# INFLUENCE OF DIETARY CONSTITUENTS UPON RENAL SIZE AND STRUCTURE.

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# TABLE OF CONTENTS.

Page

Page

## Introduction.

The morphology, both normal and pathological, of the kidney has been widely investigated. Pathologists, biochemists, physiologists, pharmacologists, and more recently, endocrinologists have all contributed much valuable information which has led to a better understanding of the structure as well as of the function of this vital organ. Many techniques and experimental approaches have been employed, and a wealth of data has been accumulated. Among these techniques may be found those of the nutritionist. Effects of both overdosages and deficiencies of numerous elements and compounds have been investigated. Unfortunately, this work has been done by so many different investigators under various conditions and for a variety of reasons, that it has proven difficult to collect and to correlate all this huge volume of experimental data concerning the effect of diet on the kidney.

It is the aim of this thesis to provide a critical review of the ef effects of dietary constituents upon renal size and structure. Many of the experimental results obtained to-date (1945) will be enumerated, correlated and commented upon, in order first to present as clear a picture as possible of what we know to-day about this subject and second, to act as a guide for further reading and experimental investigation in this field.

Each dietary factor is discussed in turn in terms of the effects of overdosage and deficiency. Summaries and conclusions are included at the close of each main section. Some original experimental work is also mentioned

In the course of the original experimental work presented here, use has been made of certain experimental surgical interventions and various hormonal substances, in order to investigate their influence on dietary alterations of renal morphology. Thus, obstruction of the pylorus has been utilized in order to deprive the animal of Chloride rather than the much slower and more tedious process whereby the animal is fed a diet deficient in Chloride for a period of several months. The Renotropic effect exhibited by several hormones has been utilized in order to produce a maximum kidney growth at the time when it was expected that the renal effects of dietary deficiencies and overdosages would appear.

In order to keep this thesis within reasonable limits, it has been impossible to mention or to review in any detail many papers dealing with experimental kidney morphology. The unavailability of recent European literature has rendered it impossible to include the results obtained by Continental scientists. Wherever possible, comprehensive reviews and other articles covering certain aspects of the subject not here discussed have been indicated. Clinical reports and articles of a similar nature have been kept down to a minimum as this review is mainly concerned with the experimental rather than clinical findings.

## Inorganic Elements.

The monograph by Shohl (1), entitled "Mineral Metabolism", furnishes a comprehensive picture of the general rôle of inorganic elements in animal nutrition and metabolism. In this study, we are primarily concerned with the effects of mineral deficiencies and overdosages upon renal morphology and physiology.

The earliest work in this field dealt with the effects of what we now term 'multiple deficiencies', those involving several dietary constituents rather than a single substance. It is only within the last decade or so, that individual mineral elements have been investigated with regard to their specific effects in biological reactions, and particular importance in organ morphology.

In 1923, Jackson and Carleton (2), in an experiment designed to produce rickets in rats, found an increase in kidney weight amounting to about 33 % above the normal value. They also noted a marked cardiac hypertrophy. The diet which they employed was not only completely defieient in mineral constituents, but also lacked vitamin and essential food substances which we consider indispensable to a well-balanced diet.

Winters, Smith and Mendel (3), using a diet similar in many respects to the above, observed a 63 % increase in renal size. They failed to confirm the increase in the size of the heart, however. Since their diet had not been purified, but consisted of natural food-stuffs, it is impossible to find any definite cause for the discrepancy in cardiac weights.

Smith and Schultz (4) repeated the experiment on young rats and found, by chemical analyses of the kidney constituents, that the increase in weight was at least largely due to a retention of mineral matter. The kidney tissue had actually increased in size at a time when the rest of the body had been suffering from a mineral deficiency. In a more detailed study, Swanson, Storvick and Smith (5) made an attempt to follow the changes occurring in the kidney when the animals were kept on a salt-free diet for a definite length of time. Their results indicated that hypertrophy of the organ occurred during the first twenty-one days of treatment, a fact which they attributed to an increased need for renal absorption of necessary mineral substances from the glomerular filtrate. The kidneys then began to regress, and when all the experimental animals were killed after ninety days, no histological abnormalities of structure were visible. It should be mentioned that the diet used by the above workers was quite different in composition than those which were used later on, being deficient in base and having a distorted Ca/P ratio, factors which are known to influence kidney size, as Brooke and Smith (6) have demonstrated since.

Swanson and Smith (7) fed a diet to rats which was deficient only in its content of mineral salts. After ninety days on this regimen, the animals were sacrificed and their organs weighed. The kidney was not among the organs which were investigated, but the authors discuss the mechanism of the changes which occurred in the size of the other organs and attribute them to an alteration in the degree of hydration. An increased water content appears to account for the heavier kidneys seen by Swanson et al. Unlike the findings of Winters and co-workers, these investigators (7) failed to notice any significant change in heart weight and size.

In a paper by Eppright and Smith (8), the trend toward the study of the effects of deficiency of each separate inorganic element, is already apparent. Up to this point in the history of nutritional investigation, use had been made of diets containing salt mixtures usually deficient in many chemical elements. Henceforth, work upon each element in turn was the case. Animals in the experiments of Eppright and Smith (8) were kept on diets which were identical in all respects, save for the inclusion of different salts in varying quantities in the salt mixtures used. The control animals for these experiments were kept upon the Osborne-Mendel Salt Mixture (9) . The kidneys of the animals receiving Sodium Chloride were quite large, while those receiving Calcium and Phosphorus compounds in excessive quantities showed many casts in the tubules. Animals receiving Potassium in the form of the Citrate together with Sodium Chloride showed an augmentation of only 15.6 % in size over the size of kidneys of normal control animals, while those getting Sodium Chloride alone alone showed the relatively enormous enlargement of 69 % above the normal values. The kidneys appeared to grow even after the animals had ceased to grow.

Before proceeding to a consideration of the effects of the individual inorganic elements upon the kidney, mention should be made of two papers dealing with the effects of prolonged water deprivation. Kudo (10) found that rats living on a low water intake for thirteen weeks developed kidneys which were 58 % larger than normal. These observations on the augmentation of kidney size by water deprivation were independently observed and confirmed by Jackson and Smith (11).

Potassium.

The effects of an overdosage of Potassium are not in any way concerned with kidney morphology, and so this review deals only with the results of a deficiency of this element.

The syndrome characteristic of Potassium deficiency almost always includes cardiac as well as renal changes. Osborne and Mendel (12) and Miller (13,14) were among the first to work with Potassium-deficient diets. They noticed a retardation of growth in their experimental animals. Leulier and Vanhems (15) actually found that death would occur after three weeks on a diet free of Potassium salts. However no report was made concerning any pathological changes which may have taken place in these animals.

The first detailed description of the effects of Potassium deprivation upon organ morphology, was given by Schrader, Prickett and Salmon (16). Only kidney changes will be mentioned here, although alterations in many other organs are described. Young rats were kept on a Potassium-free diet for a period of time ranging up to four weeks. At autopsy, extensive renal and cardiac changes were observed. The kidneys were pale and enlarged. Microscopically, "a diffuse tubular nephritis was presented. The epithelium of the tubules was markedly edematous, and had sloughed off in some cases. Pycnosis of nuclei was common. There was a considerable degree of congestion of the renal blood vessels. Little change was seen in the glomeruli." In the heart, massive erosions were seen in the myocardium and endocardium.

Grijns (17) found that the amount of Potassium necessary for normal growth was somewhere between 0.05 % and 0.016 % of the diet. Thomas, Mylon and Winternitz (18) observed severe heart lesions in rats and in hogs, kept

on a low-Potassium diet . Heppel (19), engaged in a study of the effects of Potassium-deficient diets upon the serum and muscle Potassium concentration, was the first to demonstrate that in Potassium deficiency there occurred a replacement of Muscle Potassium by Sodium. This biochemical discovery may be prehaps be associated with the production of lesions in such organs as the heart, especially those in the myocardium. Renal lesions were observed as well by this worker (19). Detailed studies concerning the relationship between the kidney and cardiovascular disease were carried out by Winternitz, Mylon, Waters and Katzenstein (20), in the course of which the results obtained by Heppel (19) were confirmed. Orent-Keiles and McCollum (21) reviewed some of the more important work in this field and reported upon their own experiments, in which they found a lowering of the Potassium content of the heart to an extent of about 30 %, while the Kidney Potassium fell 23 % below normal, in animals fed on a low-Potassium diet . Liebow, McFarland and Tennant (22), using Heppel's diet (19), gave a very complete description of the lesions which they obtained in the kidneys of mice fed upon this diet. "Some tubules in the deficient animals are greatly distended and are lined by flattened epithelium", they state, "The cytoplasm of the cells, particularly in the loops of Henle, is more coarsely granular in part and in part more vacuolated." Restoration of Potassium to the diets of the deficient animals restored their kidneys to normal. "The changes in the kidney are interpreted in part as those of hypertrophy and hyperplasia, but there is also evidence of necrosis of occasional epithelial cells and of colloid change of the cells of the papillary ducts and of proliferation of the interstitial tissue." The authors also believe that the more severe manifestations of renal damage play a small rôle in bringing about the increased weight of the organ.

