

THE PROBLEMS OF EXPERIMENTAL NEPHRITIS*

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Our present knowledge of nephritis is the result of the methods of clinical observation, pathological anatomy and experimental pathology, successively applied. By means of the first of these, Richard Bright, in 1827, demonstrated that albuminuria and dropsy had an intimate relation to certain pathological changes in the kidney. Studies in pathological anatomy during the following years led to the differentiation of several types of nephritis, and, finally, to a classification based on morphological alterations. I do not think it an exaggeration to say that clinical observation has added little of essential importance to Bright's original conception of eighty years ago, or that pathological anatomy has added little to Weigert's classification, which has been generally accepted for thirty years. Bright's views, it is true, have been amplified, certain phases of the relation of renal disease to cardiovascular disturbances have been more clearly understood, and much negative evidence concerning uremia and edema has accumulated; but little has been added by clinical methods to our knowledge of the interrelation between a kidney lesion and its manifestations. The methods of pathological anatomy have given a classification, based on careful study of the gross and minute lesions of nephritis, and with these have been correlated in a more or less satisfactory way clinical manifestations and changes in the urine. This most important period of anatomical study began in 1851, with Frerichs, who considered all forms of nephritis as stages of a single process, beginning as an acute nephritis and ending as the small granular kidney; the period terminated with Weigert, who, in 1879, demonstrated conclusively that Frerichs' stages do not represent the successive changes of a single lesion, but are distinct types of nephritis, caused by various injurious substances acting during varying periods of time, and representing the varied reactions of kidney tissue thus influenced. Weigert's view is the one held to-day. More recent studies by improved histological methods have added to our knowledge concerning certain details, especially in regard to the glomerular changes, the sequence of lesions, and certain unusual types of nephritis,

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but the methods of pathological anatomy offer no promise of an interpretation of the important problems of this many-sided disease.

The application of the experimental method to the study of renal disease is not a recent development. For many years experimental lesions of the kidney have been utilized, and with gratifying results, in the study of the sequence of the histological changes occurring in nephritis. With such studies, essentially anatomical in nature, have been combined, in recent years, investigation by methods which allow an interpretation of changes in function, upon which morphological studies throw no light. Such investigations necessarily demand the methods of chemistry and physiology; and we have witnessed in the past few years the curious spectacle of pathologists turning from the methods in which they were trained to those of the physiologist and chemist in which presumably they had, originally, little or no training. Investigation by such methods is termed "experimental pathology" merely because the pathologist, despairing of the anatomical method, has seen fit to adopt them in the study of altered function. It is to such methods that we must look for an advance in our knowledge beyond that which has been possible by the methods of clinical medicine and pathological anatomy; and if the pathologist is criticized, as frequently happens, for appropriating the methods of other sciences and for applying to the field of endeavor thus created the term "experimental pathology," it is sufficient to point out that the physiologist and the chemist, as well as the pharmacologist who shares the same methods, have with few exceptions limited themselves to the field of normal function.

That nephritis has been one of the principal objects of attack by these methods is in part due to the importance of the disease, and in part also to the fact that the kidney lends itself very readily to experimental study. And, moreover, although the results of experimental study may not always be applied to explain disease in man, it must be evident that, owing to the peculiarities of the structure and function of the kidney, results of experimentation with this organ have a very definite application. Thus, some aspects of etiology, the almost specific action of certain substances in picking out certain kidney structures, the character of acute lesions and the relation of these to chronic lesions, questions of repair and regeneration, the matter of cast formation and the source of albumin, are problems which, when elucidated by animal experiments, can readily be transcribed to explain similar problems in human nephritis. But aside from these, the experimental method offers hope, in part already realized, of a solution of the more prominent problems of renal edema, of anuria, the question of the relation of renal disturbances to

hypertension and heart hypertrophy, and the most important, though at present the most hopeless, problem of uremia.

Here I may at once call your attention to the fundamental problem of experimental nephritis, that is, the influence of the glomerulus as contrasted with the influence of the tubule. This enters into all phases of renal pathology, in some partially elucidated, but in most still a matter of doubt and speculation. The dual structure of the kidney is responsible for the difficulty which we have of interpreting the physiology as well as the pathology of this organ. We are familiar with glands in which different types of cell are concerned in the elaboration of different chemical substances, and with those in which cells are modified to produce an internal, as contrasted with an external secretion, but the kidney stands alone as an organ with two widely different structures, having for a common object the elimination of a single fluid representing the products of metabolism. This is not the place to discuss the significance of this structural peculiarity and its bearing on the function of the kidney, though it must be considered in what follows. It may be permitted, however, to point out here that the glomerulus, as has been emphasized by Beddard, is a structure without analogy elsewhere in the body except, perhaps, in the choroid plexus of the brain; and that the urinary tubule differs from all other gland tubules in its length and complexity. On these peculiarities of structure, coupled with the peculiarities of the renal circulation, depends the power which the kidney has to remove from the blood-stream the fluid and solids which constitute the urine. If we disregard the one synthetic process of which we have positive knowledge, the formation of hippuric acid from benzoic acid and glycine, the essential function of the kidney is one of elimination, with the important feature that the resulting fluid contains all of the soluble components of the blood except its protein constituents and dextrose—in a different percentage, it is true, but still the same substances.

If we accept departures from normal elimination as evidence of disturbance of kidney function, the problem of experimental nephritis is to determine the part played in this disturbance by glomerulus and tubule, respectively. This may be done by the use of physiological methods which graphically demonstrate alterations in vascular reactions and by comparing such results with those obtained by chemical study and eventually correlating both with the anatomical changes. By such studies of simple phases of the problem of nephritis, enough has been accomplished to warrant their continuance with the prospect of adding essentially to our knowledge of renal pathology.

The study of experimental nephritis may be expected, however, to do more than explain the sequence and significance of pathological changes. By producing lesions which affect only certain structures as the glomeruli or the tubules, or but certain portions of the tubules, we may expect not only to solve some doubtful points in the physiology of this organ, but also to obtain data of considerable importance to the pharmacologist and therapist, thus bringing the work home to the clinician. As a single example may be given the study of the effect of diuretics on the diseased kidney as compared with their effect on the normal. Our knowledge of the latter action is fairly complete, but we have very little knowledge of the former. The study of vascular dilatation and contraction in the kidney, the elimination of water, the general composition of the urine, the chlorid-regulating mechanism and many other points, in distinctly tubular and distinctly glomerular forms of nephritis, which we are now able to produce, should yield practical information of great value. Some information in regard to these matters we now possess, but before it can serve as working knowledge, extensive chemical and physiological studies of various forms of nephritis must be made from the pharmacological point of view.

I have gone somewhat into detail in this introduction, not only for the purpose of demonstrating the value of the study of experimental nephritis, but also for the purpose of showing that the results of such study are of interest to everyone concerned with the problems of normal and abnormal physiology—to the physiologist, the chemist, the pathologist, the pharmacologist and the clinician. And, in order to maintain interest, if it has been aroused, I shall deal briefly with the methods of inducing nephritis, the character of the acute lesions, and the relation of these to chronic lesions, attempting to set forth clearly the types of experimental lesions known as tubular and glomerular. Time thus saved will be devoted to the more interesting questions of altered function.

ETIOLOGY AND CHARACTER OF THE EXPERIMENTAL LESIONS

In speaking of the etiology of nephritis in man, excluding, of course, lesions due to the localization of bacteria, we use, owing to our inexact knowledge, the phrase "soluble toxic substances reaching the kidney through the circulation." So, in experimental nephritis a direct nephritic poison must be capable of absorption, of solution in the body fluids and of causing injury to the renal cells when given in doses so small as not to cause death through its other actions. An indirect poison acts through products formed by blood or tissue destruction, as with the hemolytic poisons; here the action on the kidney is secondary. If we exclude

Siegel's experiments on the production of nephritis by the application of cold, all forms of experimental nephritis are caused by substances falling in the above classification.

According to Sollmann, all metals, so far as they have been studied, cause nephritis, though some act only in corrosive doses or when given intravenously. Other nephrotoxic substances are aloin, coal-tar products, alcohol, anesthetics, oxalates, cantharidin, essential oils, snake venom, ricin, abrin, bacterial toxins, hemolytic poisons and nephrotoxic immune serum.

Of these some act diffusely, while others affect the tubules or the glomeruli separately. Only such as have a more or less definitely circumscribed action are of value in producing experimental nephritis. Thus in the group affecting tubular epithelium with little or no primary glomerular injury, we may place, as most important, uranium nitrate, the chromates of potassium and of ammonium, and corrosive sublimate. Of those affecting glomeruli especially, the more important are arsenic, cantharidin and snake venom. All of these latter have some slight effect on tubular epithelium, probably secondary to circulatory disturbances dependent on the glomerular injury, but the latter lesion is so marked and so evidently primary that they are usually referred to as glomerular poisons. Another agent of value in experimental work is diphtheria toxin which combines glomerular and tubular injury.

