these mononucleated leucocytes represent developmental stages of one and the same/

same type of cell. The chief function of these uninucleated cells, was to act as very active phagocytes. They cleared away all the cellular debris from the field of inflammation, and Maximow also observed that they can at times undergo further development and become transformed into fibroblasts. Although these cells in the interstitial tissue of the kidney may also act as phagocytes, I have found no evidence of this function in my series of experiments; but from what we now know of these cells, there is much in favour of the view that their function here, as elsewhere, is phagocytic. In the Kidney also, it is probable, they play an important part in new formation of fibrous tissue.

SPLEEN:

Lesions of the spleen have been commonly observed in human diphtheria. Bizzozero[6] found the Malpighian bodies were chiefly affected. There were small focal areas in which the lymphoid cells were destroyed, and there was a number of phagocytic cells which included disintegrated cells and yellow granular pigment. Oertel[50] describes changes which are almost constantly present. The pulp is congested and haemorrhages are frequent. In the pulp spaces, the cells are increased in number, the majority being leucocytes of the lymphoid type. In the Malpighian bodies, there is sometimes an accumulation in the central part, of cells with epithelioid characters, but the most constant change was a fragmentation and disintegration of the lymphoid cells of the follicle. Barbacci[3] has more recently studied the changes in the organs in diphtheria. In the spleen, he found, in addition to necrosis of some cells in the follicles, active mitosis in others. The central part of the follicle generally showed a greater degree of disintegration of its cells than the periphery. Phagocyte cells in the follicles were few in number. There was hyaline swelling of the adenoid reticulum and the small blood vessels, and blood pigment was occasionally deposited in the pulp. The lesions/

lesions of the spleen, as result of experimental diphtheria, have not been very fully studied. Flexner,[20] working with sterile diphtheria cultures, gives the results of his observations. In the follicles, there is an increase of large cells with pale vesicular nuclei, but there is no active mitosis. Later, these large cells become fewer in number owing to the occurrence of necrotic changes in them. Various forms of fragmented cells are then found in the follicles, and they originate not only from the large swollen cells, but also from lymphoid cells. Phagocytosis is never a marked feature. In the pulp, necrotic changes occur in the cells of the framework and also in leucocytes within the spaces. Large pale phagocytes are sometimes found taking up cellular detritus and blood pigment, but cells containing red blood corpuscles are rarely found. The blood vessels are not specially affected.

PERSONAL OBSERVATIONS:

In the rabbits' spleen, the lymphoid follicles are well developed, and pulp consists of spaces with well defined walls, but there is little intervascular tissue. The lesions of the Spleen vary in accordance with the intensity and rapidity of the intoxication. In the acute cases (specimens 1, 2 and 3)/

3) the characteristic feature is the extreme congestion of the organ. The pulp spaces are greatly distended and filled with blood corpuscles. At parts, there are actual haemorrhages. The basement membrane of the spaces is swollen, and the endothelial cells possess an increased amount of granular protoplasm, and their nuclei are large and pale. Many have become desquamated. The pulp spaces contain, besides red blood corpuscles, a considerable number of mononucleated leucocytes and a few polymorphs. There is nuclear debris in the spaces, resulting from disintegration of many of the leucocytes and endothelial cells. Phagocytosis has not yet become a prominent feature, although a few large phagocytes containing some blood pigment and nuclear debris are present here and there in the pulp spaces. Finally, in the pulp spaces at this period, thrombi are abundant. They are hyaline or fibrinous in consistence, and mostly give a positive reaction to Weigert's fibrin stain. They are most numerous in the pulp spaces around the follicles. Some spaces are completely blocked; in others, the thrombi are attached to the wall at one side, or lie free in the lumen, surrounded by red blood corpuscles.

The Malpighian corpuscles always suffer, but all/

all are not equally affected. The striking features are the extreme congestion of its central arteriole and capillaries, and the destruction of the lymphoid cells. This is best seen in specimen No. 3 and is shown in Plate XIX . In many follicles, the dilated capillaries replace to a great extent the lymphoid cells. Within the central arteriole and also in the lumina of many capillaries, hyaline or fibrinous thrombi are found. I have never seen a central arteriole completely blocked in consequence, although some capillaries, especially at periphery of follicle, may be occluded. The basement membrane of the capillaries is swollen, and the delicate fibrous reticulum of the follicle is in a similar condition. There is no hyaline change in the walls of the central arterioles. In all the Malpighian bodies, there is a greater or less degree of destruction of lymphoid cells. In many, this is so considerable, that only localized areas of cells remain, whose nuclei have not undergone complete disintegration. These better preserved cells show no constancy as regards their position in the follicle; at one time they lie towards the centre, at other times they are situated more peripherally. These changes in the cells of the follicle are best studied/

studied in specimens Nos. 1 and 3; in No. 2 the lymphoid follicles have not suffered so severely. If we examine a follicle, in which a great number of its lymphoid cells have evidently disappeared and been replaced by dilated capillaries, we find a large quantity of chromatin granules lying free. Many of the cells which remain, present various degenerative changes, specially characteristic in their nuclei (Plate XX .) Solidly staining nuclei are common: these may be circular or irregular in outline and they vary in size. While many are concentrated, others are larger than normal nuclei, and the cells in which they lie are swollen, with increased granularity of their protoplasm. These solidly staining nuclei undergo fragmentation, and break up into a number of smaller or larger chromatin masses. These may be retained within the cell whose protoplasm has not yet disintegrated, or occur as free masses or granules. During the fragmentation of the altered nucleus, an appearance which resembles Aster stage of Karyokinesis, is sometimes seen; but the irregularity in the form of such a fragmentation clearly indicates that it is a retrogressive and not a progressive change. These chromatin fragments stain deeply with nuclear stains, but many show metachromatic staining in eosine and methylene blue preparations. In addition to these cells, other cells show/

show different alteration of their nuclei. These cells are swollen, and nuclei are large and pale, sometimes hyaline in appearance, but more frequently small chromatin granules are still retained within a nuclear membrane, either scattered irregularly or arranged as a peripheral granular layer. These pale swollen nuclei are most abundant in the least affected follicles, and they may undergo further changes, resulting in their complete destruction. The nuclear membrane disappears, and the chromatin granules become free. Phagocytosis of the nuclear debris in the affected follicles is never a prominent feature. In many follicles, phagocyte cells are entirely wanting, in others a few are present. These are large, possess a pale ovoid or vesicular nucleus, and resemble the endothelial cells covering the delicate reticulum of the follicle. They contain a variable number of included necrotic cells and chromatin granules. Some contain red blood corpuscles and yellow granular pigment.

In subacute intoxication, on the other hand, the lesions of the lymphoid follicles are quite subsidiary, but there is the progressive development of an interesting series of phenomena in the pulp spaces. Congestion of the pulp spaces or vessels of the follicles has now to a great extent disappeared/

disappeared and thrombi are no longer found. Hyaline change in the arterial walls is uncommon. More frequently, the delicate fibrous reticulum of the follicles and the basement membrane of the capillaries and pulp spaces are swollen. The lymphoid cells in most Malpighian bodies are intact, but in a small minority, degenerative forms occur. The nuclear changes resemble those already described, but in the affected follicles there is a preponderance of cells with pale swollen nuclei. Complete disintegration of nuclei, with the occurrence of free chromatin granules, has never progressed to any degree. I have not found any evidence of increase in number of lymphoid cells by active mitotic division. Mitotic figures are no more numerous than one finds under normal conditions. The endothelium of the reticulum does not seem to be affected. I have not observed any proliferation of its cells, even in areas where the lymphoid cells are degenerated, similar to what Mallory (43) finds in typhoid fever. Phagocytosis by these cells, is no more developed in subacute intoxications than it is in the acute cases. At most, a few phagocyte cells are found within the reticular spaces of the follicle.