At approximately the same time as these studies were in progress (1941), Follis (23) reported that exercise would aggravate the cardiac lesions produced in rats by a diet deficient in Potassium. Renal changes, however, were not affected at all. An extensive study by Follis, Orent-Keiles and McCollum (24) was also published at the same time, dealing with the effects of Low-K diets upon the heart and kidneys. The diets utilized by these workers were much lower in their Potassium-content than those of their predecessors. This may account for the discrepancies in results between the various investigators. Rats weighing from 100 to 125 grmas were placed on the experimental regimen, animals being killed at varying intervals up to about one year. The microscopic pathology of both the heart and the kidneys were described in detail.

The kidneys of the Potassium-deficient animals increased greatly in size, and were paler in colour. Necrosis of the epithelial cells lining the convoluted tubules occurs, the cells becoming detached from the basement membrane and lying in the tubular lumina as pink hyaline masses. Dilatation of many tubules occurred. Casts were found in the tubular lumina after about three weeks, and several calcified epithelial cells were noted.

Follis (25) also fed a diet deficient in both Potassium and in Thiamine to rats, and found that the myocardial lesions characteristic of Potassium deficiency could be largely prevented by such a diet. The renal changes, however, were not in any way affected by the simultaneous Thiamine deficiency. Durlacher, Darrow and Winternitz (26) studied the effects of Low-Potassium diets as well as of the corticoid, Desoxycorticosterone Acetate, upon the kidney of the rat. In the animals on the deficient diet, the kidneys were found to be enlarged, and histologically, there was dilatation of the tubules with a marked hyperplasia of the lining epithelium. These changes mainly involved the

ascending loop of Henle and the collecting tubules. The rats which were fed on the Potassium-deficient diet and simultaneously treated with D.C.A. showed renal changes which were essentially similar in nature. It was also contended by these workers that the administration of a 1.5 % solution of Potassium Chloride could prevent the renal changes produced by Desoxycorticosterone Acetate overdosage. The authors regarded the kidney enlargement as being due to "hypertrophy associated with the attempt to reabsorb Potassium in the presence of a deficiency." They also demonstrated that the feeding of a high Potassium diet would bring about no demonstrable changes in the renal parenchyma. Heart lesions were shown to be aggravated as well on a diet low in Potassium while undergoing a simultaneous D.C.A. overdosage by Darrow (27). The work of Follis et al. is reviewed up to the year 1942 (28).

Magnesium.

The effects of an excessive intake of Magnesium may be attributed to an upset in the mineral balance of the organism, rather than to a specific activity of the element per se. Haag and Palmer (29) first noticed the occurrence of renal calculi in rats kept on a high Magnesium diet. Watchorn (30) studied this phenomenon in greater detail, and found that the ingestion of Magnesium Carbonate was a very effective means of producing these calculi. Kidney damage was also apparent, but it was attributed to the back pressure resulting from blockage of the ureters by the calculi, and so could not represent a true nephrosis. Most Pharmacological text-books contain ample information of the effects of high doses of Magnesium salts upon the body as a whole.

The literature dealing with the effects of Magnesium deficiency on the kidneys is much more abundant than that devoted to the symptoms of overdosage. Probably the first investigator to work with diets low in Magnesium was Leroy

(31), who found that mice kept on a Magnesium-deficient diet would not survive more than a month. McCollum and Orent (32) and Kruse, Orent and McCollum (33) carried on most of the pioneer research in this field and furnished excellent descriptions of the dramatic nervous symptoms which are peculiar to Magnesium deficient state in the rat. Orent, Kruse and McCollum (34, 35) in further publications also studied the effects of Low-Magnesium diets on the dog, and Kruse et al. (35) studied the changes in mineral metabolism which took place in Magnesium-deficiency. However, none of these publications contain any refernces to the kidney or to possible renal damage. The early work of Greenberg and Tufts (36,37) may be mentioned as an example of the necessity of standardizing the proper experimental conditions. Using diets which were much too high in their content of Magnesium, these workers failed to detect any kidney changes.

It was not until the work of Cramer (38) in England was published that a description of kidney changes in Magnesium deficiency became available. In Cramer's experiments, rats were kept on a regimen low in Magnesium but not entirely lacking in that element for a period of approximately five weeks. At autopsy, the kidneys were significantly enlarged, most of them showing alterations of a definitely pathologic nature. "Microscopically," Cramer reported, "there occurred an extensive degeneration of tubules and glomeruli, which is most marked at the junction of cortex and medulla, but extends far into the cortex. These degenerative changes were sometimes, but not always accompanied by calcareous deposits in the tubules and glomeruli, but where found they were restricted almost entirely to the junction between cortex and medulla. They are not seen, as a rule, in the degenerated tubules and glomeruli situated in the more peripheral parts of the cortex. Sometimes a def-

finite increase in the connective tissue around the degenerated tubules has been observed, but there is no evidence of an inflammatory reaction."

It should stressed once more that the diet employed by Cramer was much higher in its Magnesium-content than that used by the workers in the laboratory of McCollum. Brookfield (39) found more extensive lesions in the kidneys of rats on a similar diet. No normal tubules could be identified microscopically, while the cells lining Bowman's capsule were largely swollen or desquamated. Moore, Sholl and Hallman (40) a similar condition occurring in the kidneys of calves with a low blood Magnesium. A later report by the same workers (41) emphasized the presence of a nephritic condition, marked renal atrophy having been observed.

Schrader, Prickett and Salmon (16) could not find kidney lesions in their Magnesium-deficient animals, only mild changes being encountered in the renal tubules. Watchorn and McCance (42) maintained young rats on a diet only partly deficient in Magnesium. After three months, they observed deposits of Calcium in the tubules, which formed casts which in some instances completely obliterated the tubular lumina, thereby producing dilatation of the renal tubules above the cast. The glomeruli were essentially normal in most of these animals.

Greenberg and Tufts (43) in studies designed for the determination of the nature of low-Magnesium tetany did not describe any kidney changes. The paper of Greenberg, Lucia and Tufts (44) however, yields quite a large amount of information concerning the pathology of the kidney in cases of Magnesium deficiency. They found first of all, that renal involvement could not be

produced in animals which had been kept on an extremely low Magnesium diet, since death usually intervened before the renal manifestations could be developed. They therefore kept their animals on a diet low in Magnesium and supplemented this diet from time to time with small amounts of Magnesium Sulphate. After six weeks, the animals showed extensive calcification in their kidneys. Microscopically, Greenberg et al. found that the zone of greatest calcification was the cortico-medullary boundary. "In the initial stage the tubules are seen to contain much granular and pultaceous material. At a more advanced type of deficiency, large concretions of Calcium occur in the outer stripe of the boundary zone, crowding the adjacent tubules. The tubules containing Calcium become distended, and the epithelium atrophic." Diffuse tubular atrophy was also seen around the concretions of calcareous material.

Further studies by Tufts and Greenberg (45) showed that the Calcium content of the kidney increases as much as fifteen-fold during Magnesium deprivation. This increase in kidney Calcium is usually preceded by renal damage. A high content of Calcium in the Magnesium-deficient diet was found to aggravate the condition and bring about a much greater degree of kidney calcification (46).

Recently, Evans and Sullivan (47) found that there was no relationship between Magnesium deficiency and deficiency of one or more of the B-complex vitamins. The dermatological lesions produced by depriving the rat of either of these substances seemed to be quite identical in appearance and some investigators (42,45) had expressed belief that there was some sort of a relationship between these two deficiencies. In the course of this work, Evans and Sullivan (47) confirmed the production of renal lesions on Magnesium-deficient diets,noting extensive tubular degeneration and calcification.

The symptoms of Magnesium deficiency as they affect the organism as a whole, are discussed by Evans and Sullivan (48) in a subsequent publication.

It seems then that in Magnesium deficiency, kidney damage is first produced . The mechanism of this action is unknown. The second step consists in the calcification of the injured renal tissue. This mechanism is similar to that seen in renal calcification resulting from Vitamin D overdosage.

Sodium.