All of these cause the appearance of albumin and casts in the urine; only uranium nitrate produces edema.

Although I have, thus far, used the terms "tubular" and "glomerular" in reference to these poisons, they may more definitely be denominated, respectively, "epithelial" and "vascular" poisons. Until recently this division was made on anatomical grounds, that is, on histological evidence of degeneration, necrosis, exudation or cell proliferation, but the study of nephritis by physiological methods has brought out evidence of the existence of functional glomerular injury of extreme grade accompanied by little if any anatomical evidence of vascular lesion. These methods have also shown that nephritides due to agents formerly supposed to act only as tubular poisons, present, in the late stages of intoxication, definite evidence of vascular incompetency.

It is necessary therefore to describe briefly the lesions produced by the more important nephritic poisons. This description will be limited to those poisons especially discussed in this address. It, however, by no means exhausts the list of substances which may be used.

The anatomical changes due to uranium and to the chromates, are, in the early stages, confined essentially to the tubules, especially the

convoluted tubules, and consist of granular or fatty degeneration and definite necrosis often affecting large groups of tubules. Corrosive sublimate causes similar lesions involving especially the ascending loops of Henle and characterized also by the deposition of lime salts. In these typical forms of tubular nephritis no anatomical lesions of the glomeruli are evident in the early stage, but in the late stages an ill-defined thickening¹ of the capillary walls may sometimes be seen and evidence of vascular disturbance is shown by physiological methods.

The glomerular form of nephritis varies. Arsenic, which acts through paralysis of the capillaries, causes little or no anatomical change in the glomeruli. The capillary loops may show slight thickening, the vessels may be overfilled, and the nuclei may stain peculiarly. Exudate into the glomerular space is usually absent, though a slight amount of coagulated serum may be present. By physiological methods, however, it is shown that despite the absence of anatomical lesions, serious vascular injury is present. Tubular involvement is slight and usually difficult to demonstrate.

Cantharidin causes a glomerular nephritis involving both the tuft and the capsular space. The lesions of the capsule have been variously described as desquamative, as consisting of a leucocytic exudate and as due to the presence of epithelial cells pushed up into the capsule from the convoluted tubule. Lyon has recently emphasized this latter view and also describes degenerative changes in the convoluted tubules and ascending loops of Henle with necrosis of the latter. Functional tests demonstrate serious vascular injury.

The venom of the rattlesnake, as I have recently determined, causes a very remarkable glomerulonephritis of the exudative type. Single large doses or repeated small doses cause an exudation of serum and fibrin in both the capsular space and the glomerular tuft. This exudate is usually but not always hemorrhagic. Leucocytes are not prominent, but occasionally are present. The tubular changes are slight or entirely absent.

Diphtheria toxin is the best example of those poisons which combine both epithelial and vascular injury. Hyaline thrombi are found in the glomerular capillaries and small arterioles of the cortex in acute and intense intoxication. The vessel walls show hyaline changes and, in the later stages, cyst-like hemorrhages in the tuft (Lyon). Leucocytes are abundant in the tuft and slight necrosis may occasionally be seen (Flexner). With these changes are found extensive degenerative and necrotic lesions of the convoluted tubules and the ascending loop of Henle.

1. In the uranium lesion, Christian has described hyaline droplets in the capillary loops.

Undoubtedly, the lesion in both tubular and glomerular nephritis occurs in that portion of the kidney through which the poison is eliminated, though this has not been definitely demonstrated except in the case of uranium.²

From this, and our knowledge of the elimination of iron through the convoluted tubules, it seems probable that nephritis, due to the salts of various metals, is an indication of injury at the point of elimination. The peculiar involvement of the loops of Henle in the corrosive sublimate lesions supports this view. It is not too much to hope that by careful study of such localized lesions, experimental nephritis may eventually contribute to our knowledge, not only of altered function, but also of the normal physiology of the kidney.

The glomerular lesions likewise must be considered as a special manifestation of a general injury to capillary structures; the intensification of that action in the glomerulus being due to concentration of the poison at the point of elimination.

At present, then, we are familiar with several poisons which affect either tubule or glomerulus, respectively, the injury being recognized sometimes by anatomical changes, sometimes by functional disturbances and sometimes by both.

The futility of judging of altered glomerular function by anatomical changes alone is best illustrated by Takayasu's histological study of the kidneys utilized by Schlager and Hedinger in their investigations of disturbances of function in various forms of nephritis. This work will be discussed in detail later. Here it is sufficient to state that in arsenic and cantharidin nephritis characterized by constant and severe disturbance of vascular reactions, the glomeruli presented exudative lesions in only 2 per cent. of the kidneys examined, and this anatomical condition reached a degree comparable to the functional disturbance only in those kidneys showing total insufficiency. Proliferative lesions of tuft or capsule were not demonstrable. The only frequent lesion was increase in size of the glomerular nuclei and an indistinct outlining of the capillary walls, due, apparently, to an ill-defined thickening. The nuclear changes, moreover, occurred in tubular as well as in glomerular nephritis. Such results would appear conclusively to establish the possibility of serious

2. Schneider working with *Petromyzon fluviatilis* injected uranium solution in the muscle of the back, and also subcutaneously, and found that by the use of a fixing fluid containing potassium ferrocyanid, picric acid and hydrochloric acid, the uranium was precipitated as a brownish-yellow deposit in the epithelium of the tubules.

functional disturbance with little or no evidence of structural lesion. To this problem I shall return in the discussion of altered function.

This brief description summarizes the more important types of acute injury caused by irritants acting directly on the kidney. It remains to discuss the relation of these to the production of lesions which may be termed chronic nephritis, or are accompanied by manifestations characteristic of the chronic disease in man. The production of such a condition has been the object of nearly all work on experimental nephritis, and until recently with no success. Lyon, who worked with cantharidin, diphtheria toxin and corrosive sublimate, with the object of following acute lesions to their termination in chronic, found that acute lesions rapidly disappear and that the kidney returns to normal. Such has been the experience of many other investigators, and has incidentally served to strengthen the clinical observation that an acute nephritis, if the causative agent be no longer active, may go on to cure without the development of subacute or chronic lesions. This, however, is a phase of experimental nephritis which, in view of the very recent statement of Müller based on clinical observation and supported by the pathological studies of Löhlein, should again be investigated, and especially with regard to the matter of glomerular lesions. Müller expresses the opinion that a chronic nephritis may be the result of an acute lesion with a progressive course marked by acute exacerbations, or, on the other hand, there may be complete cessation of symptoms for many years with eventually a contracted or indurated kidney due to healing by scar formation.

Löhlein, as the result of a very careful study of selected material, has shown that many individuals dying of chronic nephritis present a definite history of an acute nephritis, followed by a quiescent period of several years, before the appearance of the chronic lesion responsible for death. His conclusions are based more especially on the kidneys of scarlet fever and acute coccus infections, in which he found inflammatory glomerular changes which seemed to be the starting-point of the fibrotic tufts and thickened glomerular capsules characteristic of the later developing chronic nephritis. Such observations are not new. Others have reported isolated instances of a chronic nephritis following the acute lesion of scarlet fever. Thus Handford describes such a condition after scarlatinal nephritis in a child 12 years old, in whom the chronic condition developed three years after the acute; and Councilman describes a chronic interstitial nephritis with heart hypertrophy following scarlet fever, in a child of 2 years. Similar findings have been reported by Leyden, Mann and others. Löhlein's extensive and thorough study, however, brings the problem once more prominently before us, and, coupled with

the observation of Müller, makes it one of much importance. It would seem possible that by the use of a substance like venom, which acts as a definite glomerular poison and causes exudation and very striking endothelial destruction, experimental evidence of chronic nephritis following a single injury could be added to the clinical and pathological evidence now at hand.

Despite this possibility, it must be admitted that the experimental study of nephritis supports the more common conception of the etiology of chronic nephritis in man, that is, that it is a gradually developing lesion due to the long-continued insidious action of some ill-defined toxic substance. With the possible exception of the recent experiments of Dickson, the results obtained have been neither constant nor of such nature as to justify the term of chronic nephritis. Certainly if we take as a criterion, a persisting lesion of the kidney characterized during life by elimination of albumin and casts, and histologically by changes involving glomeruli, tubules and connective tissue, nearly all experimental efforts can be excluded. If we include edema as a necessary corollary, chronic nephritis has not been produced experimentally. Some of the methods which have resulted in lesions approaching chronic nephritis are, however, worthy of mention. Ophüls, who investigated this subject, came to the conclusion that the best results could be obtained with lead, and, by the prolonged administration of a lead salt, he produced in guinea-pigs and dogs a definite sclerosis. The urine, however, did not contain albumin and casts. The same objection holds for experiments with many of the other metals (Petroff).