CHANGES IN THE PULP: /

CHANGES IN THE PULP:

In subacute intoxication, the distension of the pulp spaces is inconsiderable, and haemorrhages no longer occur. We have now to examine the contents of the spaces, and the reaction of the lining endothelium. In some animals, e.g., guinea-pig, a varying number of pigment containing cells is found normally in the pulp spaces; in the healthy rabbit these are very rarely observed. In animals, which do not survive beyond three days, phagocytosis by cells in the pulp spaces is never the same constant and striking feature it is in animals subjected to a longer duration of intoxication. In specimens, Nos. 9, 16, and 19, the phenomenon is seen at its minimum. These phagocyte cells present a great variety of forms, and we have now to determine the source of these cells. Two distinct types can be differentiated, large cells with pale nuclei and smaller cells with round and deeply staining nuclei. These latter are mononucleated leucocytes, and every gradation in size is seen, from a typical lymphocyte to a large hyaline corpuscle. The nuclei of the smaller forms always stain more deeply than the nuclei of the larger. In eosine and methylene blue preparations, the protoplasm retains the blue colour, but some of the larger forms show a less characteristic/

acteristic staining of their protoplasm, which may be finely granular. These mononucleated leucocytes are actively phagocytic, and even the smaller forms are seen to ingest red blood corpuscles, but it is particularly the larger cells which exhibit the most marked phagocytic properties. Mitotic figures are occasionally observed in these cells in the pulp spaces. The other type of phagocyte is a cell derived from the lining endothelium. The endothelial cells become swollen; their nuclei are larger, stain less deeply and are more vesicular in character. These cells desquamate into the pulp spaces, where they are seen as large cells with pale nuclei, and a considerable quantity of finely granular protoplasm irregular in outline. But in addition, one observes at parts active proliferation of these endothelial cells. These newly formed cells are much smaller and their nuclei stain deeply and are round or ovoid in form. These cells often exactly resemble the lymphoid cells in the spaces, and one is often forced to the conclusion that many of the mononucleated cells in the pulp spaces are locally derived from proliferated endothelial cells, which have subsequently desquamated. The main function of these phagocytes is to ingest red blood corpuscles whose/

whose vitality has probably been injured by the circulating diphtheria toxine. These phagocyte cells are represented in Plates XXI and XXII. Red blood cells in all stages of disintegration are seen. Red cells, of normal form and staining red with eosine, may be found in the process of inclusion, or may be found wholly included lying within a vacuole in the protoplasm of the phagocyte. After the red cell has been ingested, its haemoglobin soon becomes discharged, and it remains for some time longer, as an unstained globule. The haemoglobin accumulates in the form of yellow granular pigment. Sometimes, as many as six red blood corpuscles are included within one phagocyte. When the destruction of red blood corpuscles has been extensive, there is the accumulation of large granular masses of yellow pigment in the pulp. The majority of these masses lie free in the spaces, and some spaces may be entirely blocked. The pigment masses are sometimes deposited in the intervascular tissue, but the fibrous trabeculae never contain a pigmentary deposit. The pigment masses tend to accumulate, especially in the pulp spaces which lie adjacent to the fibrous trabeculae. The mode of origin of these pigment masses can be easily followed./

ed. In the pulp spaces, phagocyte cells which have ingested a number of red blood cells ultimately become transformed into globular masses of granular pigment which fuse to produce the larger masses. These phagocyte cells not only ingest red blood corpuscles, but also polymorph leucocytes. In the pulp spaces, there is an accumulation of a varying number of these leucocytes, the majority showing degeneration of their nuclei, chiefly by fragmentation. These degenerated cells are taken up by phagocytes, and several may be found in one cell along with included red cells and granular pigment. Thereafter, speedy disintegration occurs. The protoplasm condenses and stains more deeply before it finally disappears. The nucleus becomes further fragmented, and numerous chromatin granules are then found scattered irregularly throughout the phagocyte cell. In the pulp spaces of almost every specimen, all stages of this phagocytic activity can be followed. Some reference must finally be made to changes occurring in the structure of the phagocyte cells. The protoplasm frequently contains vacuoles which are devoid of contents, or within them, lie included red cells or leucocytes. The nuclei always undergo progressive diminution/

diminution of staining properties and this is particularly noticed in the deeper staining round nuclei of the mononucleated leucocytes. Its position becomes eccentric as the quantity of ingested contents increases, and it is then most frequently found pressed towards one side of the cell. The form of nucleus varies, and the variety of shapes it may assume is considerable. In the pulp, red blood corpuscles may become disintegrated without apparent inclusion within phagocyte cells. In the splenic veins, pigment carrying cells are found, having travelled in the blood stream from the pulp spaces where phagocytosis is most active. These cells are carried to the liver by the portal vein. Occasionally, fibrinous thrombi are seen to partially occlude some of the branches of the splenic vein. The great part of the pigment within the cells or accumulated in masses, gives more or less definite iron reaction (Specimens 5, 12, 13, 15.) Some pigment does not stain at all, and this is found to represent pigment derived from recently destroyed red blood corpuscles. By the use of hot hydrochloric acid, most of this pigment gives iron reaction. In the Malpighian bodies, there is no deposition of pigment masses. In a few cases, the phagocytes of the follicles contain some iron pigment.

CORROSIVE SUBLIMATE POISONING:

Rabbits and cats were used as the experimental animals, but the following is based on the results obtained with rabbits. In all, twelve rabbits were subjected to acute, subacute or chronic poisoning. The poisonous effects of various salts of Mercury have been investigated, in relation to their action on the Kidney. Corrosive sublimate has alone been used in this series of experiments, but other salts, particularly the salicylate and iodide have, in the hands of other observers, been found to produce lesions of an identical nature in the kidney.

In several experiments on cats, attempt was made to induce the poisoning, by introducing the corrosive sublimate into animal's stomach along with its food: but this method was discarded owing to the inconstancy of its results. In rabbits, the injection of ½% solution in distilled water was made either subcutaneously or intramuscularly, and the results have been fairly uniform and constant in correspondence to the amount introduced. In the majority of the animals, only one dose was given, and duration varied from 12 hours to 17 days according to the dose. In 3 animals, a chronic poisoning was/

was induced by injection of sublethal doses, and the animals survived as long as 100 days. It was found that .0164 gram corrosive sublimate per 1000 gram weight was usually fatal within 24 hours. The following examples will suffice to illustrate the general outline of the experiments:-

Rabbit No. 1: Female, wt. 1520 grams.

Dose: .0164 gram per 1000 gram wt.

Duration - 12 hours.

Rabbit No. 2: Male, wt. 1750 grams.

Dose: .015 gram per 1000 gram wt.

Duration - 65 hours.

Trace of albumin in urine on day succeeding injection. No albumin in urine contained in bladder post-mortem.

Rabbit No. 4: Female, wt. 2390 grams.

Dose: .0125 gram per 1000 grams.

Duration - 75 hours; wt. 2030 grams.

There was anuria. Diarrhoea with blood in stools. Small quantity of urine in bladder post-mortem, which contained albumin and casts (hyaline and granular.)

Rabbit No. 7: Male, wt. 1840 grams.

Dose: .0025 gram per 1000 grams on six successive days.

Duration - 132 hours: wt. 1440 grams.

On day of death albumin equalled 2 grams per litre. Red blood corpuscles and casts also present.

Rabbit No. 8: Male, wt. 2270 grams.

Dose: .0088 gram per 1000 grams.

Duration - 17½ days.

Albumen amounted to 2 grams per litre on day after injection. Gradually decreased till absent on 13th day. Hyaline and granular casts were found as late as 14th day.

Rabbit No. 9: Female, wt. 1990 grams.

14th Feb: .010 gram per 1000 grams. Albumin equalled 4½ grams per litre. Red blood corpuscles and casts (hyaline and granular.)

1st March: Urine free from albumin and casts wt. 1980 grams.

12th March: Wt. 2094 grams. .010 gram per 1000 grams.

25th March: Wt. 2150 grams. .010 gram per 1000 grams. albumin 1½ grams per litre. Casts.

29th March: Wt. 1840 grams. No albumin.

23rd April: Wt. 2094 grams.
.012 gram per 1000 grams.
Slight trace albumin.

11th May: Wt. 1755 grams. No albumin.

21st May: Wt. 1925 grams.
.013 gram per 1000 grams.
Anuria.