Sodium, in the from of its chloride, is an indispensable part of the animal diet. The early literature contains many reports of cases of Sodium Chloride poisoning. However, since many of these publications are very old and inaccurate, no attempt will be made to deal with them here. The experiments of Heinz and Haas (49) may be mentioned. Fowl given a diet containing 63 % of Sodium Chloride died within a very short time. Kidney changes are not described. Needham (50) described the effects of a chronic overdosage with Sodium Chloride. The kidney structure was altered, some breakdown in tubular structure occurring in the experimental animals, which were rats kept on a diet in which NaCl formed 17 % of the total food intake. There was also a very striking shrinkage of the glomerulus, which filled only half of the glomerular space. After seventy-two days of treatment, profound degenerative changes were visible in the kidney, especially in the tubules, most of which had lost any semblance of shape. Masses of pigment were seen in a few places, and in most of the glomeruli, the nuclei were entirely absent. It was concluded by the author that degeneration of the kidney accompanies excessive and prolonged polyuria (50).

In this laboratory, Selye (51) and Selye and Stone (52) found that

chicks were extremely sensitive to small doses of Sodium Chloride in drinking water. As little as 0.9 % Sodium Chloride in the drinking water caused fatal pathological changes in the kidneys which were large, pale and edematous, especially in the animals dying within the first ten days of the commencement of the experiment. The tubules seemed to be particularly involved. The chicks dying between the tenth and twentieth experimental days, had entered upon the chronic stage of the disease. Here, the kidneys showed evidences of developing Nephrosclerosis, accompanied by a diminution in size, and a growing irregularity of surface. The similarity of these experimentally-produced lesions with those seen in Bright's disease was stressed by the author. A discussion of the pathology of this so-called 'pullet disease' is presented by Jungherr and Levine (53).

It has been very difficult to construct diets completely free of Sodium because of the wide distribution of this inorganic element in foodstuffs. We therefore find that comparatively few papers have been published on the effects of Sodium deprivation. Mitchell and Carman (54), Miller (55) and St. John (56) were among the first to work with Sodium-deficient diets in animals. They all observed a retardation of growth, and St. John (56) reported blindness and sterility. Orent-Keiles, Robinson and McCollum (57) examined the ocular lesions in greater detail, and confirmed the occurrence of sterility in the Sodium-deficient rat. Practically all tissues seemed affected to some degree, although no specific renal lesions could be detected. Kahlenberg, Black and Forbes (58) did not observe the ocular lesions in their experimental studies, while Turpeinen (59) found no specific kidney involvement in young Sodium-deficient puppies. Orent-Keiles and McCollum (60) performed an experiment in which an extremely low-Sodium diet was employed and Follis (61)

reported upon the pathological findings in the experimental animals. There were no kidney changes after a period of time as long as six months. All these observations upon the effects of a Sodium-deficient diet upon the renal parenchyme are thus in complete accord. It may therefore be concluded that Sddium deficiency exerts no effects upon the kidney.

Calcium.

Calcification is a well-known pathological phenomenon, and is not considered in detail in this thesis. Mention must be made, however, of some dietary factors which may bring about calcification for a variety of reasons, cheif among these factors being an excessive ingestion of the element Calcium itself. Polak (62) observed renal calculi in young rats fed on a diet containing an added 2-3 % of Calcium Carbonate. Eppright and Smith (63) noted renal calcification in rats fed supplements of both Calcium and Phosphorus. Hummel and Barnes (64) reported upon the occurrence of kidney calcification in rats. Using the diet of McCay et al. (65) which had been designed to retard growth, they found that this calcification process seemed to be associated in some manner with the retardation of growth. Barnes (66) reviewed the whole subject of Calcium deposition and presented experimental data showing that $Ca_2HPO_4$ caused more extensive calcification than did $CaCO_3$, which is the Calcium salt usually employed as a source of this mineral element. The added Phosphorus seemed to increase the extent of the calcification. In the kidney, the medullàa was the common site of the process. The protein intake had also been found to influence the degree of calcification, a low protein diet being most conducive to this process.

The work of Greenberg and co-workers has clarified to a great extent the physiological effects of Calcium, as well as the pathological changes

taking place in Calcium deficiency. Boelter and Greenberg (67) noted the occurrence of hemorrhages in the kidneys. However, these hemorrhages were not specific for this organ, since they were of widespread occurrence in the body. Further work by Greenberg et al. (68,69,70) failed to mention any specific renal involvement as a result of the feeding of Low-Calcium diets to rats, and it may safely be concluded that no renal changes occur under these dietary conditions.

Phosphorus.

Addition of large amounts of Sodium Phosphate to the diets of young rats has been found to bring about a large increase in renal weight by McKay, McKay and Addis (71). As has previously been mentioned, Eppright and Smith (63) found extensive calcification of the kidneys in rats fed on a diet high in the content of both Calcium and Phosphorus. Harris and Innes (72) found necrosis of the tubular epithelium in rats and rabbits fed on a high phosphate diet. A high Calcium intake simultaneously. tended to aggravate the condition The intake of Vitamin D also seems to have been involved. In general, it has been very difficult to exclude the effects of this substance in any consideration of the effects of varying the amounts of Calcium and Phosphorus in the diet. In this study, an attempt has been made to classify certain experimental reports under the general heading of the mineral element involved or under Vitamin D. This is admittedly rather artificial, but it seems to be of some value in allocating the principal effects of each of these nutritional factors to their proper places in this discussion.

MacKay and Oliver (73) administered a high Phosphate diet to rats for a period of seven weeks. At autopsy, the kidneys presented a greyish appearance. The tubules were lined by hyperplastic regenerated epithelial giant

cells in the outer medullary zones. Many lumina were filled with calcareous deposits. The lesions seemed to originate at the terminal portions of the proximal convoluted tubules, after which they underwent calcification and interstitial fibrosis.

Schneider and Steenbock (74), working with a Low-Phosphorus diet, noted the formation of renal calculi in their experimental animals. Chemical analyses of these calculi showed them to be composed of Calcium Citrate(75). Day and McCollum (76) fed rats on a diet extremely deficient in Phosphorus, and Follis et al. (77) reported the pathological findings at autopsy. No renal calculi could be found by these workers. They explained the contrary results of Schneider and Steenbock (74) on the basis of the relatively high content of Calcium of their experimental diets. A study of the effects of Phosphorus deficiency in dogs was carried out by Freeman and MacLean (78). The kidneys of these animals were found to be quite normal at the close of the experimental period. It is therefore safe to conclude that a dietary regimen low in Phosphorus does not affect the size or structure of the kidney.

Chlorine.

Once again, mention must be made of the very important rôle of Sodium Chloride in nutrition. It has been notoriously difficult to construct Low-Chloride diets. Nevertheless, some work has been done on the effects of Chloride-deprivation upon the rat. Orent-Keiles and co-workers (57), Marquis (79) and Greenberg and Cuthbertson (80) studied this problem, but none of them reported any kidney lesions. Voris and Thacker (81) and Thacker (82), in the course of biochemical studies on the effects of Low-Chloride diets, could not find any kidney involvement either. Very recently, however, Cuthbertson and Greenberg (83) reported extensive renal damage in rats on a diet

extremely deficient in Chloride. These lesions began in the convoluted tubules and eventually involved the whole nephron. The damaged nephrons may be replaced by connective tissue. The chloride content of the kidneys of the animals on this type of diet has been found to be sharply reduced. No final conclusions as to the effect of Chloride deficiency upon the kidney can therefore be made until further data is made available.

Miscellaneous.

Among the many experiments upon the effects of various inorganic substances upon the kidney, only one representative need be mentioned here. Stier and Hayman (84) injected Ringer's Solution intraperitoneally in order to provoke diuresis. They expected that the kidney would increase in weight due to the "work-hypertrophy" which it would be forced to undergo. However, no such enlargement occurred, although in unilaterally nephrectomized rats, the kidneys of the animals receiving injections of the Ringer's Solution seemed to be significantly enlarged.

Follis (85) summarized the general effects of mineral deficiencies in animals, and a comprehensive review which includes the effects of rare inorganic elements not mentioned here, can be found there.

Summary and Conclusions.

1. A deficiency of mineral substances in the diet tends to cause a hypertrophy of the kidney . This may be due either to a "work-hypertrophy" occasioned by the increased need for tubular resopption of mineral elements in the glomerular filtrate, or may be caused by a concentration of minerals in the kidney parenchyma as a measure of conservation by the body of these vital substances.

2. Potassium deficiency produces renal and cardiac lesions, which may be accompanied by hypertrophy of these organs, and which may be due to the low concentration of this important constituent of intracellular fluid. A slight degree of calcification and cast formation may sometimes be noted.