The experiments of Ehrlich and of Levaditi with vinylamin show that the primary necrosis of the papilla of the kidney caused by this substance may be followed by cortical injury with increase of connective tissue and considerable contraction. In a few of these experiments, in which mice were used, edema, hypertrophy of the left ventricle and albuminuric retinitis were observed, with characteristic changes in the urine. Such changes, however, were not constant. The value of these experiments, moreover, is slight, for the diffuse nephritis followed destructive lesions of the papilla leading to mechanical obstruction, and were not due to a primary injury of cortical structures caused by a circulating poison, though it must be admitted that Lindemann has described the production of such injuries by the use of this substance.

Occasional positive results have been obtained with a variety of substances, as cantharidin (Aufrecht), oxalic acid and oxamid (Ebstein and Nicolaier), potassium chromate (Ophüls), and uranium nitrate (Siegel). I have myself found, in the course of a study which had for its object

the production of edema in the dog, a typical contracted granular kidney as the result of continued injections of potassium chromate and nephrotoxic immune serum. Chronic lesions, however, cannot be produced constantly by such methods and occasional positive findings, in view of the frequency of spontaneous lesions, must be regarded with suspicion. Or, to look at it in another way, these occasional positive results may have been due to the accidental presence of some secondary factor, as some metabolic or circulatory disturbance, necessary to the production of chronic nephritis. It was with this possibility in mind that Dr. Haven Emerson investigated experimentally the relation of circulatory disturbances to chronic nephritis. He recognized that, while a variety of causes are known to be responsible for, or contribute to, chronic interstitial changes in various tissues, there is almost constantly associated with them a circulatory disturbance, usually a venous congestion. It might be objected that such a disturbance is the result and not the cause of productive lesions in man, but Emerson's experiments are nevertheless of value, in that this hypothesis was, for the first time, investigated. The influence of vasodilators and vasoconstrictors was tested by inhalation and by subcutaneous and intravenous injection. Inhalation experiments during a period of half a year caused the appearance of degenerative parenchymatous lesions with slight connective tissue changes. Though these experiments were few in number, the results, due apparently to disturbances of circulation and nutrition, suggest that with this background, the long-continued administration of a renal irritant in small doses might result in the fairly constant production of chronic nephritis. In this connection Caro's observation that nephritis occurs in cats five to eight days after extirpation of the thyroid is suggestive.

In other words, the evidence at hand supports the theory that chronic nephritis should readily be produced as the result of an irritant action associated with, or causing, circulatory and nutritional disturbances. This is in accordance with our clinical and pathological knowledge of chronic nephritis in man.

In accord with this view, also, is Bradford's suggestion that the many failures to produce chronic nephritis are probably due to the fact that we have no irritant capable of causing in animals a condition analogous to acute nephritis with edema as seen in man. This statement was made in 1904. Such a substance we now possess in uranium nitrate, which, as Richter showed in 1905, causes a very definite acute tubular nephritis with the occurrence, when an excess of water is administered, of edema of the subcutaneous tissues and accumulations of fluid in the serous cavities of the body. Uranium nitrate has come into general use

as one of the most satisfactory of nephritic poisons, and Dickson, during the past year, has shown that its prolonged administration causes chronic nephritis in a large percentage of the animals treated. Unfortunately, his choice of experimental animals did not allow a study of edema. If rabbits, in which edema is readily produced, had been used instead of guinea-pigs, and the animals placed under conditions favorable to the production of edema, it is possible that his results would have been the most satisfactory yet reported. As it is, he has shown (1) that prolonged administration of uranium nitrate causes a progressive "subchronic" nephritis; (2) that a series of six or seven acute attacks results in extensive fibrotic changes, with, in some instances, granular atrophy and associated polyuria; (3) that single injections not infrequently cause more or less severe fibrosis with occasionally granular atrophy; and (4) fluid, in small amounts was found in the serous cavities of a few animals.

These experiments are of great importance in connection with what has been said about the influence of circulatory disturbances in the production of chronic nephritis. Uranium nitrate, in addition to its very decided action on renal epithelium, also causes very definite vascular disturbances. Several investigators have been forced to this conclusion, as Heineke and Meyerstein and Dickson. Recently, I have called attention to the necessity of assuming such an action in order to explain certain phases of the edema caused by this substance. Final proof of this vascular injury is furnished by Schlayer and his associates, who have shown, by physiological methods, that although uranium primarily affects the tubules, there occurs a stage of glomerular injury characterized by dilatation of the vessels and decreased permeability. This will be discussed later in connection with edema, but these observations serve here to indicate the value of uranium in combining the toxic effects apparently necessary to the production of chronic nephritis by causing not only structural changes, but circulatory disturbances also.

Thus may be summarized briefly the methods which have been employed in producing nephritis experimentally, the character of the acute lesions, and the relation of these to chronic conditions. Such a statement is necessary as a preliminary to the discussion of functional disturbances.

FUNCTIONAL DISTURBANCE

The study of anatomical changes in experimental lesions adds little to our knowledge obtained by the investigation of human material. By applying physiological methods on the other hand, we may correlate disturbance of function with any state of anatomical change and thus obtain information which clinical and pathological studies fail to give.

The kidney lends itself, perhaps more than any other organ, to investigation by physiological methods. The very abundant blood-supply with its intimate relation to the function of the kidney, the close relation of function to general blood-pressure and the influence of the circulation on diuresis, are conditions which readily allow the application of methods, the results of which may be graphically registered. Changes in kidney volume dependent on general blood-pressure or on the influence of its own independent vasomotor system may be measured by the oncometer, and the results for the normal compared with those in animals with experimental nephritis. Likewise a simultaneous study of diuresis allows of the determination of the changes in the elimination of fluid. The injection of various substances influencing blood-pressure or diuresis yields information concerning the reaction of the kidney to these stimuli, and by their use it is possible to differentiate between the disturbances due to a glomerular and to a tubular nephritis. Further information concerning disturbances of function due to tubular or to glomerular lesions, respectively, may be gained by the use of phloridzin, and by correlated studies of the protein and salt elimination. Some information, as the result of such investigations, especially in regard to diuresis, is offered by pharmacological studies, but the most comprehensive study of this kind has been made by clinicians, by Schlayer and his associates, and deals particularly with the vascular reactions in the two types of nephritis.

Their work is based on the assumption that the vascular reactions of glomerular nephritis should differ from those of tubular nephritis and that this difference should be readily determined by the action of certain stimuli, the effect of which would be to cause either contraction or dilatation of the vessels. These changes, through decrease or increase of the kidney volume, would be readily recognized with the aid of the oncometer. It was necessary to choose stimuli the effect of which would be but transient and which would cause no injurious after-effects, thus allowing a series of observations on the same animal within a comparatively short space of time. Furthermore, as the conditions of experiment were such that observation on the same animal before and after the development of nephritis could be made only in short-period experiments, it was necessary to demonstrate that these stimuli exerted a constant effect on normal animals.

To test the capacity of the vessels to contract they used sensory stimulation (tobacco smoke in the nose or transient suffocation) as an example of effect through the vasomotor center, and adrenalin as an example of the effect of peripheral contraction. Each of these methods produced a transient diminution of kidney-volume with an increase at the same time in general blood-pressure. Caffein and strong salt solution were used for the purpose of producing dilatation of the renal vessels. In connection with all these conditions the relation of diuresis to vascular changes and the power of phloridzin to cause glycosuria were also studied.

In brief, the study was one of the reaction of the renal vessels to various stimuli and the relation on the one hand to general blood-pressure and on the other to diuresis.

Necessarily, much depended on the uniformity of the control experiments, and for this reason rabbits of the same breed and similar weight were chosen, and with the exception of adrenalin, all substances were injected in definite ratio to body weight, and all but phloridzin, intravenously. Sensory stimulus and adrenalin (1 drop of 1 per cent. solution in 0.5 c.c. normal salt solution) increase blood-pressure with a corresponding fall in kidney-volume. In each instance this effect is transient, the normal condition being resumed in a very short space of time. On the other hand, 5 per cent. salt solution (5 c.c. per kilo) and 5 per cent. caffeine solution (2 c.c. per 1.5 kilo) cause a marked dilatation of the renal vessels with strong pulsation and immediate diuresis, the general blood-pressure remaining unchanged. At the end of the experiment, phloridzin was given subcutaneously; this caused a moderate diuresis with glycosuria but without increase in kidney volume or in general blood pressure.