26th May: Dead. Wt. 1472 grams. In bladder post-mortem, small quantity of urine which contained albumin (2 grams per litre). Numerous hyaline and granular casts and a few red blood corpuscles.

URINE OF POISONED ANIMALS:

Albumin was constantly found in the urine passed on day after administration of dose, except in those cases where small sublethal doses were given. In some, there was anuria. The amount of albumin varied from a trace to 7 grams per litre. In the following days, albumin gradually diminished and was usually absent on the day or days preceding animal's death. The urine did not commonly contain red blood corpuscles, but in a few cases they were numerous. Granular casts were always more abundant than the pale hyaline variety. Sugar was never found.

Diarrhoea was rare in rabbits. It was present in two cases only, and was accompanied by passage of considerable quantity of blood. In cats, poisoned by/

by introduction of poison along with the food, diarrhoea was present in all.

POST-MORTEM EXAMINATION:

The serous cavities were free from transuded fluid. The lungs frequently showed numerous minute subpleural haemorrhages, and there was more or less oedema. The veins were always distended and in one case, Inferior Vena Cava was occupied by an ante-mortem thrombus. Right cavities of heart were distended and filled with fluid blood and varying quantity of post mortem clot. Left ventricle was usually contracted and empty. Small haemorrhages on pericardial surface were noted in a few cases. Liver and spleen in acute poisoning were swollen and congested; in chronic poisoning, they showed no special affection beyond occasional increase in size of spleen. In the stomach, it was frequent to find small submucous haemorrhages in lower third of organ, and in one animal, the stomach was distended by a large red blood clot. In rabbit No. 9, which survived 101 days, a small ulcer was found on anterior stomach wall, near the pylorus. In the large intestine and especially in rectum, small submucous haemorrhages were common, although animal during life had not suffered from diarrhoea.

Large/

Large areas of the mucous membrane of the large intestine were gangrenous in the two animals in which diarrhoea was present.

The appearance of the Kidney varied according to the intensity and duration of poisoning. In acute cases, kidneys are enlarged and surface is finely mottled. On section, medulla is dark venous in colour, and the cortex is swollen and pale with injection of interlobular vessels. There is no haemorrhage into the mucous membrane of the pelvis. In subacute and chronic cases, swelling of cortex is less marked and vessels are not unduly congested. In the boundary layer, greyish opaque lines are found to radiate towards the surface along the lines of the medullary rays. In some cases, as in rabbit No. 9, whole cortex shows numerous similar areas irregularly distributed. These are calcified areas and are hard and gritty to the touch. On the addition of weak hydrochloric acid, there is ebullition of gas. I have never observed atrophy of the cortex, even in poisoning of long duration.

LITERATURE:

That disease of the kidney could be produced by Mercurial salts in the treatment of Syphilis, has been recognised since the 16th Century. Overbeck[51] regarded the presence of albumin in the urine during the course of Mercurial treatment as due to a simple catarrh of the kidney.

Pavy[52] investigated the physiological action of white precipitate upon the kidneys, experimentally in animals. He found the cortex was highly striated, due to the presence of white columns radiating from base of medulla to the surface of the organ. These white columns were shown to be blocked tubules in which there was a deposition of phosphate of Calcium. The Malpighian bodies were not implicated. These results of Pavy were confirmed by Saikowsky.[59] More recently, this subject has been investigated by Kaufmann,[32] Leutert,[39] Klemperer,[37] Karvonen[31] and by Harnack and Küstermann.[28] Karvonen subjected rabbits and dogs to acute and subacute mercurial poisoning. He used Corrosive Sublimate and Salicylate of Mercury. In the acute cases, the glomerular capillaries were congested, with sometimes, the passage of albumin and blood into the capsular space. In subacute poisoning, glomerular changes were more marked. The glomerular and capsular epithelium is often desquamated/

desquamated, many capillary loops are devoid of nuclei, and there is emigration of leucocytes into the capsular space. A great part of the literature on this subject resolves itself into a discussion on the nature of the calcified masses which are found in the tubules. After the injection of a poisonous dose of a mercurial salt, while a great number of tubules may appear normal, small groups of tubules here and there are necrotic. This necrosis is quite marked after 24 hours; in other less affected tubules, cells are swollen and in various stages of disintegration. Kaufmann has described the occurrence of wide spread capillary thrombosis in all the organs, in acute mercurial poisoning of animals and also of man. The capillaries were blocked by red thrombi produced by the fusion of red blood corpuscles into a homogeneous mass. Kaufmann attributes this thrombus formation to direct injury of the red blood corpuscles by Mercury, or to the liberation of fibrin ferment, whereby coagulation of blood occurs in the minutest vessels, with consequent stasis and fusion of red cells in the larger vessels. The necrosis which occurs in the tubules of the kidney, Kaufmann regards as an anaemic necrosis, and he finds the capillaries which correspond to the necrotic areas are

no longer permeable. Other observers have never seen the thrombosis of the vessels, described by Kaufmann, and most therefore believe that the necrosis is produced by a direct action of the circulating or secreted Mercury upon the cells. In the necrotic tubules, lime salts are subsequently deposited. Accordiny to Kaufmann and others, it is particularly the convoluted tubules which are affected in man, whereas in rabbits, the straight tubules of the medulla always suffer to a greater extent than the convoluted tubules. Virchow(61) maintained that the lime was deposited in the lumina of the tubules, and then secondarily in the epithelium. He regarded the process as analagous to lime metastasis which occurs when tumours or destructive processes in bone lead to an overloading of the blood with lime salts. Klemperer agreed with the views of Virchow. Kaufmann, on the other hand, finds deposition of lime only in necrotic cells, and Prévost(55) has come to a similar conclusion. The experimental results of Leutert are different and he recognises two distinct processes of calcification. He finds that lime salts are deposited in injured, but still functionating epithelial cells, and considers it as an abnormal secretion process.

In/

In addition there is calcification of totally necrosed cells and of casts produced by their fusion. Leutert thus distinguishes a separation of lime salts by still functionating parts, and a pure deposit in necrosed parts. Karvonen's views are in complete harmony with Leutert's. There is no unanimity of opinion as regards the source of the lime. Virchow and Prévost believe that a direct decalcification of the bones is occasioned by the Mercurial salts. Prévost describes hyperaemia of the bone marrow, decrease of lime in the bones, and an increase of the amount in the blood. This has not been confirmed. On the other hand, Klemperer finds the amount of lime in the blood is decreased in Mercury poisoning and Binet[5] finds there is no increase of the output of calcium and phosphorus in the urine. Harnack and Küsterman have described diffuse fatty changes in the epithelial cells, but as they used cats in their investigations, their observations on this point are valueless. Changes in the interstitial tissue will be referred to later. In man, Mercury poisoning produces lesions similar to those found in experimental animals. Prévost describes calcareous deposits in the tubules of the cortex and interstitial changes in the kidneys of a man/

man who survived four weeks. Many other cases are on record, and the lesions have been chiefly in the secreting cells of the cortex, the glomeruli being rarely affected. Thus Griffon[25] describes the glomeruli as apparently healthy in a patient who succumbed on the 21st day. On the other hand, Canuet and Pilliet[10] make mention of the occurrence of glomerulitis and desquamative nephritis in a patient dead on the 10th day, but no detailed description of the glomerular changes is given.

PERSONAL OBSERVATIONS ON CHANGES IN THE KIDNEYS OF RABBITS POISONED BY CORROSIVE SUBLIMATE.

In fresh sections, stained with Sudan or Osmic Acid, fatty degeneration in the epithelial cells is never a marked feature. In acute poisoning, it frequently is entirely absent, and in chronic cases, no fat may be found, or only minute granules occurring irregularly in the cells of some secreting tubules, and more rarely in the collecting tubule cells. The necrotic changes which occur in the cells are unaccompanied as a rule by any fatty metamorphosis. The most diffuse example of fatty degeneration was found in animal No. 2, in which the duration of the poisoning was 65 hours. Here, numerous fat granules and globules are present, especially in the cells of ascending limb of Henle in the medulla, and to a less extent in the convoluted tubule cells in the superficial part of the cortex. In the collecting tubule cells, fat is rarely found.