3. Kidney damage may be produced by a diet low in its content of Magnesium, provided there is sufficient Magnesium present to insure the animal's survival for the length of time necessary for the developement of the renal changes. Calcification of the damaged tubules occurs as a result of an upset in the normal Ca/Mg ratio brought about by the Magnesium-deficient diet. This derangement of the physiologically-important mineral balance brings about a general increase in the concentration of Calcium salts in many of the bodily organs, the kidney being especially affected.

4. Overdosage with Sodium produces renal lesions similar to those seen in Nephrosclerosis. The tubules are particularly affected, though shrinkage of the glomeruli has been reported.

5. Sodium-deficient diets have no effect upon the kidney.

6. An excess of Calcium in the diet produces renal calculi, as well as renal calcification. This action is aggravated by a simultaneous high phosphate intake. No specific changes are seen to occur in the kidney on Calcium-deficient diets. ((The intake of Vitamin D (Calciferol) is very important in work with Calcium diets, as is also the level of the parathyroid hormone.))

7. A high Phosphorus intake may cause renal calcification by upsetting the Ca/P balance. Low-Phosphorus diets exert no effects upon the kidney.

8. A deficiency in Chloride may cause kidney damage, as described in a very recent report, which is, however, rather sketchy. Further information must be forthcoming before a final decision can be arrived at concerning the effect of a Chloride deficiency upon the kidney. Alkalosis which invariably occurs on a Low-Chloride diet, must be reckoned with in any appraisal of the situation.

## EXPERIMENTAL SECTION.

The following experiments were undertaken for the purpose of ascertaining whether or not the effects of overdosage with Desoxycorticosterone Acetate upon the kidney are due to a specific pharmacological action of this substance, or whether this action is mediated through the lowered Potassium concentration in the blood, brought about by the hormone. If this latter alternative holds, then the lowering of the Potassium concentration of the plasma by means other than the use of D.C.A., the feeding of a Low-Potassium diet, for example, ought to produce the same general pathological picture. The simplest manner in which this could possibly be done, is to autopsy the animals after having kept them on the diet for about a month's time, and then to weigh the kidneys and heart and use the extent of the enlargement of these organs as a measure of the degree of pathological involvement.

### Historical Introduction.

The effects of the endocrine glands upon the kidney and heart have been intensively studied only during the past few years. In 1939, Thomson (86) outlined some of the relationships between the studies of Urology and Endocrinology. Selye (87,88,89,90,91), Selye and Friedman (92), Pfeiffer et al. (93), Kochakian (94), Crabtree (95), Korenchevsky and Ross (96), Ludden, Kruger and Wright (97) and Welsh and co-workers (98) were among many workers who firmly established the concept of the renotropic action of various steroid substances, chief among them, the testoid hormones. Selye (99) reviewed the effects of deficiencies as well as of excesses of the various hormones produced by the

endocrine glands, upon the kidney. Another comprehensive review on this subject is that of Friedman (100). Further experimental work by Selye et al. (101,102) brought to light the renotropic action of an anterior lobe preparation, and also demonstrated that the simultaneous administration of Thyroxine would greatly augment this effect. In the experiments reported below, it was decided to employ Methyl Testosterone as a renotropic agent, in order to investigate the effects of Low-Potassium diets upon this action, common to most testoids (103).

Selye (185) produced experimental Nephrosclerosis by overdosage with the salt-active corticoid, Desoxycorticosterone Acetate. Subsequently, it was found that the simultaneous administration of Sodium Chloride would greatly sensitize the animals to the nephrosclerotic effect of D.C.A. It was also found that extirpation of one kidney would act as an additional sensitizing agent (104). It is for these reasons that all the D.C.A.-treated animals in the experiments which follow, were unilaterally nephrectomized and kept upon 1 % saline as drinking water. Further work by Selye and co-workers (105,106, 107,108,109) has contributed to a very great extent to our understanding of the mechanism of action of D.C.A. overdosage upon the kidney, heart and vascular system in general. As a result of all this work, the point of view has been adopted that the retention of Sodium is responsible for the changes seen in D.C.A. overdosage(184).

It has also been found by Selye and co-workers (110,102) that the administration of an anterior lobe extract to suitably sensitized animals (i.e., unilaterally nephrectomized and kept on 1 % saline), would produce Nephrosclerosis and the other symptoms of what Selye has termed the "Diseases of Adaptation." On the other hand, Selye and Rowley (111,112) discovered that the renotropic

steroid, Methyl Testosterone could prevent the Nephrosclerosis produced by D.C.A. overdosage. For this reason, all animals used in this series of experiments have been castrate males or females, since it was feared that the endogenous production of testoids by the male would interfere with the experimental results.

Durlacher, Darrow and Winternitz (26) studied the effects of D.C.A. as well as those of a Low-Potassium Diet upon the kidney, and concluded that the drug did not produce renal lesions per se, but acted through the medium of the Low Potassium concentration of the blood, which it produces. These authors also claimed that the administration of a 1 % solution of Potassium Chloride could completely prevent these manifestations of D.C.A. overdosage. In this latter claim, they were supported by the work of Darrow and Miller(113) and Darrow (27) who studied the effects of this substance upon the heart.

In this laboratory, however, it has been impossible to confirm the beneficial effect of Potassium salts upon the lesions produced by D.C.A. overdosage. Masson and Beland (114), working with mice, and Selye and co-workers (115,184) working with rats, could not detect any protective action at all. As it has been pointed out by these workers, the results of Darrow et al. (26) were simply based upon a compilation of data concerning renal size and weight, without the inclusion of a precise description of pathological changes. Also, it must be remembered that at that time (1942), the action of D.C.A. in producing Nephrosclerosis, cardiac hypertrophy and lesions, Periarteritis Nodosa and all the other changes characteristic of an overdosage with this substance, had as yet not been discovered by Selye and co-workers. The lesions of Potassium deficiency, as described by Follis et al. (24) and others, do not seem to be identical with those seen and described by Selye et al. (107,109).

## Experimental Diets.

The experimental Low-Potassium diets employed in these experiments were modifications of those used by Durlacher , Darrow and Winternitz (26). The Normal-Potassium Diet consisted of the same ingredients that went to make up the Low-Potassium ration, with the addition of 1 gm. of Potassium Chloride for every 100 gms. of diet. The Low-Potassium diet contained less than 1.5 meq. of Potassium per 100 gms. of diet, while the Normal-Potassium diet contained 22.5 meq. per 100 gms. The Purina diet used in some of these experiments contained 15.5 meq. per 100 gms. (Courtesy of Mrs. E.M. Sylvester, who kindly performed these analyses). The actual composition of the Low-Potassium diet follows :-

| | | | |
|---|---|---|---|
| Dextrin (Commercial White) | 35 gms. | Brewer's Yeast (Dried) | 4 gms. |
| Dextrose (Commercial) | 30 " | Salt Mixture | 4 " |
| Crisco (Vegetable Fat) | 10 " | Cod Liver Oil (Ayerst) | 1 " |
| Lactalbumin (Labco 15-42) | 16 " | α- Tocopherol (Synkavit) | 1 mg. |

The Salt Mixture employed, contained the following substances :-

| | | | |
|---|---|---|---|
| Sodium Chloride | 20 % | Ferric Citrate | 2 % |
| Calcium Carbonate | 35 % | Zinc Sulphate | 0.2 % |
| Calcium Triphosphate | 25 % | Manganese Sulphate | 0.1 % |
| Magnesium Sulphate | 15 % | Sodium Iodide | 0.1 % |

In the Potassium diet given to the control animals, 25 % Potassium Chloride was given in this Salt Mixture, mainly at the expense of the Sodium Chloride, which the animals were receiving in adequate amounts in their drinking water. The ingredients were uniformly mixed and rolled into pellets, which were fed to the animals.

T A B L E 1 .

| Treatment | Purina Diet | Normal Potassium Diet. | Low Potassium Diet. |
|---|---|---|---|
| No. of Animals | 12 | 5 | 13 |
| Average Final Body Weight. | 110 (64-144) | 88 (48-109) | 74 (56-90) |
| Average Kidney Weight. (2). | 1.278 (0.834-1.476) | 1.023 (0.812-1.246) | 1.282 (0.901-2.018) |
| Av. Ky. Wt. as % Av. Body Wt. | 1.16 (0.99-1.42) | 1.16 (1.04-1.69) | 1.73 (1.50-2.43) |
| Average Heart Weight. | 0.464 (0.321-0.578) | 0.414 (0.289-0.483) | 0.402 (0.310-0.571) |
| Av. Ht. Wt. as % Av. Body Wt. | 0.42 (0.29-0.53) | 0.47 | 0.54 |

Experiment No. 1 :- "Effect of a Diet Low in Potassium upon Renal Size and Structure."