These results were always obtained with normal animals, and the degree of reaction with each stimulus was practically the same. With such observations as controls, a study was undertaken of animals with various forms and differing stages of toxic nephritis. Potassium chromate and corrosive sublimate were used for the production of tubular nephritis, and arsenic, cantharidin and diphtheria toxin for vascular nephritis.

Schlayer's opinion concerning tubular nephritis is based on 21 experiments with chromate and 15 with corrosive sublimate animals. The reactions to the various stimuli in the early stages of nephritis so produced do not differ markedly from the normal. It was found that the animals eliminated a larger amount of urine than do normal animals, which is in accord with Weber's observations, and also that diuretics led to a still greater flow, as had also previously been demonstrated by Hellin and Spiro. The vascular reactions differed from the normal only in degree; the power of the vessels to contract after sensory stimulus and adrenalin was slightly increased and the power to dilate was greater also, to about the same extent. Phloridzin acted as normally, that is, caused polyuria and glycosuria.

The results with corrosive sublimate were similar except that the polyuria before the administration of diuretics was not so marked. In both forms, epithelial lesions were very prominent, but no anatomical changes were evident in the glomeruli. In short, the early stages of a tubular nephritis with albuminuria and cast secretion and severe anatomical changes in the tubular epithelium offer no physiological or anatomical evidence of vascular injury.

Before taking up the late stages of tubular nephritis, the reactions of vascular nephritis, for the sake of sharp contrast, may be described. Cantharidin and arsenic nephritis offer the best examples of this type. Severe vascular disturbances come on very quickly. In cantharidin nephritis, the early polyuria characteristic of the chromate lesion is absent. Within four to eight hours the effect of sensory stimulus and

adrenalin is much less than in the normal, and after the administration of diuretics the power of the vessels to dilate decreases and with it diuresis. As the nephritis proceeds to severer degree, or if larger doses of the irritant be given, the power to contract after sensory stimulus and adrenalin becomes minimal and dilatation and diuresis become slight or cease entirely. Under such circumstances phloridzin produces no diuresis and no glycosuria.

The lesions due to arsenic are similar to those of cantharidin except that the general blood-pressure falls more quickly and remains at a lower level. This is to be explained by a greater peripheral capillary injury or perhaps by more intense action on the vasomotor center.

This comparison is very instructive. A tubular nephritis with extensive epithelial destruction and a urine rich in albumin and casts give no physiological evidence of vascular disturbance except a slight polyuria and a slightly heightened response to vascular stimuli. On the other hand, in a glomerular nephritis with little or no evidence of anatomical injury to either tubules or glomeruli, and with comparatively slight albuminuria and cast excretion, we find that the capacity of the vessels to contract and dilate is greatly altered, and with this a corresponding inhibition of diuresis, which may go on to total insufficiency.

These observations demonstrate for the first time the possibility of primary injury to glomeruli and tubules, respectively, and offer a sound experimental basis for the conception of a vascular as contrasted with a tubular nephritis.

But how, ask those who object to the direct application of experimental evidence to the problem of human pathology, is this to help us in explaining the majority of renal lesions in man? We admit its value from a pharmacological point of view. We admit also the possibility of primary glomerular injury and primary epithelial injury, and also that occasionally the glomerular lesion, as in scarlatinal nephritis, may remain the predominating lesion, and, on the other hand, that the acute renal lesions of certain intoxications, as cholera, eclampsia, and to a certain extent of diphtheria, may be purely epithelial lesions; but what is the bearing of this experimental evidence on those forms of nephritis in which both glomeruli and tubules are involved, and, most frequently, it would seem, the tubules first and more seriously? This question is a proper one, and while it cannot be fully met as yet, it is, I believe, answered in part by the studies which Schlager and his associates have made of the later stages of tubular nephritis. They find that the late stages occupy a middle position between early tubular and typical vascular nephritis, and in severe forms may simulate the latter. The reaction

to sensory stimulus and adrenalin remains practically normal, but the power of dilatation and diuresis, after the administration of diuretics, decreases gradually, and in severe late stages, that is, after two to four days, dilatation is very slight or absent and diuresis does not occur. Phloridzin no longer causes glycosuria. These changes may be accompanied by slight histological alterations in the glomeruli, but the condition is, essentially, a functional glomerular disturbance following tubular injury. That this secondary glomerular involvement is a true vascular disturbance and not the result of compression of the glomeruli, due to the retention of urine in tubules blocked by casts, Schlayer and Hedinger have shown by experiments in which the ureters were ligated. Under such conditions no vascular disturbance resulted. Thus, these investigators have demonstrated not only tubular and vascular nephritis as experimental conditions, but have shown that the former may develop into the latter. The relation, however, of the late glomerular changes to the early epithelial changes cannot be explained without more complete experimental evidence. That the late vascular injury is due to the original poison is doubtful, but the possibility must be considered, in view of the fact that in Schlayer's experiments with diphtheria toxin, a gradually developing nephritis of the tubular type passed, after only twenty hours, into the typical vascular type. Again, it is possible that the tubular nephritis may cause the development of secondary poisons, consequent on metabolic disturbances in other organs, and capable of affecting the glomeruli. In this connection must also be considered the matter of the "give and take" of renal function recently emphasized by McCrae. This theory assumes the possibility of the glomeruli taking over in part at least the function of the tubules. It is possible that substances normally passing through the tubular epithelium are, when the latter is destroyed, eliminated by the glomeruli, the endothelial cells of which may be more susceptible to injury by such substances than is the tubular epithelium.

These are some of the problems suggested by Schlayer's work, which await the verdict of further experimentation by physiological methods. During the past year I have been interested in certain phases of these problems, and have repeated Schlayer's experiments, using the dog rather than the rabbit, because of the more stable circulatory mechanism of the former.³ The vascular reactions of the two types of nephritis, observed in the rabbit, I have found to occur also in the dog. The tubular form likewise develops into the atypical vascular form.

3. Pearce, R. M., Hill, M. C., and Eisenbrey, A. B.: Experimental Acute Nephritis; The Vascular Reactions and the Elimination of Nitrogen. *Jour. Exper. Med.*, 1910, xii, No. 2.

Additional evidence of the distinction between tubular and vascular nephritis is offered by chemical studies which, with the assistance of Dr. Miner C. Hill, were carried out in connection with the experiments just mentioned.³ These depend on our knowledge that most, if not all, of the urinary nitrogen is eliminated through the tubules, and on the assumption that in tubular nephritis this elimination would be diminished. Daily estimations of the total nitrogen elimination in animals with tubular and glomerular nephritis, due to uranium nitrate and arsenic, respectively, were made. It was found that in the tubular nephritis a decrease of nitrogen equal to 9 to 14 per cent. of the normal elimination occurs, while in the glomerular form this decrease does not occur. Indeed, the arsenic animals showed an increased elimination varying from 7 to 16 per cent., demonstrating that the tubules not only were not injured, but also that they were able to care for the augmented output of nitrogen consequent on the increased metabolism due to arsenic.

These observations are of twofold interest. In the first place, the work with arsenic offers additional evidence of the possibility of producing a glomerular disturbance without affecting the function of the tubules, and, on the other hand, the diminished excretion of nitrogen in tubular nephritis⁴ indicate the possibility of a retention leading to a disturbance, not only of the glomerulus in the "give and take" of kidney function, but responsible perhaps for some of the more general manifestations of nephritis.

Here may be introduced also other evidence, of an entirely novel nature, which is of value in the differentiation of tubular and glomerular nephritis, and which would appear to be of definite physiological importance in the matter of normal tubule function. I refer to my recent investigations of the depressor substance of dog's urine, and I do this with some hesitation, as the application of the observation to the nephritis of man is not at all clear.

Elsewhere, in a discussion of the influence of kidney extracts on the blood-pressure, I have described the very striking depressor influence of dog's urine, when injected intravenously into other dogs. At that time this observation was of interest only in that it appeared to indicate that the similar depressor influence exerted by extracts of the dog's kidney was due to the content of urine which could not be removed.

My interest in this peculiar manifestation was again aroused by a chance observation made during the course of a recent study of diuresis

4. Siegel also describes this decreased elimination of nitrogen in uranium nephritis, and Green, in a recent study of chromate nephritis, found a decrease of 20 per cent.

in the pathological kidney. Dog's urine, on account of its very decided depressor influence, from which the animal quickly recovers, was used in this work as a means of rapidly lowering the blood-pressure.