BLOOD VESSELS:

In acute poisoning, dilatation of the blood vessels, especially of the larger veins at junction of medulla and cortex, was always present to a marked degree; in subacute and chronic poisoning, congestion/

gestion of vessels was never so noteworthy. Within the larger veins, it was frequent to find masses of a finely granular, faintly staining substance, but the presence of fibrinous threads was rarely observed. Increase in number of leucocytes within the vessels was not observed, and no haemorrhages occurred in the interstitial substance or into the tubules.

MALPIGHIAN BODIES:

Corrosive sublimate, in my series of experiments, never produced any characteristic changes in the glomeruli. Karvonen describes the frequent occurrence of desquamation of glomerular epithelium and the passage of red blood corpuscles and leucocytes into the capsular space, and in chronic cases an increase of connective tissue in the glomerular tuft. In my acute cases, the congestion of the capillaries was considerable, but in subacute and chronic cases, any signs of congestion were usually absent. Thrombi within the glomerular capillaries or afferent arterioles, such as have been described in acute diphtheria toxine poisoning, were entirely wanting. Kaufmann has laid much stress on the occurrence of thrombi of fused red cells in the capillaries of the kidney and other organs in acute mercury poisoning, and advances this as the explanation/

tion of the necrotic changes. Such fusion of red cells, I only found rarely, in isolated capillaries of the tuft, and complete occlusion of the lumen never resulted. There was no evidence of the presence of any such extensive capillary thrombosis as a factor in the production of the cellular lesions. Hyaline changes in the capillary walls were absent, although in acute cases, some of the capillary loops were separated by an oedoematous transudation. The nuclei of the endothelial cells were always well preserved, stained deeply and remained attached to the basement membrane. Swelling of these cells, and desquamation into the capillary lumen was not common, but it was occasionally observed that a desquamated and swollen endothelial cell almost entirely filled the lumen. There was no leucocyte accumulation in the vessels of the tuft, and emigration into the capsular space was a feature which was absent. The glomerular epithelium, especially in the acute cases, showed swelling of its nuclei and increased granularity of its protoplasm, but it was rare to find any desquamated cells. At times, there appeared to be increase in the number of these cells, between the capillary loops. Bowman's Capsule was occasionally slightly swollen, but there was never proliferation/

proliferation or desquamation of its endothelium. These cells, in the majority of cases, showed no reaction; at most a few showed swelling of their protoplasm, but nuclei were always well preserved. The most characteristic change in relation to the glomeruli, was the almost constant occurrence of granular debris in the capsular space. The quantity varied in different capsular spaces: in some cases, as in Experiment 4, duration of intoxicating being 75 hours, the greater part of the space is filled with this granular material, and the tuft is compressed and pushed towards its root (Plate XXIV.) The source of this material might be an albuminous transudation from the capillary vessels, or it might result from granular disintegration of cells at the neck and origin of the convoluted tubules. It was present when there was entire absence of any congestion of the tuft. From a study of the various cases, I regard its main source to be a granular disintegration of those cells which line the neck of the capsule. Disintegration of desquamated glomerular or capsular cells was not a source of it. In the minimal degrees of accumulation of this material, it always occurred in the vicinity of the origin of the tubule, and this was the more characteristic, if/

if the section showed the continuity of the space with the tubular lumen. The material was in the form of round granular masses, which corresponded in appearance to similar masses in the lumina of the tubules derived from secreting cells. The cellular origin was further proved by the frequent presence in these masses of pale nuclei or chromatin granules. The larger accumulations were evidently produced by the fusion of separate masses, and the evidence of such a fusion could often be demonstrated. Granular debris of an identical appearance accumulated in the lumina of the convoluted tubules in consequence of disintegration of secreting cells. Deposition of lime salts was sometimes observed in the granular debris within the capsular space. Red blood corpuscles were never found in the capsular spaces. In the chronic cases, when many tubules, secreting and collecting, had become blocked with calcified masses, one anticipated atrophic changes in many glomeruli. I have never seen any atrophied glomeruli with fibrous transformation. Even in animal No. 9, which was subjected to chronic poisoning and lived 101 days, and in which there is most diffuse blocking of the tubules, the glomeruli are all apparently healthy and unaffected. The only effect which the extensive blocking of the tubules seems/

seems to produce, is the occasional dilatation of the capsular space; but it is quite possible that in consequence of complete or partial blocking of convoluted tubules, some of the debris resulting from the granular disintegration of the cells near its origin, may be pushed along a line of less resistance into the capsular space.

CHANGES IN THE TUBULES:

A noteworthy feature is the irregular affection of the secreting tubules. While many tubules are comparatively normal, others have undergone necrosis. Necrotic changes are well marked at the end of twelve hours. There are two types of necrosis seen. The cells of the most severely affected tubules, die en masse and fuse to form deeply staining finely granular, or almost homogeneous masses. This corresponds to what Weigert has described as coagulative necrosis. On the other hand, tubules which have been less affected show every gradation, from granular swelling to complete granular disintegration. The most vulnerable tubules are the ascending limbs of Henle, especially those parts which lie in the medullary rays of the cortex and in the upper part of medulla. The parts deeper down in the medulla are always less gravely affected. In acute poisoning/

poisoning, and if no calcification has yet occurred in the necrotic masses, as seen in Exp. No. 1 and No. 3, the great majority of the ascending limbs have, in the situations mentioned, undergone coagulative necrosis (Plate XXIII.) This type of necrosis is almost entirely limited to these tubules. It is rare to find any extensive coagulative necrosis of the convoluted tubules. The cells in which this type of necrosis occurs, become slightly swollen, become detached from the basement membrane and fuse to form masses which occlude the lumen. These masses are commonly granular, but may be almost homogeneous in appearance. They stain deeply with haematoxylin in fresh specimens; in paraffin sections stained with haematein and eosine, they give a deep red reaction. The nuclear changes are characteristic. They rapidly disappear, so that those masses may be entirely devoid of any nuclei. At other parts, nuclei are still present. These are condensed and stain solidly, and the further transformation seems to be a rapid solution of the chromatic substance, leading to complete disappearance. Fragmentation of these solidly staining nuclei, as has been noted in diphtheria toxine experiments, is uncommon. Only rarely can the presence/

ence of small chromatic masses or granules be detected in the fused necrotic cells. The ascending tubules, whose cells have not thus rapidly necrosed, show changes similar to those now to be described in the convoluted tubules. While all ascending limbs appear to be affected to a greater or less degree, many of the convoluted tubules may entirely escape. Reference has already been made to this point, and the possible explanations discussed. These experiments however, show that the secreting tubules of Henle are more vulnerable than the convoluted tubules to the poisonous action of Mercury, as they are to other poisons and toxines which have been studied. In inflammatory conditions in the human kidney, a similar vulnerability is shown by these tubules. The convoluted tubules which are affected show all grades of granular disintegration. Some are found whose cells are swollen and granular and lumen is completely occluded. In more advanced stages, cells become vacuolated, and they break up into a granular mass which accumulates in the lumen. Some tubules may be seen, in which there are no longer any cells attached to the basement membrane, and lumen is filled with a granular debris similar in character and staining properties to that which accumulates in the capsular space. On the other hand, cells more/

more or less affected are still attached to the wall, and the lumen is occupied by cells whose cell membrane is still intact, but whose protoplasm has for the most disappeared. When many such altered cells are found in the lumen, a net-like arrangement with round meshes is displayed. In addition, single necrotic cells with homogeneous or finely granular condensed protoplasm, and with condensed or fragmented nuclei, also occur in the lumen. The nuclei of the convoluted tubules do not exhibit any striking changes. Many are normally stained, even though protoplasm may be in advanced state of disintegration. They may be slightly decreased in size and stain more deeply, but solidly staining condensed nuclei are rare, except in single necrotic cells discharged into the lumen. Others are enlarged, swollen and pale, showing deficiency of chromatin. These nuclei disappear by a process of Karyolysis, and scarcely ever does one see a change resulting in Karyorhexis. In chronic poisoning, the changes in the cells are of this granular, disintegrative type, the cells which have undergone coagulative necrosis being now all calcified. Mitotic figures and evidences of regeneration of cells are rarer than in diphtheria toxin poisoning, but are not entirely absent.