Female rats, weighing between 50 and 100 gms. were divided into three groups. Group 1 was fed the Purina Fox Chow normally employed in this laboratory, and served as absolute controls. The second group received the Normal-Potassium Diet, while the third experimental group was maintained on the Low-Potassium ration. The animals were killed after thirty days, their kidneys and hearts fixed in Suza Fixative and in 10 % Formalin and weighed. They were sectioned, stained and examined histologically. The animals which died during the course of the experiment were not included in the tables presented here, which accounts for the difference in the number of animals considered in each group.

As can be seen from the accompanying table, (Table 1 ), an augmentation of kidney and heart weight occurs after the feeding of a diet low in Potassium. The animals receiving the Low-Potassium diet did not grow as well as did the others, and so comparison between actual renal and cardiac weights is unprofitable. If we examine the figures for the average kidney weight as percentage of the average final body weight, we find that whereas groups on Purina and on the synthetic Normal-Potassium diets are practically identical, there is a significant increase in the Low-Potassium group. We may also notice that very little overlapping occurs between the groups 1 and 2 on the one hand and group 3 on the other. The hearts are enlarged but not to the same striking degree.

The results obtained here are generally similar to those of Durlacher, Darrow and Winternitz (26), who worked with much older animals, and therefore, published data which did not show the full potentialities of the kidney-enlarging effect of Low-Potassium diets.

Histologically, it was found that the kidneys and hearts of the groups on Purina and the Normal-Potassium diets were essentially normal in appearance. In the third group, kidney necrosis was observed in one animal, while the kidney of another exhibited a granular surface, and two other rats showed hearts in which the beginning of myocardial infarction could be detected.

Experiment No. 2 :- "Effect of a Diet Low in Potassium upon Renal Size and Structure. II. Effect of Unilateral Nephrectomy and 1 % NaCl solution as Drinking Water."

The experimental procedure was identical to the one used in the preceding experiment, except that all the animals underwent unilateral nephrectomy at the beginning of the experimental period, and were kept on drinking water containing 1 % NaCl.

T A B L E 2 .

| Treatment | Purina Diet | Normal Potassium Diet | Low Potassium Diet. |
|---|---|---|---|
| No. of Animals | 3 | 15 | 16 |
| Average Final Body Weight. | 135 (114-163) | 102 (78-129) | 96 (45-139) |
| Average Kidney Weight. (1). | 1.132 (1.002-1.268) | 0.854 (0.658-1.032) | 1.149 (0.792-1.630) |
| Av. Ky. Wt. as % Av. Body Wt. | 0.85 (0.78-0,88) | 0.85 (0.68-1.12) | 1.24 (0.91-1.52) |
| Average Heart Weight. | 0.593 (0.493-0.696) | 0.482 (0.398-0.596) | 0.437 (0.317-0.550) |
| Av. Ht. Wt. as % Av. Body Wt. | 0.44 (0.42-0.46) | 0.47 (0.39-0.50) | 0.51 (0.33-0.62) |

Here, as in the first table, the kidney enlargement on a Low-Potassium diet is readily discerned, although there is a greater degree of overlap between the experimental groups. The hearts are also enlarged. The kidneys, as can be seen, all underwent a considerable degree of compensatory hypertrophy, although they did not quite attain the size which two kidneys would have in the normal, non-nephrectomized animal treated with the various methods shown here. It seems , then that the surgical intervantion of unilateral nephrectomy and the administration of saline did not exert any specific influences one way or another, in this experimental arrangement. This observation may perhaps serve to differentiate to some degree, the difference between animals kept on D.C.A. which respond to this treatment by increased sensitization, and the animals of the present series, kept on a Low-Potassium diet, which do not respond similarly.

Microscopically, the kidneys and hearts of the animals of the first two groups were normal, with one exception, in which renal damage was evident. In three rats on the Low-Potassium diet, evidences of Nephrosclerosis were noted. There was dilatation of the tubules in two other members of this group, while myocarditis was seen in several animals as well as signs of cardiac infection.

T A B L E 3 .

| Treatment | Purina Diet | Normal Potassium Diet. | Low Potassium Diet. |
|---|---|---|---|
| No. of Animals | 8 | 10 | 10 |
| Average Final Body Weight. | 109 (70-145) | 104 (75-135) | 110 (82-176) |
| Average Kidney Weight. (1). | 1.017 (0.698-1.348) | 1.016 (0.735-1.236) | 1.341 (0.974-2.152) |
| Av. Ky. Wt. as % Av. Body Wt. | 1.06 (0.79-1.26) | 0.99 (0.88-1.16) | 1.23 (1.13-1.38) |
| Av. Heart Weight | 0.517 (0.335-0.623) | 0.510 (0.362-0.640) | 0.526 (0.385-0.721) |
| Av. Ht. Wt. as % Body Wt. | 0.47 (0.41-0.53) | 0.49 (0.40-0.59) | 0.50 (0.46-0.52) |

Experiment No. 3 :- "Effect of a Diet Low in Potassium upon Renal Size and Structure. III. Effect of Administration of Desoxycorticosterone Acetate in Unilaterally Nephrectomized Rats on 1 % NaCl given as Drinking Water."

The experimental procedure employed was identical to that employed in Experiment No.2, above, in addition to which each animal was implanted sub-

cutaneously with a 40 mg. pellet of D.C.A. at the commencement of the experimental period. As can be seen from Table 3, the enlargement of the kidney in animals on the Low-Potassium diet is once again evident. The heart, however, enlarged by the D.C.A. treatment alone, does not exhibit an increased hypertrophy as a result of the Low-Potassium regimen. This might inddicate that the low plasma Potassium was responsible for this effect. Such an interpretation, however, cannot be valid for the kidneys, since these organs do apparently get larger as a result of the two stimuli, the D.C.A. and the deficient diet, than they do after D.C.A. treatment alone. However, there is some overlap between the values in the two groups and no conclusions can be drawn.

Microscopically, four animals in the group, receiving the Normal-Potassium diet showed cardiac damage, usually involving only the myocardium. Hydronephrosis was seen in one animal in this group, while another showed traces of renal lesions. In the group on the Low-Potassium diet, four animals showed myocardial lesions, accompanied by evidences of Nephrosclerosis. An additional animal showed lesions in the renal pelvis.

As has already been mentioned in the historical introduction to these experiments, Selye and co-workers were able to elicit a number of characteristic pathological maifestations in the dog, monkey, rats and other animal species by D.C.A. overdosage. The lack of widespread lesions in these animals, treated with D.C.A. for one month, is therefore very puzzling. A clue to the solution of this problem may be found in the analysis of the experimental diet, which contained as much as 65 % carbohydrate, while the ordinary Purina diet used in this laboratory in the D.C.A.-overdosage experiments contained only a maximum of 45 % of this type of foodstuff. Recently, there have been indications that carbohydrate inhibits the production of Nephrosclerosis

and other symptoms of carbohydrate overdosage. Thus it is quite possible that the low incidence of these types of lesions in the animals in the present experimental series, may be attributed to the high carbohydrate content of the diet.

Experiment No. 4 :- "Effect of a Diet Low in Potassium upon Renal Size and Structure. IV. Effect of Administration of Methyl Testosterone in Unilaterally Nephrectomized Rats on 1 % NaCl as Drinking Water."

The procedure which was followed here was quite identical to that of the previous experiment, which the exception of the subcutaneous implantation of a 50 mg. pellet of Methyl Testosterone instead of the D.C.A.

T A B L E 4 .

| Treatment | Purina Diet | Normal Potassium Diet. | Low Potassium Diet. |
|---|---|---|---|
| No. of Animals | 4 | 15 | 10 |
| Average Final Body Weight. | 96 (75-106) | 86 (57-120) | 90 (60-136) |
| Average Kidney Weight. (1). | 0.870 (0.781-0.918) | 0.889 (0.675-1.204) | 1.306 (1.031-1.712) |
| Av. Ky. Wt. as % Av. Body Wt. | 0.92 (0.82-1.04) | 1.05 (0.88-1.66) | 1.49 (1.05-1.93) |
| Average Heart Weight. | 0.415 (0.322-0.453) | 0.402 (0.312-0.571) | 0.444 (0.377-0.619) |
| Av. Ht. Wt. as % Av. Body Wt. | 0.43 (0.41-0.46) | 0.47 (0.41-0.56) | 0.52 (0.37-0.64) |

A glance at Table 4 reveals first of all, the renotropic action of Methyl Testosterone, when compared to the results of Table 2. The most surprising point, however, is the large size of the kidneys in the Low-Potassium group. The heart, which is not stimulated by renotropic agents, does not undergo any changes other than the hypertrophy occasioned by the Low-Potassium rations.