It served most satisfactorily for this purpose and never failed with a large series of normal urines. Early in the investigation, however, it was observed that the urine from an animal in the third day of a chromate nephritis failed to cause the usual depressor effect. This chance observation led to the routine investigation of the urine of animals with various forms of experimental nephritis. As a result it was found that the depressor substance disappeared about the third to the fifth day from the urine of those animals suffering from renal lesions characterized by extensive tubular injury and persisted after the administration of substances causing glomerular injury with little or no tubular change.⁵

This difference suggests that in the tubular lesion of chromate and uranium nephritis, which is characterized by extensive epithelial destruction, some substance normally eliminated is retained, while in the glomerular nephritis, caused by arsenic and cantharidin, this retention does not occur. The elimination of the depressor substance would appear, therefore, to be a function of the tubular epithelium. This view is supported by a study of the effect produced by normal urine as compared with that passed at the height of diuresis. Thus, in one animal, the urine obtained from the bladder at the time of inserting the cannula, caused a drop of pressure of 64 mm. Hg., whereas at the height of caffein diuresis, the drop was but 30 mm. In another animal the figures were 60, 32 and 16 for (1) the normal urine, (2) the beginning and (3) the height of diuresis, respectively. This indicates that the increased glomerular filtrate either dilutes the depressor substance eliminated by the tubule, or it passes through the tubules so rapidly that this substance is not added in the usual amount.

In animals with experimental nephritis of the tubular type the disappearance of the depressor substance⁶ from the urine is frequently asso-

5. Pearce, R. M.: Concerning the Depressor Substance of Dog's Urine and its Disappearance in Certain Forms of Experimental Acute Nephritis. *Jour. Exper. Med.*, 1910, xii, No. 2.

6. Concerning the exact nature of this depressor substance I have no knowledge. It dialyzes slowly, is not destroyed by boiling for a few minutes but does disappear after prolonged heating. It can, however, be completely precipitated from the urine in impure form by large amounts of alcohol. The precipitate thus obtained, when dried and brought back to original volume with distilled water, has a depressor effect equal to that of the untreated urine, while the filtrate evaporated at room temperature to original volume has no effect whatever. The precipitate is not a single substance, but contains phosphates, chlorids and sulphates and has a very small nitrogen content.

ciated with a lowering of the blood-pressure, which would appear to indicate that the retained depressor substance may have a definite effect on the general blood-pressure. This observation is not, however, based on blood-pressure determinations on the same animal, before and after the development of nephritis, but by contrasting the pressure in animals with tubular nephritis with that in normal animals. It may, as is true in glomerular nephritis, be due to some other factor affecting the vascular system generally.

Investigations now in progress will, I hope, throw more light on the nature of this depressor substance, and, I trust, on the significance of its disappearance from the urine. At present the latter is of importance, as a manifestation of tubular nephritis, as contrasted with glomerular nephritis; as an indication of possible normal tubule function; and possibly, also, as an explanation of certain conditions of low arterial tension in man. Concerning the latter we have little information, for clinical studies have been confined largely to the condition of hypertension. It is of interest, however, that in the disturbances following too great experimental reduction of the dog's kidney, a condition of acute renal insufficiency, Janeway has demonstrated a definite fall in general pressure. If it could be shown also that the depressor substance disappears from the urine of these animals we would have a very substantial basis for a theory of acute renal insufficiency of tubular origin leading to hypotension.

It is perhaps needless to say that such observations have apparently no bearing on the hypertension of scarlatinal nephritis or that of the interstitial type of chronic nephritis. Also, one cannot assume that the experimental conditions here described hold for human nephritis. At present they must be considered merely as interesting experimental observations concerning the influence of the kidney on blood-pressure, and although it brings to this subject some confusion and uncertainty, future investigations may add unexpected knowledge, perhaps, in the direction of a better understanding of tubule functions.

EDEMA

As edema is, in many ways, the most striking manifestation of certain forms of nephritis in man, it is natural that it should be considered in a discussion of experimental nephritis. I will not attempt, however, in this connection to present the conflicting views concerning the physiology of lymph formation or the general pathology of edema, which are admirably set forth in Meltzer's lectures on this subject, but will limit myself to the recent studies due to the stimulus of Richter's demonstration that acute uranium nephritis in animals is accompanied by edema.

The older literature contains much experimental evidence concerning the importance of hydremic plethora or of vascular injury (Cohnheim and Lichtheim, Magnus, Albu) in the production of renal edema, but as this is for the most part based on transfusion experiments in which large amounts of fluid were used, or experiments on dead or nephrectomized animals, it is not entirely satisfactory, as the conditions are too artificial. The results of such experiments are based on the absence of kidney function rather than on the influence of the altered function of the diseased kidney. Only uranium nephritis, of the various forms of experimental renal disease, is accompanied by a spontaneous edema, and thus offers experimental conditions analogous to nephritis in man.

The more important theories of renal edema may be briefly stated. On the one hand are those who support the importance of hydremic plethora as enunciated by Grainger Stewart and Bartels, but more or less modified by later investigations, as those of Roth-Schultz and others. On the other hand, are those who consider hydremic plethora of secondary importance, and, following Cohnheim, ascribe to the injury of peripheral capillary blood-vessels, the important rôle. With this theory is closely associated that of Senator, who presupposes injury of the renal vessels as well as of the peripheral vessels.

There is a tendency to bring these explanations together, giving each its share in a theory which ascribes the cause of edema to the combined influence of renal vessel injury and peripheral (cutaneous) vessel injury, the former leading to retention of water or salts, or both, and the latter responsible for the increased permeability of the capillaries at the site of the local accumulation of fluid. In brief, the problem has become essentially that of the relative importance of vascular injury, hydremia and salt retention. Since the demonstration of the value of uranium nitrate⁷ for the production of a nephritis with edema, the influence of these factors has been extensively reinvestigated.

7. It is a matter of local interest that, although macroscopic evidence of renal injury due to uranium was observed by Leconte in 1854, the first carefully recorded observations on uranium nephritis were from Professor Chittenden's laboratory at New Haven, and in 1889, in a communication from this laboratory, Professor Chittenden and Dr. Alexander Lambert of this city first described ascites in connection with uranium poisoning. Woroschilsky in the following year, in a communication from the pharmacological institute at Dorpat described, accurately, diffuse edema of the skin and subcutaneous tissues and the accumulation of fluid in the serous cavities of the body. These observations were, however, either overlooked, or their importance not appreciated, for it was not until 1905, when Richter's communication appeared, that the importance of this experimental lesion was generally recognized.

Richter found that rabbits receiving subcutaneously small doses of uranium nitrate and at the same time 100 c.c. of water daily by stomach-tube, developed a well-marked edema of the subcutaneous tissue with the accumulation of considerable amounts of fluid in the serous cavities. This edema, it is true, differs in two respects from that of nephritis in man:

1. There is a greater tendency for the fluid to accumulate in the serous cavities and subcutaneous tissues than in the skin proper. This is probably due to histological differences between the skin of man and the rabbit, but is not of great importance, for the widespread edema involving the subcutaneous tissues of the abdomen and thorax and frequently extending to the neck, head and extremities, is sufficient evidence of general edema.

2. The fluid is richer in albumin and tends to clot more readily than is the case in man. There is, however, no evidence that this fluid is of inflammatory origin; the high albumin content is probably to be explained by the acute character of the lesion, and in this regard approaches the character of the fluid in the edema of scarlatinal nephritis.

Despite these slight differences the picture is sufficiently similar to the edema of man to be considered a true experimental nephritic edema, and is so regarded by the large number of investigators who have confirmed Richter's observation.

The studies of uranium edema fall into two groups: those bearing on the question of water and salt retention, and those dealing with vascular injury. The first group includes experiments in which artificial plethoric hydremia is produced and those in which the salt content of the body fluids is increased by administration of sodium chlorid. The second group includes physiological studies of the renal vessels in uranium nephritis and also the study of the influence of vascular poisons in those forms of experimental nephritis not ordinarily accompanied by edema.

The literature of water and salt retention in nephritis, which is voluminous and most confusing and contradictory, need not be summarized. The matters of greatest strife are (1) whether salt retention or water retention is primary; (2) if the salt retention is primary, whether it is a true tissue retention or secondary to vascular lesions which render the glomeruli less permeable to the salt. In either case the water retention is considered to occur as a result of the salt retention. The third possibility is that both water and salt are retained simultaneously as the result of glomerular injury.

The experimental evidence, based on altered kidney function in animals, which was at hand previous to the study of uranium edema, may be illustrated by two types of experiment. Beck and Glucinski, as well as Lepine, had demonstrated that temporary ligation of the ureter of one kidney was followed by a lessened

elimination of chlorids as compared with the opposite sound kidney, thus favoring apparently the theory of decreased renal permeability. On the other hand, Castaigne showed that, although there is a diminished chlorid excretion in dogs with experimental nephritis, as compared with normal dogs, this difference was not observed if the respective animals received salt solution in the renal artery. In other words, if the salt was brought to the kidney, the kidney could excrete it. In other experiments normal and nephritic animals were bled and the blood replaced by saline solution. Shortly afterward 200 c.c. of blood taken from the renal artery of each showed the salt content to be less in the animal with nephritis. These experiments are usually quoted as evidence of primary retention of chlorids in the tissues. It must be borne in mind, however, that in these experiments the renal lesion was not one accompanied by a spontaneous edema.