COLLECTING TUBULES:

The cells of these never undergo necrosis in this condition. At most, they undergo swelling with increased granularity of the protoplasm. The smaller collecting tubules in their medullary rays are most affected. The cells of these tubules also exhibit phagocytic tendencies towards the necrotic cells in their lumen, and many contain small granules of yellow pigment. These have probably been derived from red blood corpuscles, but they do not give iron reaction.

CALCIFICATION AND CAST FORMATION:

Calcification may be present at the end of 30 hours, and in poisoning of longer duration, it is almost constantly present beyond this period. However, in animal No. 3, which lived 72 hours, no calcification of necrotic cells has yet occurred, although present in all other cases. Deposition of lime salts always occurs earliest in the fused necrotic cells of ascending limbs of Henle, and those parts of the tubules situated in the medullary rays in the deep part of cortex may alone be calcified, as shown in animal No. 6, in which duration of poisoning was 120 hours. In animals which have received only one dose of poison, all the calcified masses/

masses are found in the region of the medullary rays (Plate XXV.) with the exception of a few beneath the capsule and around some glomeruli. Karvonen, Leutert and Prévost have described calcium deposition in swollen granular cells which are still functionating. They regard it as an abnormal secretion process. This, I found, to be exceedingly rare. Occasionally, granules of lime were found in a secreting cells whose nucleus had not yet disappeared, but the deposition almost invariably occurs in the cells which have undergone the coagulative necrosis, and in the granular masses which result from the granular disintegration of the secreting cells. I have never observed calcification of nuclei, as these had almost all disappeared previous to onset of calcification. The lime salts are deposited at first, as granules which, later, fuse to form a solid calcified mass which may completely fill the lumen. Such large calcified masses are most frequent in ascending limbs of Henle, but when the animal has been subjected to chronic poisoning, by the injection of repeated doses, a great number of convoluted tubules are in a similar condition. The basement membrane may still remain intact, but it commonly also becomes calcified and fuses with the mass/

mass in the tubule. Calcification is also seen in the granular debris contained in the lumina of the convoluted tubules, and more rarely, in that accumulated in the capsular space. It is especially common to find, in the collecting tubes of the medullary rays, calcification of necrotic cells and granular casts. There may be only a small calcified mass, but not infrequently the lumen is entirely occpuied by a solid mass, and the lining epithelium is flattened and compressed. Most observers mention that in rabbits, the calcification occurs in the straight tubules of the medulla, and to a less extent in the convoluted tubules of the cortex. My results are not in accordance with this observation. Once the lumen of a straight tubule is blocked by the presence of a calcified mass, the subsequent calcification of its epithelial cells occurs, but any direct calcification of collecting tubule cells probably does not occur. In paraffin sections, the calcified masses stain most characteristically with haematein, in haematein and eosine, or rubin and orange preparations. With van Gieson's stain, the necrotic cells stain of a characteristic yellow colour. By the addition of dilute hydrochloric acid, the calcium salts are dissolved with the ebullition of gas bubbles, and there remains an amorphous structure/

structure which forms the basis of these calcified masses. The lime salts which are present, are chiefly phosphate and carbonate, partly perhaps in combination with organic substances. The deposit is amorphous and granular. A crystalline deposit, I have never observed. Formation of calcified masses occurs whenever there has been necrotic changes in the tubular cells. It is well seen in the rabbit's kidney in the experimental study of infarction. It is also due to the action of other poisons and toxines, as demonstrated in the diphtheria toxine series of experiments. The source of the lime is regarded by many, - Prévost, Koniger, Virchow, Kobert and von Noorden - as a direct decalcification of bone, due to mercury poisoning. The amount of lime normally present in the tissues and blood is probably sufficient to account for that which is precipitated. I have made no observations on the quantity of lime in the blood and urine in this condition.

In the collecting tubules of the medullary rays, and to a much less extent in the larger tubules in the medulla, there is abundant formation of other casts. The great majority of these are cellular in origin. The pale hyaline or more fibrillated casts,/

casts, which one finds so commonly in the tubules in diphtheria toxine experiments, and which one regards as a transudation from the blood vessels, are here comparatively few in number. The casts are uniformly granular in appearance, or commonly are more colloid in consistence and vacuolated. All transitions may be seen, and their derivation from the fusion of necrotic cells or protoplasmic debris can be easily traced. In this condition, it is rare to find emigrated leucocytes either free in the lumen, or attached to casts. Dilatation of the straight tubules in the cortex, and to a less extent of the convoluted tubules, is of almost constant occurrence in chronic poisoning. Animal No. 9 shows extensive development of this, in consequence of presence of numerous calcified casts, and it is seen in a more irregularly distributed fashion in Nos. 6, and 7. The basement membrane of the tubules in some cases, is markedly swollen.

INTERSTITIAL TISSUE:

Karvonen, in acute mercury poisoning in rabbits, found an infiltration of the interstitial tissue with round cells almost constantly present after the second day. Leutert also mentions a leucocyte emigration around the calcified tubules, and Klemperer commonly/

commonly encountered a similar interstitial infiltration. Harnack and Küstermann only found it in one case in their studies on acute mercury poisoning in cats. Karvonen describes the accumulation of these mononucleated leucocytes around necrotic and calcified tubules, and some had emigrated into the tubules. He also describes in chronic poisoning, the transition of these cells to connective tissue cells, and the subsequent formation of fibrous tissue which replaces the atrophic tubules. In acute poisoning, I found considerable dilatation of intertubular capillaries where these had not been compressed by swollen tubules, and it was frequent also to observe the occurrence of some oedœma in the interstitial tissue, but emigration of leucocytes, such as has been described in diphtheria toxine kidneys, was constantly absent. At most, around some calcified tubules, a few mononucleated leucocytes accumulated, and in animal No. 6, around an area of calcified tubules, there was the only marked degree of leucocyte emigration in relation to a vein in the deep part of the cortex, which was observed in this series of experiments. In chronic poisoning of long duration, the majority of the calcified masses showed little or no reaction of interstitial tissue around them. In animal No. 9, the formation

of/

of some definite fibrous tissue localized around some of the masses may be seen. The cells present all the characters of connective tissue cells, and no transition of emigrated mononucleated cells to connective tissue cells was observed.

SPLEEN:

In acute poisoning, there is always congestion of the pulp, which shows variations in intensity in different cases. It was most marked in animal No. 3, in which duration of intoxication was 72 hours. In striking contrast to the effects of diphtheria toxine, is the absence of lesions in the Malpighian bodies. These may be encroached upon and compressed by the dilated pulp spaces, so that they appear smaller than normal, but otherwise, they show little or no affection. The capillaries at the periphery may be congested, but the lymphoid cells are well preserved. At most, a few cells show necrotic changes, and a small amount of nuclear debris is sometimes to be found, especially towards the periphery of the corpuscle. In animal No. 1, dead in 12 hours, evidences of this are seen, and some of the nuclei show metachromatic staining, but phagocyte cells containing nuclear fragments are wanting. The large endothelial cells of the Malpighian corpuscles are frequently swollen, and the number of cells/

cells with large, round, pale, vesicular nuclei is frequently increased. In the acute cases, the pulp spaces are dilated and basement membrane is swollen, but in the chronic cases, the dilatation of these spaces is inconsiderable. The characteristic changes in the splenic pulp are in relation to the destruction of red blood corpuscles. Even at the end of 12 hours, large phagocyte cells containing red blood corpuscles or granular pigment, are numerous in the pulp spaces. Corrosive sublimate, like many other poisons, exerts an injurious effect upon many red blood corpuscles, and their ultimate disintegration and destruction is accomplished by phagocyte cells. This phagocytosis is displayed chiefly by the endothelial cells of splenic pulp, and to a less extent by the vascular endothelium in other organs. In these experiments, pigment containing cells were found in the liver, many of which had come from the spleen, but phagocytosis of red cells by the endothelium of the intralobular capillaries was frequently observed. The phenomena of phagocytosis in the spleen have been fully described in the consideration of the action of diphtheria toxine, and no further detailed discussion is requisite. The phagocyte cells, in mercury poisoning/