Histological examination yielded the following results :-
Kidneys and hearts of Purina-fed animals were quite normal, the former showing some evidence of renotropic stimulation. One animal possessed an accumulation of colloid droplets in the convoluted tubules of the kidney. These colloid droplets were also seen in kidneys of two animals on the Normal-Potassium diet. Renal infarcts were demonstrable in two other animals of this group. Two rats on the Potassium-deficient diet showed a Lipid Nephrosis accompanied by a degree of nephrosclerotic change and hypertrophy. In addition, two other animals exhibited Nephrosclerosis in this group. Tubular dilatation was seen in a fifth. Foci of myocarditis were seen in four animals of this group.

Since Selye and Rowley (111,112) have shown that the administration of Methyl Testosterone prevents D.C.A.-induced Nephrosclerosis, it would seem quite logical to expect the same action in the case of the lesions of Low-K diets, that is, if we assume that the viewpoint of Durlacher et al. (26) is correct. However, as has been pointed out above, the incidence of renal lesions in this entire series of experiments seems to be highest in the groups receiving the Normal-Potassium and Low-Potassium diets and implanted with Methyl Testosterone.

During the course of this entire series of experiments, blood was taken from most of the animals for chemical determinations. Determination of Sodium were carried out according to the method of McCance and Shipp (116), Potassium

determinations according to Jacob and Hoffman's (117) modifications of the method of Kramer and Tisdall (118) and Chlorides according to Van Slyke (119). (The author wishes to thank Mrs. E.M. Sylvester most sincerely, for the chemical determinations which she carried out and which are presented below.)

T A B L E 5 .

| Blood Constituents. | Serum Na. No. | Meq. | Serum Cl. No. | Meq. | Na/Cl No. | | Serum K No. | Meq. |
|---|---|---|---|---|---|---|---|---|
| Purina. 1 % NaCl. | 3 | 136 | 3 | 94 | 3 | 1.44 | 3 | 5.68 |
| Normal-K. 1 % NaCl. | 8 | 139 | 7 | 90 | 7 | 1.54 | 7 | 5.66 |
| Low-K. 1 % NaCl. | 9 | 136 | 7 | 85 | 7 | 1.62 | 7 | 3.23 |
| Purina. 1 % NaCl. Methyl Testosterone. | 4 | 137 | 4 | 94 | 4 | 1.45 | 4 | 5.24 |
| Normal-K. 1 % NaCl. Methyl Testosterone. | 11 | 138 | 11 | 91 | 11 | 1.53 | 11 | 5.32 |
| Low-K. 1 % NaCl. Methyl Testosterone. | 6 | 134 | 6 | 87 | 6 | 1.54 | 6 | 3.13 |
| Purina. 1 % NaCl. D. C. A. | 12 | 148 | 12 | 86 | 12 | 1.72 | 12 | 3.08 |
| Normal-K. 1 % NaCl. D. C. A. | 6 | 163 | 6 | 85 | 6 | 1.90 | 6 | 4.40 |
| Low-K. 1 % NaCl. D. C. A. | 1 | 155 | 1 | 75 | 1 | 2.07 | 1 | 2.15 |

The lowering of the Potassium concentration is quite comparable in the three groups. The results obtained by Selye, Rowley and Hall (120) regarding the inactivity of Methyl Testosterone upon the level of the plasma Potassium has been confirmed. The hypochloremic effect of D.C.A. manifests itself to some extent as well. In short, the changes in the blood chemistry brought about

by the feeding of Low-Potassium diets are very similar to those produced by prolonged treatment with large doses of Desoxycorticosterone Acetate.

## Summary and Conclusions.

It is very difficult to draw many conclusions from the foregoing set of experiments as mortality among the experimental animals was very high, and individual variation was very great. Nevertheless, the following points may be noted :-

1. An increase in both renal and cardiac weight occurs after the prolonged feeding of a diet low in Potassium. This hypertrophy is of a pathological nature, and is often accompanied by lesions in the kidney tubules and myocardium.

2. Partial nephrectomy and drinking water containing 1 % NaCl does not seem to sensitize the animals to the injurious effects of Low-Potassium diets.

3. D.C.A. and Low-Potassium diets, if given simultaneously will augment the renal hypertrophy somewhat,beyond the usual hypertrophy in animals getting either of these treatment alone. The heart size seems to depend upon the Potassium level of the blood; a low K concentration producing cardiac hypertrophy.

4. The renotropic action of Methyl Testosterone is manifested in animals receiving the Low-Potassium diet, the two kidney enlarging stimuli being additive.

5. Methyl Testosterone does not protect the rat from the heart and kidney lesions brought about by an inadequate intake of Potassium in the diet.

6. The changes in blood chemistry produced by the feeding of a Potassium-deficient diet are similar to those seen in D.C.A.-treated animals.

## Pyloric Obstruction.

If we wished to produce an experimental deficiency in Chloride in an animal, the approach of the nutritionist would obviously be to place the animal on a Chloride-deficient diet, in order to deplete its endogenous stores of this mineral substance. As we have seen, this is usually a long and tedious procedure, and does not yield conclusive results in the end. It therefore occurred to us, that in order to produce a rapid and acute loss of Chloride from the body, we could use the drastic method of surgical intervention.

It is a well-known fact that in cases of clinical pyloric or high intestinal obstruction, the body is rapidly depleted of its stores of Chloride. An alkalosis sets in, as the $CO_2$-combining power of the blood rises. The Chloride is lost to the organism by secretion into the gastric juice, largely as H Cl. Similaf conditions obtain in excessive vomiting and gastric tetany. A very readable account of the theoretical background of these phenomena may be found in the monograph by Gamble (121).

The clinical literature is replete with papers dealing with the various effects of pyloric obstruction upon the other organs of the body, upon the blood chemistry and upon the organism as a whole. Most of the pathological changes which occur as a result of this condition may be encountered in the kidney, and so fall within the scope of this study. It is believed by many that these renal lesions are secondary to the disturbances in the normal chemical composition of the blood. One of the characteristic changes whihh occur in the blood picture following pyloric or high intestinal obstruction is a marked lowering in the concentration of Chloride ion. The performance

of a pyloric ligation in rats therefore ought to produce a Chloride deficiency. We may now proceed to a discussion of the historical developement of this phenomenon, which since it produces a Chloride deficiency, may rightly be placed immediately following "effects of Chloride deficiency upon the kidney" in this review.

Nazari (122) in 1904, observed a definite pathological change in the kidneys of two cases of gastric tetany. He noted calcareous deposits in the convoluted tubules and in the ascending loop of Henle. In experimental pyloric obstruction in the dog, Hartwell, Hoguet and Beekman (123,124) noted a degeneration of the tubular epithelium. South and Hardt (125) observed a cloudy swelling in the kidney, though Draper (126) and Stone et al. (127) found kidneys which were histologically quite normal, in the course of their studies on numerous clinical cases of pyloric obstruction.

A decided impairment of kidney function as measured by the standard tests, following pyloric obstruction, was claimed by McQuarrie and Whipple (128), while Tileston and Comfort (129) found a rapid increase in the non-protein nitrogen concentration in the blood after the developement of this condition. Cooke, Rodenbaugh and Whipple (130) confirmed this work in their experiments on dogs, where they observed a 300 % increase in the N.P.N. above normal. Louria (131), in a study of seven clinical cases, reported high blood Urea figures.

In six cases of severe pyloric and duodenal obstruction which terminated fatally, Brown and co-workers (132) found evidence of severe toxic nephritis. Degenerative changes occurred in the tubular epithelium while granular and fatty degeneration were in evidence throughout the renal parenchyme.

Zeman, Friedman and Mann (133) presented a detailed pathological description of kidney lesions found in four clinical cases of pyloric obstruction. They pointed out the similarity of these lesions to those seen in poisoning with Mercuric Chloride (corrosive sublimate). "Degeneration of the cells lining the spiral and terminal straight portions of the proximal convoluted tubules " were reported. Pyloric obstruction was performed on a series of cats was performed by the same workers (133), and necrosis followed by calcification was seen. Microscopically, they observed a diffuse degeneration of the renal tubules, starting in the terminal portions and ascending to those portions of the tubules adjacent to the glomeruli.

Fox and co-workers (134) found symptoms of acute nephritis following obstruction of the small intestine. Others reported cases of renal damage after pyloric or high intestinal obstruction usually accompanied by a hypochloremia (135,136,137,138,139,140,141,142). The above workers attributed these renal lesions, which were marked by calcification, mainly to the hypochloremia and to the resulting alkalosis. Perez-Castro (141) found that the administration of Sodium Chloride would prevent the deposition of Calcium in the renal parenchyme. This action was attributed to the replacement of the lost Chloride.