In the early work on uranium edema it was found that the administration of water in excess was essential for the development of a frank edema, though occasionally, as in Georgopoulos's series, a slight or moderate grade of edema occurs in unwatered animals.

Richter took up the question of the relation of hydremia to salt retention. He has found that if both salt and water are administered to animals a greater edema is produced than with water alone. On the other hand, salt without water has no power to increase the hydrops, and if salt is given with half the amount of water usually administered, the edema is not appreciably greater than in those receiving water only. On these observations and the demonstration that chlorid retention occurs in other forms of experimental nephritis without the occurrence of edema, Richter concludes that water retention is more important than salt retention.

Georgopoulos has utilized uranium nephritis to determine the matter of chlorid retention by direct quantitative analysis of the body fluids and tissues. His conclusions are very definitely stated as follows:

In uranium, as well as in cantharidin nephritis, no constant relation exists between the water and salt excretion; more water than salt is retained, thus leading to a decrease in the chlorid content of the blood. This indicates that water retention is dependent on a primary disturbance of the water-eliminating power of the kidney and is not secondary to chlorid retention. Moreover, an increase of chlorids in the tissues with a reduction of chlorid concentration of the blood could not be demonstrated in animals, with or without edema.

Schirokauer, in a similar investigation, found that in edema, although the tissues had a salt content greater than normal, it was no greater than the salt increase in the blood and in the hydropic fluid of the body cavities. The increased salt content of the tissue does not therefore support the theory of primary salt retention, but indicates rather that in the process of transudation the salts and water leave the vessel in the same percentage relation, one to the other, as they occur in the blood. Other important studies are those of Bence concerning the altered distribution of water in the body and of Heineke

and Meyerstein dealing with salt and water relations. The results of the latter, in that they indicate that salt retention may precede water retention, are not in accord with the other investigations quoted, but I have, I believe, sufficiently illustrated the value of uranium nephritis in the study of this phase of experimental edema and also shown that the bulk of evidence does not support the theory of primary salt retention.

Of even greater interest are the recent experimental observations concerning the importance of vascular injury. It was early recognized that, although the administration of water in excess was necessary for the development of uranium edema, this was not the essential factor, for the administration of water with or without salt to animals with chromium, aloin, cantharidin and other forms of nephritis did not cause edema, despite the fact that in some of these forms, as chromium nephritis, the histological changes are practically the same as those of the uranium disease. Such observations naturally recalled the early experiments of Cohnheim and Lichtheim concerning the importance of vascular injury, due to various forms of irritation of the skin, and those of Magnus, in which vascular poisons, as arsenic, chloroform and ether were used, and suggested the possibility of an action of uranium, or of substances formed during the course of nephritis, on the blood vessels, both renal and peripheral. Several investigators (Blanck, Heineke and Meyerstein, Georgopoulos and Pearce) have expressed opinions to this effect. It remained, however, for Schlayer and his associates, Hedinger and Takayasu, to demonstrate by physiological methods a functional disturbance of the renal vessels in uranium nephritis, which disturbance, apparently, is an important factor in the production of edema. Uranium nephritis, it may be again emphasized, is anatomically of the type of tubular nephritis and characterized by extensive destruction, even to necrosis of complete tubules, and by abundant elimination of albumin and casts. Anatomical changes in the glomeruli, aside from slight thickening of the capillaries, the outlines of which are more or less indistinct, are not evident. Schlayer attacked uranium nephritis by the same methods which had served to differentiate tubular and vascular nephritis. It was found that in the early stages as well as in the late stages, uranium gives the reactions of a true tubular nephritis, of the type of the chromate or the corrosive sublimate disease. It has, however, an intermediate stage which differs strikingly from both the pure tubular and the pure vascular forms and which Schlayer has observed in no other form of nephritis except once in that form due to diphtheria. I may repeat, in order to present this peculiar reaction more clearly, that the characteristic feature of vascular nephritis is the failure of dilatation

of the vessels with little or no diuresis after the administration of diuretics. These manifestations also occur in the late stages of tubular nephritis. They occur also at the end in uranum nephritis, but preceding it is an intermediate stage, during which the administration of 5 per cent. sodium chlorid causes extreme dilatation with strong pulsation but no corresponding diuresis. This stage develops thirty-six to forty-eight hours after the onset of the experimental disease at a time when no edema is evident, but when the urine is decreased in amount as compared with the preliminary polyuria. The vessels react to contraction stimuli strongly, the blood-pressure shows no change, the power of dilatation of the vessels is maintained but is unaccompanied by flow of urine. This occurrence, which was observed in fourteen animals, would appear to be definite evidence of decreased permeability of the glomerular vessels, marking a pre-edemic stage, during which a retention of water and salt occurs. Later, when the renal dilatation fails, the capillaries of the general circulation presumably become permeable and edema develops.

This phenomenon has one peculiar phase. Some minutes after the inhibition of diuresis caused by the salt injection a few drops of urine are excreted, but no further improvement in the flow of urine occurs. If, after a lapse of twenty minutes or so, caffein is injected, the kidney volume, which has fallen, increases to the maximum attained after the previous salt injection, and a slight or moderate diuresis occurs. This diuresis is not so great as normal caffein diuresis but is more prolonged, and the kidney-volume does not return to its normal level. That the production of diuresis under these circumstances is peculiar to caffein was shown by the fact that if the injections were reversed, caffein given first and followed by salt, each produced the same effect as before. Also it was impossible to cause diuresis by the administration of other diuretic substances, as urea, dextrose and sodium sulphate, though all cause dilatation of the blood-vessels.

That caffein alone produces diuresis in this stage is of interest pharmacologically, as Schlayer has pointed out, in that it supports those observations which ascribe its activity to a purely secretory process. Also from a therapeutic point of view it is well known that the action of caffein in nephritis in man may differ from that of the saline diuretics.

Several objections might be raised to the view that the essential lesion in edema is a diminished glomerular permeability. All of these, however, are met by Schlayer's carefully controlled experiments. It might be objected that the strong sodium chlorid solution itself produces the glomerular injury. That salt is harmful to the normal kidney has been frequently demonstrated, and Castaigne and Rathery have shown that the injection of normal salt solution into rabbits with injured kidneys causes an increase in albumin elimination. Against this objection we have the observation of Schlayer that urea and dextrose, certainly non-toxic in the doses used, had the same effect as

strong salt solution. Other objections, as that based on the theory of primary salt retention and the assumption that the body had almost reached its limit of salt fixation at the beginning of the experiment, and that the half-gram of salt injected was sufficient to bind the water so that no diuresis could occur, are met by experiments in which three-hundredths of a gram of salt produced about the same decrease in diuresis as did the half-gram, which should not be the case if these objections were valid. The objection that a salt retention associated with an early increased permeability of the peripheral vessels might account for the edema is met by experiments which show that no edema could be produced, during this intermediate stage, by transfusing the tissues with salt solution, whereas it could readily be produced in the final stage. In brief, the control experiments indicate that the increased permeability of the peripheral vessels follows, and is presumably the result of the glomerular impermeability.

One must admit the importance of Schlayer's observation concerning this peculiar condition of the renal vascular system in the intermediate stage of uranium nephritis; a functional disturbance which occurs only in that form of experimental nephritis which leads to edema. It is the strong point of a theory of edema which reconciles many of the conflicting views on this subject. Decreased glomerular permeability, occurring primarily and causing a retention of water and salts with secondarily an increased permeability of the peripheral blood-vessels is a convincing theory, and perhaps more than a theory, when the experimental work on which it is based is considered. Certainly the experimental evidence which Schlayer offers shows that, if either of these factors is absent, no edema occurs.

Concerning the importance of these factors, I have reached similar conclusions as the result of a study somewhat different in nature. Accepting Schlayer's opinion that a vascular lesion is essential to the production of edema I have attempted to produce edema in true tubular nephritis by the administration of vascular poisons. The relative importance of hydremia and vascular and renal injury was also studied. Potassium chromate was used to produce a type of experimental nephritis almost exclusively tubular and not accompanied by edema. For the production of vascular injury, rattlesnake venom, ricin and arsenic, all well-known vascular poisons, were utilized. Water administered by stomach-tube, in amounts of 100 c.c. daily, brought about a condition of plethoric hydremia. A large number of rabbits were used; some received all three of these substances, some only one, and others various combinations of two; thus all possibilities were controlled.