poisoning, are mainly derived from proliferated and desquamated endothelium, but evidences of the phagocytic properties of the mononucleated leucocytes are not so noteworthy as in the diphtheria toxine experiments, and they are never in such abundance. There is also less accumulation of damaged polymorphs, but it is quite common, however, to find ingestion of these cells by phagocytes. In animals, which have received only one dose of poison, and survived for varying periods, the phagocytosis of red cells and accumulation of pigment were most prominent in those in which a fatal issue resulted in the earlier days of the poisoning. On the other hand, if the animal be subjected to repeated doses, then there is a progressive increase in the quantity of accumulated pigment in the pulp. This is well seen in animal No. 9 and Plate XXVI, where numerous large granular masses of pigment completely fill many of the spaces, particularly those in the vicinity of the fibrous trabeculae. In the sections treated with ferrocyanide of potassium and hydrochloric acid, one finds that the pigment does not give the iron reaction unless the duration of the poisoning has lasted several days. In the chronic cases, the greater part of pigment gives this reaction. No hyaline/

hyaline thrombi were ever found in the vessels of the spleen, but in a few dilated pulp spaces, fibrinous threads were occasionally seen.

CANTHARIDIN POISONING:

Thirteen animals were used in this series of observations, and they were subjected to acute and subacute poisoning. A solution of the cantharidin crystals was made, either in olive oil or in acetic ether, and this was injected intramuscularly. The poisonous effects were much more speedily produced by the ethereal solution. As much as 3 cc. of acetic ether was used in some cases, but control experiments were made with acetic ether alone, and showed that the lesions, which were produced in the kidneys, resulted from the action of the Cantharidin, and the control animals which were subjected to similar quantities of ether, showed no symptoms. Identical lesions were produced by the olive oil solution. The duration of poisoning varied from 6 hours to 3 days, when a single dose was given. Other animals, which received several sublethal doses, survived as late as the 14th day. Attempts were made to establish more chronic poisoning, but these yielded no additional results. I have failed to obtain, as with/

with diphtheria toxine, the development of chronic progressive lesions in the kidney, subsequent to an acute inflammatory process. The following examples will suffice to explain the outline of the experiments.

Animal No. 1: Male, wt. 1780 grams.

Dose: 3 m.gr. cantharidin dissolved in 3 cc. acetic ether.

Duration of poisoning was 6 hours.

Animal No. 2: Male, wt. 1670 grams.

Dose: 10 m.gr. in olive oil. Lived 10 hrs.

Animal No. 5: Male, wt. 1680 grams.

Dose: 6 m.gr. Cantharidin in olive oil. lived 17 hours.

Animal No. 6: Male, wt. 1750 grams.

Dose: .2 m.gr. in 1 c.c. acetic ether. Lived 25 hours.

Animal No. 7: Female, wt. 1825 grams.

Dose: .5 m.gr. in 1 c.c. acetic ether.

In urine, albumin and red blood corpuscles, which persisted till death at end of 67 hours. Wt. 1415 grams. Albumin equalled 6½ grams per litre.

Animal No. 9: Female, wt. 1780 grams.

17th Sept: .5 m. gr. in 1 c.c. acetic ether.

22nd Sept: .5 m. gr. in 1 c.c. acetic ether, wt., 1500 grams.

25th Sept: Dead; wt. 1190 grams.

Albumin and red blood corpuscles present in urine after 2nd injection. A few granular casts.

Animal No. 11: Female, wt. 1930 grams.

17th Sept: .5 m. gr. in 1 c.c. acetic ether.

22nd Sept: .5 m. gr. in 1 c.c. " " wt., 1800 grams.

30th Sept: Dead; wt. 1300 grams.

After the first injection, albumin amounted to 4½ grams per litre. Only a few red blood corpuscles present in urine. Albumin gradually diminished until absent on 22nd September. After 2nd injection, albumin reappeared and estimation showed 7 grams per litre. Red blood corpuscles numerous.

EXAMINATION OF URINE:

Animals, which died within 24 hours, suffered from anuria. At the autopsy, the bladder in these cases, was empty and contracted. In some, it contained a small quantity of urine containing albumin and red blood corpuscles. When duration of poisoning was longer, albumin was always found in the urine, along with numerous red and white blood corpuscles. If a fatal issue did not result, the abnormal/

normal constituents disappeared in a few days, and animal was restored to its normal health. Casts were never abundant, and these were mainly hyaline and granular in character.

POST MORTEM APPEARANCES:

The kidneys were enlarged, and in the acute cases, small haemorrhages were common around and in the capsule. The cortex was swollen, and was markedly congested. No thrombi were ever found in the renal vessels. In all cases, there was absence of blood clots in the pelvis of kidney, ureter and bladder. Small submucous haemorrhages were frequently found in the bladder, but no where else in the urinary tract. The liver was dark red in colour, being filled with venous blood. The spleen was congested. Recent submucous haemorrhages in the lower one third of stomach were of constant occurrence in acute poisoning. They sometimes reached the size of a crown piece, but in only one case, animal No. 10, which survived 10½ days, was there the formation of an ulcer. The lungs were congested, and subpleural haemorrhages had often occurred. The right cavities of the heart were distended with fluid blood or post mortem clots; the left were usually empty and left ventricle was firm and contracted/

LITERATURE ON CANTHARIDIN POISONING:

Cornil and Brault[12] subjected rabbits to acute and subacute poisoning. They injected .005 to .01 gram cantharidin, subcutaneously. In animals, which died in a few hours, characteristic lesions were present in the Malpighian Bodies. The lesions were advanced at end of one hour. The tuft only occupied half or even one third of capsular space, and is compressed by a membranous exudate in which are imbedded nuclei in variable numbers. The nuclei are large and vesicular. They regarded these nuclei as the swollen nuclei of emigrated lymphocytes. The capsular endothelium is not affected so early. Later, the cells become swollen and are desquamated. The convoluted tubules were swollen and the cells showed granular disintegration and vacuolation with the accumulation of granular debris in their lumina. Lymphoid cells and red blood corpuscles were also found within the tubules. When poisoning only lasted ½-1½ hours, the glomeruli and convoluted tubules were alone affected, but after this period, the collecting tubules also suffer. The cells become granular and desquamate into the lumen. In slow poisoning, by the injection of small doses every 2 or 3 days, Cornil and Brault found that/

that by the 4th day, the glomerular tuft occupies its space and exudate has almost disappeared, but there were numerous mitotic figures in the convoluted tubule cells and in the intravascular endothelium. In chronic intoxication, there was accumulation of round cells along the arteries. Cornil says that cantharidin first attacks the glomerular capillaries and there is an accumulation of white blood corpuscles which emigrate into the capsular space. The epithelial lesions occur later. Browicz[8] found in acute poisoning, enlargement of the glomeruli with the accumulation in the capsular spaces of finely granular and hyaline masses which contained no nuclei. Similar masses were found in the tubules, and a few lymphocytes had emigrated into interstitial tissue. Aufrecht[1] has described the occurrence of an interstitial nephritis in an animal subjected to a daily dose of .0025 gram for several weeks. Eliaschoff subjected rabbits to acute poisoning by subcutaneous injection of .01 gram cantharidin, dissolved in acetic ether. The glomerular changes corresponded to those described by Cornil. There was an albuminous exudation, and an emigration of white blood corpuscles into the capsular spaces. These cells become swollen to twice the size of normal white corpuscles, and their nuclei are large and pale./

pale. There was no desquamation of glomerular or capsular epithelium. All the tubules are affected with the exception of the descending limbs of Henle. There is granular disintegration of cell protoplasm. The most resistent tubules according to Eliaschoff, are ascending limbs of boundary layer. Nowhere was there an emigration of white blood corpuscles into the interstitial tissue. Welch poisoned white rats with cantharidin and found in some cases proliferation of endothelium of glomerular capillaries; in others the proliferation of Bowman's capsular endothelium was more marked. In acute cases, there was occasionally the presence of a few secreting cells in glomerular spaces.