An excellent review of the whole question may be found in the clinical report of Martz (143), wherein three cases of persistent vomiting associated with pyloric obstruction were presented. Extensive peritubular deposits of Calcium in the vicinity of the convoluted tubules were seen. The mineral was located immediately beneath the tubular epithelium, between the Tunica Propria and the epithelial cells of the tubules, but not in the capillaries. The

pathogenesis of the kidney calcification is discussed. The author (143) feels that the severe alkalosis produced by the loss of Chloride ion after pyloric obstruction is the direct cause. The alkalinity of the blood reduced the solubility of the Calcium ions. In order to conserve fixed base, the kidney kept on secreting a strongly acid urine. This made the cell fluids of the kidney even more alkaline than they had been before and brought about the precipitation of the blood Calcium in the renal parenchyme. This interpretation was supported by the work of many experimental and clinical studies (144,145,146,147,148,149,150,151). Recently, McLetchie (152) noted regenerative as well as degenerative processes going on in kidneys after pyloric obstruction. These alterations were confined to the ascending portion of Henle's loop and the distal convoluted tubules. The most striking histological phenomenon, however, was the formation of communications between the tubules and adjacent venules. The author believes that there may be a special physiological reason for these communications, relating to the function of the kidney under these special pathological conditions.

The actual cause of death following pyloric or high intestinal obstruction has been the goal of many clinical and experimental studies. Among the factors which have been implicated are the adrenal cortex and the high Potassium level of the blood. Since the salt-active, Desoxycorticosterone-like hormones of the adrenal cortex regulate the level of the blood Potassium, these two factors are obviously interrelated, and will be dealt with here.

The Adrenal Cortex.

The evidence for the involvement of the adrenal cortex in the syndrome produced by pyloric or high intestinal obstruction falls under two main headings:-

there are those conditions in which actual morphological changes occur in the adrenal cortex and there are the functional manifestations of adrenal cortical involvement, such as the occurrence of hyperpotassemia and hypochloremia, as well as the pronounced rise in the concentration of non-protein-nitrogen in the blood, all of which would serve to indicate a deficiency of the secretions of this endocrine gland.

In 1936, Wohl, Burns and Clark (153) performed a series of experiments in order to determine whether there were any morphological changes in the adrenal cortex after pyloric obstruction. Twelve dogs were operated upon, and blood chemistry studies were made. Histologically, "the Zona Glomerulosa was well demarcated from the Zona Fasciculata: the former presented a 'moth-eaten'appearance with loss of cell contours and shrunken nuclei, while the latter had a granular cytoplasm apparently devoid of lipid material." Saline treatment inhibited the lipid loss normally occasioned by the operation.

Next, Wohl, Burns and Pfeiffer (154) administered adrenal cortical extract to dogs after experimental high intestinal obstruction in attempt to alleviate the symptoms of that condition. They demonstrated two facts. First of all they demonstrated that there were many features in common between acute obstruction and adrenal insufficiency; secondly, they showed that the combined administration of physiological saline and cortical extract counteracted the toxemia of high intestinal obstruction. Unfortunately, a detailed report of their autopsy findings is lacking.

The problem of the rôle of the hyperpotassemia of pyloric obstruction in bringing about death, was discussed in detail by Scudder, Zwemer and Truszkowski (155). They felt that "the Potassium rise is ascribed to some combination of dehydration, tissue breakdown, to action of bacterial toxins, with consequent adrenal and renal dysfunction resulting in inadequate Pot-

assium elimination." They explain the beneficial action of Sodium Chloride administration, observed by numerous workers (153,156,157,158,159), by the lowering of the blood Potassium which it effects, by means of diluting the blood and by increasing the excretion of Potassium salts by the kidney. The clinical results of Scudder, Zwemer and Whipple (160), covering a multitude of cases, argue for the identification of the high blood Potassium level with the lethal agent in pyloric and high intestinal obstruction. Cutler and Pijoan (161) and Knight and Slome (162) subscribed to this interpretation as well. Recently, however, a large group of investigators (163,164,165,166) have presented convincing evidence that the hyperpotassemia cannot possibly be considered as the toxic agent in obstruction of the pylorus of duodenum. Besser (167) reviews the confusing literature on this topic up to the year 1940, pointing out the various factors which may be involved in causation of death following obstruction.

Among the more recent literature on the subject, is a report by O'Donovan and Murphy (168) on two cases of what they termed "extrarenal uremia". resulting from pyloric obstruction. They defined this condition as "a clinical state characterized by elevation of the blood nitrogen, dehydration due to loss of extracellular fluid and electrolytes, normal or low blood pressure, and oliguria which may progress to anuria." They recommended subcutaneous or intravenous infusions of Sodium Chloride. Noth and Wilbur (169) had pointed out the beneficial action of NaCl in the treatment of the chemical changes occurring in the blood after pyloric obstruction several years previously.

Heuer and Andrus (170) injected an aqueous extract of high intestinal loops, which proved fatal after some time to the experimental animals, dogs

in this case. These aqueous extracts presumably contained the toxin postulated by several workers in this field as the actual lethal agent. The injection of adrenal cortical extract greatly prolonged the lives of these animals. A blood transfusion, administered together with the hormonal extract, enhanced this life-prolonging effect.

Very recently, Tuerkischer and Wertheimer (171), studying the effect of adrenalectomy upon gastric secretion, found that the secretion of gastric juice greatly diminished in this condition. The gastric secretions were collected from stomachs which had been tied off at the pylorus according to the method of Roe and Dyer (172). Neither Desoxycorticosterone Acetate nor Sodium Chloride alone, could restore the normal secretory activity of the gastric mucosa. Adrenal cortex extract, containing the sugar-active substances as well as the salt-active ones, was effective in restoring the normal gastric secretion in these adrenalectomized rats. These experiments, therefore, point to a possible relationship between the gastric mucosa and the sugar-active, Corticosterone-like hormones of the adrenal cortex. Of what importance this may prove to be in the event of chemical changes in the blood resulting from pyloric or high intestinal obstruction, we cannot as yet say.

Desoxycorticosterone Acetate.

The effects of Desoxycorticosterone upon electrolyte metabolism are fully discussed in the recent review by Swingle and Remington (173). For the purposes of this discussion on pyloric obstruction and the chemical changes which this condition brings about in the blood, it is sufficient to stress three main properties of this steroid :-

1. It depresses the blood Potassium to a marked degree.

2. It also lowers the blood Urea level to a great extent.

3. It increases the volume of the plasma.

The latter effect of Desoxycorticosterone has been well illustrated by the experiments of Fine, Fuchs and Mark (174) who delayed the marked fall in plasma volume after the experimental distention of the small intestine, by the intravenous administration of this substance.

In this laboratory, the effects of Desoxycorticosterone Acetate upon the blood chemistry has been studied in various experimental animals, especially the rat. As the results obtained from these studies have a direct bearing upon the problem of pyloric obstruction, they will now be considered.

In 1940, Selye (175) showed that pre-treatment of mice and rats with Desoxycorticosterone Acetate prolongs the life of the animal after bilateral nephrectomy. In a more detailed report, Selye and Nielsen (176) demonstrated that the administration of this steroid would reduce the non-protein nitrogen of the blood from an average of 204 mgs.% to an average of only 170 mgs.%, in animals which had been bilaterally nephrectomized and which were bled for chemical blood determinations thirty-two hours post-operatively. Dosne (177) investigated the optimum conditions under which this anti-uremic effect of D.C.A. is manifest.

In an experiment on the effects of overdosage with Desoxycorticosterone Acetate on the intact rat, Selye and Dosne (178) found a striking decline in blood and serum chlorides. This hypochloremia was found to be due mainly to a decrease in chloride of the erythrocytes (179). In the hypophysectomized rat, Selye and Bassett (180) found no significant decrease in blood chlorides

after the administration of large amounts of D.C.A., though a marked diuresis, accompanied by an increased excretion of chlorides was provoked. Selye and Dosne (181,182) also studied the effects of other hormones administered together with D.C.A. upon the blood chloride picture. The hypochloremic effect of large doses of this agent have recently been confirmed once more in this laboratory (183,184). In the latter study (184), it was noted that the administration of Sodium Chloride along with the D.C.A., instead of raising the level of the blood and plasma chloride concentration, actually lowers it still further, so that the lowest blood chlorides were obtained in the animals of this experimental group. It is interesting, but rather puzzling to learn that this hypochloremic effect does not obtain in the chick (185), which is so very sensitive to the Nephrosclerosis-producing action of this salt-active adrenal hormone. In a paper recently published by Cluxton et al. (186), mention is made of the fact that blood chlorides tend to be low in patients suffering from Cushing's Disease, which is believed to be caused by an overproduction of adrenal cortical hormones (187).