It was found that edema resulted only when the three factors of renal injury, vascular injury and hydremia were present. No one of these factors acting alone and no combination of two was sufficient to cause edema. The experiments in which venom was used were particularly valuable in that evidence of injury of the renal vessels as well as of the peripheral vessels, was offered by easily demonstrable hemorrhagic lesions of these structures.

From this summary it is readily seen that the study of experimental nephritis has added much to our knowledge of the relative importance of glomerular injury, hydremia, salt retention and peripheral vessel injury in the production of edema. This knowledge has been obtained in the only way possible, that is, by the study of a form of experimental nephritis accompanied by spontaneous edema.

TOXIC SUBSTANCES IN EDEMA

In connection with the phases of experimental nephritis just discussed, the next problem is the determination, if possible, of the character or nature of the substance or substances concerned in the production of the vascular lesions, both renal and peripheral, but especially the latter, in nephritic edema. Clinical and pathological studies offer no assistance. The early appearance of the prominent glomerular lesion of scarlet fever naturally suggests that the products of the etiological agent of scarlet fever are responsible for this lesion and possibly also, as Senator has suggested, for the vascular lesions of the associated edema of the skin; but in the absence of definite knowledge of the etiology of scarlet fever or of its toxic products, no conclusions can be drawn. Likewise in certain infections, as with the pneumococcus and streptococcus (Councilman), in which capsular and intracapillary glomerular lesions are sometimes seen, the toxic products of the infecting organism may be considered responsible for the renal lesion. On the other hand, in those forms of chronic nephritis in which edema most frequently occurs, the etiology is obscure, the relation of parenchymatous to vascular lesions uncertain, and therefore conclusions are impossible. Even though it be granted that the general vascular lesions of the acute forms of glomerular nephritis are due to the poisons of the primary disease, our lack of knowledge of the toxic factors in chronic nephritis leaves no explanation for peripheral vascular lesions. The study of experimental lesions of the kidney has thrown little light on this problem, but certain observations with the serum of animals with experimental nephritis are very suggestive of the mode of development of peripheral vessel injury. Thus Heineke found that rabbits with chromate nephritis, which is not characterized by

edema, developed edema when injected with the serum of animals with uranium nephritis. This phenomenon, since confirmed by Blanck, who, however, finds that it is not a constant occurrence, suggests that in the serum of animals with nephritis substances occur which operate to produce edema. Two explanations seem possible: either the retention, as the result of kidney insufficiency, of substances which act as lymphagogues of the second order; or the injurious action on the endothelium, of some substance or substances causing an alteration in its permeability to fluids. The latter of these explanations is naturally more in accord with the experiments of Schlayer and his associates and with those which I have described. In a later series of experiments with Meyerstein, Heineke supports the theory of injurious action on blood-vessels. In this study is reported the production of edema in 64 per cent. of chromate animals receiving uranium serum intravenously; but edema was also found in an equal number receiving normal rabbit serum. As chromate nephritis, in the absence of serum injection, does not cause edema, it is suggested that the serum in both instances had some injurious effect on the blood-vessels. Of similar import are the results obtained by Georgopulos, who produced a moderate edema by injecting nephrectomized rabbits with the serum of animals suffering with uranium nephritis. In some of my own experiments with chromate nephritis I have found it possible to produce in the rabbit moderate grades of edema by injecting an alien serum (dog), and an edema equal to that of uranium nephritis, by using nephrotoxic immune serum (dog).

Despite the difficulty of explaining Heineke's results with normal serum, the various observations presented suggest very strongly the presence in the serum of nephritis, of elements acting on vascular endothelium. Whether such substances are the retained products of metabolism or whether they are substances formed anew, in the course of nephritis, or are possibly due to disturbances in those organs characterized by internal secretion, it is impossible to say.

Such observations must fall in the same category as those of Lindemann, Bierry, Sawyer and myself, concerning the power which the serum of various forms of nephritis (chromate, uranium, spontaneous, and that due to nephrotoxic immune serum) has when injected intravenously, of causing albuminuria and cast excretion in normal animals. The effect of the serum in each group of observations suggests the influence of the common factor, the renal disturbance, but unfortunately, while suggestive, the observations are as yet of so indefinite a character that they cannot be applied to human renal pathology. They would appear, however, to form a promising basis for future experimental investigation.

THE STUDY OF CHRONIC RENAL INSUFFICIENCY

In this presentation I have thus far limited my discussion to those problems to which have been applied methods which offer a functional conception of the acute disturbances in nephritis. For this reason I have considered only those lesions to which may be applied the term "nephritis" without fear of contradiction. Such lesions are, for the most part, those of acute nephritis, and thus the problems of chronic nephritis, as uremia, hypertension, and heart hypertrophy, have necessarily been excluded. The experimental investigation of these latter phases of nephritis, because of the inability to produce constantly chronic lesions, has been attempted by means of the so-called reduction experiments in which, by operation, the kidney substance has been reduced to a minimum compatible with life. Such experiments have yielded information of much interest, and, although strictly speaking, they represent the effect of insufficient function, rather than the effect of a true nephritis, they may, I think, be discussed here in connection with the general problems of renal pathology.

DISTURBANCES OF METABOLISM AND UREMIA

By the study of the metabolism in animals with experimental nephritis one might expect to obtain information concerning disturbances of elimination, or of the influence of the kidney lesion on general metabolism, and thus throw some light on the conditions determining the development of uremia. Such studies do offer some information of early or mild disturbances manifested by diminished nitrogen elimination (Siegel, Green) but in the severer lesions, the early occurrence of vomiting and diarrhea with inability to ingest, retain or utilize properly the food administered, all symptoms evidently of renal insufficiency, so disturb the nitrogen equilibrium that metabolism studies are impossible. This is true, not only of experimental nephritis, but also of those procedures by which the renal substance is greatly reduced by successive extirpations. Such experiments have therefore added but little to our knowledge of disturbances of metabolism as obtained by clinical studies. The reduction experiments bear particularly on the influence of the kidney on general metabolism. In the first important investigation of this subject, that of Bradford, the conclusion was reached that slight reduction was followed by an increase in the elimination of water, but no change in the solids; on the other hand, an increase in total solids was found to occur after the removal of three-fourths of the total kidney substance; an absolute increase when food was taken and a relative increase when the gastrointestinal disturbances were present. As the blood and tissues under the latter circumstances showed an increase in nitrogenous extractives, Brad-

ford concluded that these disturbances were due, not to retention of products of normal metabolism, but to an increased tissue catabolism, affecting especially the muscles.

Recently Bainbridge and Beddard have repeated these experiments. They find that the increase of nitrogen elimination is not constant and occurs only during the last few days of life when the animals show a loss of 22 per cent. of body weight, the result of gastrointestinal disturbances and loss of appetite. They conclude, therefore, that the kidney has no influence on nitrogenous metabolism, and that the disturbance of nitrogen elimination is to be ascribed to inanition, and is similar to that occurring in fasting animals. My own experiments on this subject led to conclusions in entire accord with those of Bainbridge and Beddard. It would therefore appear very probable that mere reduction of kidney substance, even to a minimum compatible with life, does not lead to disturbances of metabolic function capable of being utilized in the explanation of uremia. Likewise these experiments indicate the improbability of the presence of an internal secretion of the kidney capable of influencing general metabolism.

It is evident, however, that although under such circumstances there is no disturbance of general metabolism which may be recognized by chemical examination of the urine, the very striking gastro-intestinal disturbances must be explained through some fault of kidney function. As these disturbances occur also in experimental nephritis of the tubular type (uranium, chromium and corrosive sublimate) and not at all or to but a slight extent in the vascular form (arsenic), they would appear to be due to a fault of tubule function, and the natural inference is that these disturbances are to be explained by a vicarious elimination into the gastro-intestinal canal of toxic products normally eliminated by the kidneys, and presumably are the manifestations of experimental uremia.

Some support of such a theory is offered by clinical studies of uremia by Von Noorden and his associates, who have found such a vicarious elimination, with an increase of ammonia nitrogen, to occur especially in the so-called uremic diarrhea.

In one of my early investigations I tested this theory as far as fecal nitrogen is concerned in animals with kidney reduction, but with negative results. More recently, with the assistance of Dr. Hill, I have estimated the total nitrogen elimination in urine and feces in a group of animals with various forms of experimental nephritis. A constant decrease in urinary nitrogen varying from 9 to 14 per cent. was noted in the tubular form of nephritis, during the few days preceding the development of gastro-intestinal disturbance, but at no time was the fecal

nitrogen appreciably altered. Siegel in similar experiments has also found the same drop in urinary nitrogen without an increase in fecal nitrogen.