PERSONAL OBSERVATIONS ON CANTHARIDIN POISONING:

The congestion of the blood vessels is extreme, but no haemorrhages are found, except in the submucous tissue of the pelvis in some cases. There is rupture of capillaries into the tubules, especially into the larger collecting tubules of the medulla. In the subacute cases, the congestion of the vessels, is less prominent. In fresh specimens, there is absence of fat in the rapidly fatal cases; in slower poisoning, some fatty granules may be found in ascending limbs and in collecting tubule cells, but this change is never extensive.

GLOMERULAR CHANGES:

These differ according to the intensity of the poisoning. In animals, which do not survive beyond 10 hours (specimens 1 and 2), almost all the glomerular tufts are compressed, and the capsular spaces are filled by a mosaic like structure. This appearance is produced by the accumulation of cells which are closely packed. (Plate XXVII.) The cell membrane is retained, but the greater part of the protoplasm has disappeared, so that the cells resemble clear vacuoles containing some scattered granules in their interior. The nucleus is still present in many. It occupies the centre of the cell, is swollen and pale, possessing a distinct nuclear membrane. In a few cells, the nucleus is condensed and stains deeply. According to line of section, the space may appear completely filled with these cells. Similar cells are seen in the convoluted tubules. They may lie in the lumen or still be attached to basement membrane. When the section shows continuity of tubular lumen and capsular space, as seen in Plate XXVII., these parts are occupied by a similar and continuous mass of cells. In some, the cells of the first part of the convoluted tubule are detached and accumulate within the capsular space. These/

These cells correspond to those described by Cornil and Eliaschoff as occurring in the capsular spaces, in their cases of acute poisoning. They regarded them as lymphoid cells, which had emigrated into the spaces and become greatly swollen by the imbibition of fluid. I find no accumulation of mononucleated leucocytes within the glomerular capillaries, and cannot regard these cells as swollen emigrated white cells. They are identical in appearance to cells which are found in the convoluted tubules, where their origin from secreting cells can be demonstrated. These cells then must be pushed back into the capsular spaces from the lumina of the convoluted tubules, but it is difficult to explain this phenomenon on physical grounds. The tubule may have been blocked at a lower level or compressed by dilated vessels. The nuclei of the capillary endothelium are intact and attached to the basement membrane and there is no accumulation of leucocytes within the vessels. There is no desquamation of glomerular epithelium. Bowman's capsule is swollen, but its lining endothelium is unaffected. In animals which survived beyond 10 hours, the capsular spaces are found to be devoid of contents, and the tuft occupies the greater part of the space, but the congestion of its capillaries is/

is never considerable. No hyaline thrombi are found in the capillaries. Here and there, there is slight swelling of basement membrane. The endothelium is in most cases unaffected, and desquamation of its cells is rare. Mitotic figures are occasionally seen in cells still attached to basement membrane. A few polymorphs are seen, some with fragmented nuclei. Glomerular epithelium may show slight swelling of its protoplasm, but otherwise undergoes no changes. Bowman's capsule is swollen, but its endothelium, even in subacute poisoning, neither proliferates nor desquamates. I have never found red blood corpuscles in capsular spaces.

TUBULAR CHANGES: (Plate XXVIII)

In the rapid poisoning cases (Specimens 1 and 2), there is most diffuse necrosis of the secreting cells. The tubules are dilated, and there is entire disintegration of inner portion of cell, so that only the basal part remains attached to basement membrane. In this basal portion, which is granular and stains deeply, the nucleus is frequently retained. It may be normal in appearance, but condensed deeply staining forms are common. In other tubules, the cells are swollen and pale, and resemble/

resemble those cells, which accumulate in the capsular spaces. The ascending limbs of Henle seem to be the most profoundly altered. While similar changes are seen in these tubules as occur in the convoluted tubules, some show appearance similar to coagulative necrosis. The cells are fused to form a finely granular mass and their nuclei are condensed, stain solidly, and may show fragmentation. When the duration of the poisoning exceeded 10 hours, the necrotic changes in the cells become progressively less marked. The secreting cells are swollen and granular, but complete disintegration of cells is now not common, though still to be found especially in ascending limbs. The nuclear changes in the subacute cases do not differ from those described in diphtheria toxine experiments. The basement membrane of the tubules is swollen.

The collecting tubules are also affected, particularly in short duration of poisoning. Cells are swollen and granular, and in the larger tubules are frequently desquamated into the lumen. The nuclei present various degenerative forms. They may be swollen and pale, or appear as condensed bizarre figures. In the large tubules in the medulla, polymorph leucocytes are frequently found emigrating between adjacent cells. In subacute poisoning, beyond the 4th day, mitotic figures are seen in/

in secreting tubules and also in the collecting, but these are never very numerous. The cells of the collecting tubules here also are phagocytic and contain yellow granular pigment, and ingested necrotic cells. Casts are found in the collecting tubules, most abundantly in the smaller tubules in the medullary rays. These are chiefly granular casts, with adherent or included necrotic cells. Vacuolated casts similar in appearance and origin to those described in the diphtheria toxine and mercury experiments, also occur. The rupture of capillaries in medulla has produced a haemorrhage into some large collecting tubules. There may be fibrinous threads, with red and white corpuscles entangled in their meshes. It is rare to find blood corpuscles in the convoluted tubules. I have only once observed calcification of a few necrotic cells in the ascending limbs. This occurred in an animal which survived 10½ days.

CHANGES IN INTERSTITIAL TISSUE:

Reference has been already made to the extreme congestion of intertubular capillaries. In acute poisoning, there is also some oedœma. Emigration of leucocytes is a rare phenomenon. A small localized collection of lymphocytes around a dilated vein has been observed in two subacute cases. A hyaline swelling of the walls of some arterioles in the cortex, is seen in some specimens. (e.g. 7 & 10)

SPLEEN:

The changes in the spleen in cantharidin poisoning are so closely analagous to those seen in Mercury poisoning that no detailed description is necessary. Here again, the most noteworthy feature is the ingestion of red blood corpuscles by phagocyte cells in the pulp, and the accumulation of masses of granular pigment. The Malpighian bodies are not specially affected. They are frequently compressed by the dilated pulp spaces, but their lymphoid cells do not suffer to any greater extent than in corrosive sublimate poisoning. The congestion of the spleen, with the occurrence of haemorrhages in the pulp, is the special characteristic of rapid poisoning (Specimen No. 1), but phagocytosis of red cells is a later phenomenon. When the duration of the poisoning has been less than 10 hours, a few phagocyte cells containing red blood corpuscles or granular pigment may be found in the splenic pulp, but it is only when the duration of poisoning has exceeded this period that they become numerous ~~in number~~. At the end of 17 hours (Specimen No. 5) phagocytosis is in active progress. When the animal has been subjected to several doses, there is a progressive increase in the quantity of pigment in the pulp (Specimen No. 11.) There is a greater accumulation of hyaline/

hyaline mononucleated leucocytes in the pulp spaces than was observed in mercury experiments. These present all gradations in size and are actively phagocytic. Mitotic figures are found in some. The proliferation of pulp endothelium is very marked, and the number of free phagocyte cells derived therefrom, is very considerable. In several cases (e.g., Specimen No. 9), large giant cells lie free in the pulp spaces and are phagocytic in character. They contain as many as 7 or 8 large pale ovoid nuclei, and in some cases they appear to be produced by the fusion of desquamated endothelial cells. The amount of pigment giving iron reaction is greater, the longer the duration of poisoning (Specimen No. 11).

GENERAL SUMMARY AND CONCLUSIONS.

When the research was undertaken, its object was to study the early inflammatory changes in the different tissues of the kidney, and to ascertain by the use of various toxines and poisons the correlation between the vascular and other lesions. We are now in a position to briefly summarise the results which we have obtained by the use of diphtheria toxine, corrosive sublimate and cantharidin in the investigation of these questions. In addition to the study of the acute inflammatory lesions, I had also hoped to be able to follow the development of changes of a more chronic nature, which, one anticipated, might be consequent on and consecutive to the acute lesions. How far I have succeeded in this endeavour, has been already adequately discussed. Suffice it to say, that in no case and by no variation of the experimental method, have I been able to produce and follow the evolution of changes at all analagous to those which we find in subacute and chronic diffuse nephritis in man. That such lesions may be secondary to an acute inflammation of the kidney, cannot be gainsaid, and reference has been made in a previous part of this paper to this point. But/

But my results point to the fact that in animals at least, the kidney is restored to its normal integrity after the subsidence of the acute inflammatory phenomena, and if it be permissible to draw any conclusions therefrom, one might with reasonable assurance affirm that in man also, acute inflammatory changes in the kidney may entirely disappear without the further development of subacute or chronic lesions, if the noxious agent be no longer in operation. This is a recognised fact, and indirectly, it emphasizes the importance of the continuous action of some toxic substance in the slow insidious origin of the great majority of the cases of subacute and chronic nephritis.

Of the toxines and poisons which I have used in my experiments, diphtheria toxine alone produces distinctive vascular lesions, and these, in the kidney, are localized in the small arterioles of the cortex, and glomerular capillaries. In these vessels, there is extensive formation of hyaline thrombi, and this is only observed in acute and intense intoxication. In acute corrosive sublimate and cantharidin poisoning, on the other hand, there is never intravascular thrombosis, not even the formation of red thrombi produced by the fusion of red blood corpuscles, to which Kaufmann attaches so much/

much importance in the explanation of the necrotic lesions in acute mercurial poisoning. While all these substances induce a most marked congestion of the blood vessels, haemorrhages in the interstitial tissue or into the tubules of the cortex have been entirely wanting; but in the diphtheria toxine experiments mention has been made of the occasional rupture of the capillaries in the mucous membrane of the pelvis. Haemorrhage into the collecting tubules of the medulla is of more common occurrence, and has been especially observed in acute cantharidin poisoning. Hyaline changes in the vessels are almost constantly present in subacute diphtheria intoxication, but are absent in corrosive sublimate and cantharidin poisoning. The vessels, which are particularly affected, are the glomerular capillaries and small arterioles; but whereas occlusion of the capillary lumen has never been observed in consequence, the swelling of the small arterial walls may lead to their obliteration. When we come to review the glomerular lesions, we are at once struck by the fact that the glomeruli enjoy a great immunity, especially in corrosive sublimate and cantharidin poisoning. These two poisons affect especially the secreting cells and do not appear to produce any marked affection of the glomeruli./

uli. Cornil and Eliaschoff have characterised the lesions in cantharidin poisoning as a glomerulinephritis, but I find the changes are open to a different interpretation. The cells which accumulate in the capsular spaces in acute poisoning, they regarded as swollen emigrated leucocytes, but I have shown that their origin is different. In subacute diphtheria intoxication, the glomerular changes are interesting, and the formation of the haemorrhagic cyst-like spaces in the tuft has been elsewhere fully studied. While swelling of the capillary endothelium of the glomerular vessels may be observed in poisoning by these various substances, yet in corrosive sublimate and cantharidin poisoning, it is rare to find any extensive desquamation of these cells, as is so commonly observed in diphtheria intoxication. Much stress has been laid on the occurrence of active proliferation of the capillary endothelium by Langhaus and Councilman, but I have failed to find much evidence of this, although a few mitotic figures have been observed in subacute cantharidin poisoning. The endothelial cells of the glomerular vessels, like endothelium elsewhere, are phagocytic. The glomerular epithelium is always swollen and granular, and there is frequently increase/

crease in the number of its cells. The nuclear richness of many glomeruli, according to my observations, is dependent on proliferation of glomerular epithelium. In diphtheria intoxication alone, is there an accumulation of leucocytes in the vessels. These are numerous in the tufts, and the majority show degenerative changes in their nuclei. In no case, has there been active proliferation of capsular endothelium, either in the acute or subacute cases. In all cases, the lesions of the secreting tubules have been the prominent feature and these must be regarded as due to the direct action of the circulating toxine or poison upon them. These are always most intense in the acute cases; and in diphtheria intoxication, although ~~Although~~ the influence of the vascular lesions cannot be entirely ignored, these play an unimportant and subsidiary part in the production of the cell lesions. Are these substances excreted by the glomeruli, or are they excreted by the secreting cells of the tubules? If they were excreted by the glomeruli, the cells might then be affected by their passage along the lumen of the tubules in the urine. The nature of the lesions is in opposition to this hypothesis. In acute cantharidin poisoning, however, the cells of many/

many secreting tubules are completely disintegrated with the exception of their basal portions, so that in this case, the cells would appear to be affected by the poison in the lumen; but this is the only evidence which I have found in favour of this hypothesis. The ascending limbs of Henle are the least resistent of all the tubules, to the action of these substances, and this is brought into evidence particularly in the cases of rapid intoxication or poisoning. Then one finds the most diffuse necrotic changes in these tubules, whereas the convoluted tubules of the cortex suffer to a much less extent. No more need be said regarding the simple degenerative changes in the cells, but the necrotic changes deserve further notice. Two distinct types of necrosis occur. In corrosive sublimate poisoning, we have a good example of the coagulation form of necrosis, which is almost absolutely localized to the ascending limbs of Henle in the medullary rays. In acute diphtheria intoxication and acute cantharidin poisoning some of ascending limbs may present a similar type of necrosis. One of the chief characteristics of this form of necrosis is the rapid disappearance of the nuclei. The other type of necrosis is disintegrative in nature, and affects particularly the convoluted tubules. Acute diphtheria intoxication/

toxication and acute cantharidin poisoning supply us with the best examples of this form, and the nuclear changes are especially interesting. Here we have a demonstration of the fact that the protoplasm of the cell is more sensitive to injurious agents than the nucleus. While the nucleus may disappear by a simple solution of its chromatin, yet in acute diphtheria intoxication, the necrotic cells present the most varied examples of nuclear fragmentation (Karyorhexis.)

Regeneration of cells, both of the collecting and secreting tubules, is found in all cases, except the most acute. Special attention was directed to the phagocytic properties displayed by the various tubules, but the cells of the collecting tubules alone possess this function. They ingest necrotic cells, and some contain granular pigment evidently derived from red blood corpuscles. From our observations on the origin and formation of casts, we have found that while some are formed by the coagulation of an intratubular transudate, the great majority in the subacute cases arise by the granular disintegration or colloid transformation of secreting cells.

Only in diphtheria intoxication, have I encountered a more or less constant emigration of mononucleated/

nucleated leucocytes in relation to the vessels of the cortex. New formation of fibrous tissue in the kidney has been almost entirely wanting. In chronic mercury poisoning, calcified areas of tubules may induce no reaction of the interstitial tissue; around some, however, there is a slight proliferation of fibrous tissue.

Destruction of red blood corpuscles by phagocytes in the splenic pulp spaces has been a constant and noteworthy feature in all cases. In diphtheria intoxication, the polymorph leucocytes are also damaged by the circulating toxine, and many degenerated forms are found in the blood vessels of the various organs. They accumulate in the splenic pulp spaces, and their ultimate destruction is effected by phagocytes. In corrosive sublimate and cantharidin poisoning, this is also observed, but not to the same degree. The cells which act as phagocytes in the pulp spaces are mononucleated leucocytes and the endothelial cells which line these spaces. Many other poisons, which exert a pernicious effect on the red cells, lead to the development of similar phenomena in the splenic pulp. It will be sufficient to refer to some recent work by Muir in this connection. He has found that in rabbits subjected to the injection of small quantities of living cultures/

tures of staphylococcus pyogenes aureus and also in many of the infections of man, there is extensive destruction of red blood corpuscles and leucocytes by phagocytes in the splenic pulp. I have observed the same phenomena in rabbits, in which a chronic intoxication had been induced by the injection of sterile cultures of staphylococcus pyogenes aureus.

To Professor Greenfield, I am much indebted, not only for suggesting the line of this work, but also for much valuable assistance in its prosecution.

All the drawings and photo-micrographs are the work of Mr Richard Muir, of the Pathology Department, and to him also I am deeply indebted for the great care and trouble he has taken in their execution.

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