Metabolism studies, therefore, indicate that the alimentary disturbances are not due to vicarious elimination of nitrogenous substances into the intestine, or, on the other hand, to diminished absorption of such bodies therefrom. It may be possible, as I have suggested elsewhere, that toxic substances, non-nitrogenous in nature, which cause irritation by elimination into the intestines, are responsible for this disturbance; or that, accumulating in the blood, they act either through the central nervous system, or locally on the tissues with which they come in contact.

This problem I consider one of the most important offered for solution by experimental nephritis. The gastro-intestinal disturbances with the associated respiratory and circulatory disturbances, and, not infrequently, a period of unconsciousness, essentially coma, for several hours before death, constitutes a syndrome characteristic of renal insufficiency, and presumably, of experimental uremia. It is not too much to assume that the determination of the factors responsible for this experimental condition may explain some phases of uremia in man.

HYPERTENSION AND HEART HYPERTROPHY

The hypertension and left-sided heart hypertrophy so characteristic of the atrophic form of chronic nephritis have led to numerous investigations having for their object the experimental reproduction of these conditions. It is but natural, in view of the fact that the chief characteristic of the human lesion is an atrophy of the kidney leading presumably to diminished function, that the experimental methods should at first be largely those causing a considerable diminution of the functional area of the kidney and at the same time allowing a survival of the animal for long periods of time. As the acute forms of toxic experimental nephritis cannot obviously be utilized for this purpose, and as atrophic forms of chronic nephritis cannot be reproduced with any constancy, the method employed has been that of gradual reduction of the kidney substance, by successive operations, to a minimum compatible with the life. Although conditions analogous to those accompanying the interstitial type of chronic nephritis in man have occasionally been observed as heart hypertrophy by Paoli, and an increased amount of dilute urine by Bradford, the exact study of this problem begins with the observations of Pässler and Heineke, who, in 1905, attempted for the first time to study the changes in blood-pressure by direct manometric observations. These investigators found that after the removal of a considerable portion of

the kidney substance, approximately two-thirds to three-fourths, by successive operations, a rise of blood-pressure occurred which was permanent and associated with cardiac hypertrophy and the elimination of an increased amount of urine of lowered specific gravity. This result was not constant, but occurred in about 25 per cent. of the animals which survived, by at least four weeks, a considerable reduction of the kidney substance. In such it was observed also that arterial spasm with further rise of blood-pressure quickly followed stimuli which in normal animals would produce little effect. These observations suggest that the heart hypertrophy is due to increased work resulting from the circulatory disturbances caused by the tendency to arterial spasm, and that the vascular spasm is due in its turn to the effect of retained toxic substances.

The determination of the blood-pressure in these experiments was by direct measurement in the femoral artery; single readings were made before operation and one or more after operation. Although the differences noted, varying from 15 to 29 mm. Hg with an average of 21.5, are quite definite, they are open to objection, as Theodore C. Janeway has pointed out, on account of the well-known normal variations in pressure which occur from time to time. To obtain more definite information of the changes from day to day, Janeway has utilized in such experiments the universally accepted clinical method of determining blood-pressure. He has modified the Riva-Rocci cuff so that it may be applied to the fore leg of the dog, and the pressure determined with a minimum of error, estimated at about 10 to 15 mm. This method of measurement he has used on animals in which the renal substance had been reduced by Carrel's method of ligating several of the branches of the renal artery. Observations on such animals, in some instances covering a period of fifteen months, show, as compared with the normal readings before operation, a decided increase in pressure; thus in one animal was observed an increase from the average normal pressure of 90 mm. to an average of 125 mm. after 100 days; in another an increase from 117 to 150 mm. The maximum and minimum pressures of the respective daily observations showed also the same relative increase.

From a consideration of the experiments of Pässler and Heineke and of Janeway, one cannot but conclude that a condition of experimental hypertension of renal origin is brought about as a result of the reduction of kidney substance. Such experiments, however, as yet offer no explanation of the mechanism by which the hypertension arises. It can hardly be due, in the extirpation experiments, to the influence on function of mere reduction of kidney tissue, for as I have shown, the "factor of safety" for the kidney is such that one-half of one kidney appears to be

sufficient for the proper elimination of nitrogen and presumably also for other solids. Nor in the ligation experiments of Carrel and Janeway can it be due to the mechanical effects of the reduction of the kidney circulation, for, as Ludwig has shown, complete ligation of the renal arteries is not followed by permanent increase in the general blood-pressure. The single anatomical condition which is unavoidable and follows all forms of injury is a varying degree of infarction-necrosis. This is slight in amount in the "polar" excisions of Sampson and myself, somewhat greater in the "wedge" excision of other investigators, and from the nature of the injury must reach its maximum in the ligation experiments of Carrel and Janeway. In itself this infarction cannot be responsible for hypertension, but the persistent albuminuria in Janeway's dogs indicates that it may be responsible for the development of a true nephritis which, of course, adds to the factor of diminution of functional area that of altered function. Similarly in the extirpation experiments, the irritation of sutures in the pelvis of the kidney, causes occasionally the localization of the colon bacillus with infection of the infarcted tissue and the development of a pyelonephritis (Sampson and Pearce), which must exert an injurious action on the remaining kidney substance, and as time goes on, lead through attempts at repair to a more or less chronic lesion.

I have gone into this matter somewhat critically because, although the results of reduction experiments are striking, the procedures by which they are obtained are not such as involve only a single factor, but bring several forms of kidney injury into play; that is, reduction of functional substance and productive, atrophic and vascular changes accompanied by the elimination of albumin and casts. In other words, a chronic lesion of the kidney, characterized by hypertension, heart hypertrophy and increased flow of dilute urine is produced, and this may be considered as an experimental disease analogous to certain phases of chronic renal disease in man, but it gives us no facts which explain the etiology of the vascular disturbances of the latter. The production, however, of hypertension experimentally is no small gain, and it is to be hoped that in future investigations the various factors involved in the experimental disease may be analyzed and controlled, and that the essential etiology of experimental renal hypertension established.

There is one aspect of these studies which is of considerable theoretical importance. Pässler and Heineke state that although an increased flow of urine of lowered specific gravity usually accompanies the experimental heart hypertrophy, it may occur in the absence of hypertrophy and hypertension. This would indicate that polyuria is independent of

increased blood-pressure, and is of interest in connection with Loeb's hypothesis of the influence of a glomerular reflex in the production of hypertension. This is based on the frequency with which hypertension in man is accompanied by glomerular lesions (Schmidt), and on the physiological law that the functional power of the kidney depends on the rate of blood-flow through the glomerulus. Loeb assumes that with greatly increased capillary resistance within the diseased glomerulus, the increase of flow due to local vasodilatation is insufficient for the needs of the kidney, and that the glomerulus sends a call beyond the local vasomotor system which, reaching the cerebrospinal centers, causes a reflex splanchnic vasoconstriction and thus increases the general blood-pressure so that a normal flow through the altered glomerulus results. This hypothesis might well be applied to explain the results of reduction experiments. The demands of water elimination on the greatly reduced number of glomeruli in the persisting kidney fragment might readily excite a reflex splanchnic constriction to aid in the proper elimination of water. Thus would be explained the increased blood-pressure, and by its continuance the eventual heart hypertrophy. This attractive hypothesis cannot at present receive support from reduction experiments if polyuria without increased blood-pressure, as observed by Pässler and Heineke, is found to be a frequent condition. Their experiments, however, were made on a comparatively small number of animals, and the investigation of this hypothesis should be an important phase of future studies of the reduction of kidney substance.

As all forms of experimental reduction of kidney substance are characterized by loss of glomeruli and by either increased blood-pressure or polyuria, or both, and frequently by heart hypertrophy; and, on the other hand, as hypertension does not occur in the presence of a normal splanchnic circulation, it would seem possible, by properly planned reduction experiments, either to disprove or to establish Loeb's hypothesis and thus to clarify to some extent the at present confusing theories of hypertension in nephritis.

Several other aspects of this phase of renal disturbance might be discussed, as the influence of a possible internal secretion of the kidney on blood-pressure and the matter of the presence of blood-pressure-raising substances in the serum of nephritis; but to such problems the study of the acute forms of experimental nephritis has little application, and the results of the study of experimental chronic lesions, thus far obtained, are either contradictory or entirely negative.

In concluding this presentation, I admit that I have neglected several important phases of experimental renal pathology and have treated others

in a more or less incomplete way. Such omissions have been intentional, as I have preferred to emphasize those problems to which have been applied methods which offer a functional conception of disturbance in nephritis, and which tend to distinguish between the results of tubular as contrasted with glomerular lesions and to show the relation of these to some of the more important manifestations of renal disease. To such a conception, supplementing the older anatomical knowledge, we must look for the ultimate solution of the problems of nephritis.

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