## EXPERIMENTAL SECTION.

### Introduction.

In the following experiments, constant use has been made of the surgical procedure of pyloric or high intestinal obstruction. The albino rat was the ahimal employed in all experiments described here. A description of the operative technique is now given.

### Technique of Pyloric or High Intestinak Obstruction.

The experimental animals were kept on a diet of Purina Fox Chow (Ralston Purina Co.,Woodstock,Ont.) and tap water. They were fasted for a period of about twelve hours immediately prior to the operation. In many cases, the stomach still contained some food at the time of operation, however.

The operation was carried out under Ether anesthesia, boiled instruments being employed. The abdomen was swabbed with alcohol, and a ventral mid-line incision made, extending caudally from the area of the Xiphoid Process for almost two centimeters. By means of gentle pressure exerted by the fingers on either side of the incision, the stomach was forced out through the opening. It was held between the fingers, care being taken not to touch it with any metal instrument, and a ligature of heavy black sewing thread passed around the duodenum about 0.5 cms. distal to the pylorus, care being taken not to damage the gastro-duodenal artery and its many branches. The ligature was then gently tightened so as to completely occlude the lumen of the duodenum.

During the first attempt to produce the symptoms of intestinal or pyloric obstruction, it was found that post-operatively, the gastric contents tended to spill over into the esophagus as the stomach filled up with its own secretions. This regurgitation of gastric juice produced peptic hemorrhagic esophagitis.

In order to remedy this situation, it was decided to employ the same procedure as Selye (188) had used in his study concerning the experimental production of peptic hemorrhagic esophagitis; to occlude the cardiac sphincter as well as the pylorus. This was accomplished by placing a ligature of heavy black sewing thread around the esophageal-cardiac junction, care being taken not to ligate any arteries and veins in the vicinity. The ligature was tightened until the esophageal lumen was completely occluded at the cardia. The stomach was then replaced in the abdominal cavity and the incision sewed up with individual sutures. About three or four stitches were required. A slight amount of bleeding occurred during the course of stomach manipulation. This was checked by the application of a small wad of absorbent cotton to the gastric surface.

Post-operative Treatment.

Following the operation, the animals were placed in a cage without access to either food or water. As the animal could not swallow its own saliva, due to the ligation of the cardia, this tended to accumulate around the mouth and nose, thereby hindering breathing. Small wads of absorbent cotton rolled around match-sticks were inserted between the teeth into the oral cavity and the accumulations of saliva removed from time to time.

Post-operative Observations.

The animals were quite alert after awakening from anesthesia. As time went on, however, they grew much more quiescent, and sat hunched up in a corner of the cage. The nose and whiskers became caked with dried blood. The animals became cold; the drop in body temperature was quite apparent upon handling. Just prior to death, some of them went into clonic convulsions.

Autopsy Findings.

In some experiments, blood was taken by severing the jugular vein at the time of killing. Determinations of Sodium were carried out according to the method of McCance and Shipp (116), Potassium determinations according to Jacobs and Hoffman (117), Chlorides according to van Slyke (119), N.P.N.s according to Folin and Wu (189) and Hemoglobin according to the photo-electric method of Evelyn (190). (The author of this study is exceedingly grateful to Mrs. E.M. Sylvester who carried out the chemical determinations reported in this section.)

At autopsy, the stomach presented a bloated appearance, being full of fluid visible through the semi-transparent mucosa, which was greatly distended. The intestines, liver, spleen, heart and kidneys were macroscopically, quite normal in appearance.

In cases in which the stomach contents were taken for analysis, the stomach was removed by sectioning of the duodenum below the pyloric ligature and of the esophagus above the cardiac ligature. The stomach was then punctured so as to allow its contents to run through a funnel into a test-tube.

Experiment No. 5 :- "Effect of Simultaneous Bilateral Nephrectomy upon the Length of Survival of the Rat following Pyloric Obstruction."

It is a well-known fact that following bilateral or total nephrectomy. animals will rapidly develope uremia and die shortly after. From the work of Selye (175), Durlacher and Darrow (191) and others, we know that the 100 gm. rat will survive about 60-70 hours on the average, after the removal of both kidneys. Just as in the case of pyloric obstruction, many theories have been advanced to explain the cause of death. Some of the factors which have been mentioned as possible lethal agents are a high blood Potassium, a high N.P.M. bringing about an Azotemia, and other chemical events which also figure very prominently, as we have already seen, in attempts to explain the cause of death following pyloric or high intestinal obstruction. Hence we decided to examine the effect of bilateral nephrectomy upon the length of survival following pyloric obstruction.

Two groups, each consisting of six rats, weighing between 100 and 150 grams, of the female sex, were employed. Pyloric obstruction was performed upon the members of both groups, the time of operation being noted. In addition to this treatment, the animals in the second group underwent a total nephrectomy at the same time.

The time of death of the individual experimental animals was recorded, with an accuracy of plus or minus one hour, except in the event of several rats which died during the course of the night, in which case average values equal to one-half the time between the last observation on the evening prior to death and the first observation in the morning were recorded. The results obtained are presented in Table 6, which follows.

TABLE 6.

| Number | | 1 | 2 | 3 | 4 | 5 | 6 | Average |
|---|---|---|---|---|---|---|---|---|
| Group 1 | Body Wt. | 98 | 116 | 104 | 113 | 106 | 102 | 106.5 |
| Pyloric Obstruction | Survival Time | 42 | 42 | 40 | 60 | 60 | 36 | 46.9 |
| Group 2 | Body Wt. | 104 | 108 | 103 | 102 | 103 | 116 | 106.0 |
| Pyl. Obstr. & Ky. exp.t. | Survival Time | 24 | 28 | 25 | 34 | 28 | 29 | 28.1 |

There is thus a difference of 18.8 hours between the groups. It is therefore concluded, as a result of this experiment, that simultaneous pyloric obstruction and bilateral nephrectomy shortens the life of the rat to a significant degree, as compared with the survival time following pyloric obstruction alone.

Experiment No. 6 :- "Effect of Desoxycorticosterone Acetate upon the Survival of the Rat following Pyloric Obstruction."

As mentioned above (175,176), the administration of Desoxycorticosterone Acetate prolongs the life of the rat following total nephrectomy. Since the changes in the blood chemistry after pyloric or high intestinal obstruction and those occurring in bilaterally nephrectomized rats are similar, it was considered interesting to determine the effects of pre-treatment with D.C.A. upon the survival time after pyloric ligation.

Fourteen female rats, weighing about 150 gms. on the average, were divided into two groups of seven animals each. The first group, the controls, remained untreated, while each animal in the second group was implanted with

T A B L E 7.

| R A T S | | A | B | C | D | E | F | G | H | I | J | K | L | M | N | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group 1 | Body Wt. | 202 | 182 | 191 | 210 | 205 | 197 | 188 | 150 | 159 | 164 | 130 | 149 | 142 | 165 | 152.2 |
| Pyloric Obstruction | Surv. Time | 46 | 54 | 59 | 52 | 67 | --- | 43 | 67 | --- | 55 | 67 | 54 | 66 | 68 | 58.3 |
| Group 2 | Body Wt. | 211 | 190 | 209 | 162 | 203 | 162 | 185 | 179 | 200 | 198 | 187 | 165 | 170 | 198 | 150.6 |
| Pyl. Ob. &DCA. | Survival Time | 71 | 83 | 82 | 82 | 68 | 75 | 76 | 80 | 80 | --- | 75 | 79 | 79 | 79 | 77.9 * |

a 40 mg. pellet of Desoxycorticosterone Acetate, subcutaneously, in the region of the shoulder blades of the rat.

After two weeks of this treatment, when the average weight of the rats had reached about 200 gms., they all underwent a pyloric obstruction, in the manner outlined above, the time of operation being noted. The time of survival of these animals is presented in Table 7.

Eighty hours post-operatively, there still remained seven of the animals in the D.C.A.-treated groups, all the others having expired previously. These animals were killed, and the experiment terminated. The time of survival of these seven rats is therefore given in the table as 80-83 hours. It can therefore be seen that the average value for the survival time of the animals treated with the hormone was really greater than the recorded figure, since seven of the survivors were executed, instead of being allowed to live until death resulted from pyloric obstruction. Nevertheless, as can be seen from Table 7, there is a difference of 19.5 hours between the two groups, even under these unfavorable conditions. It may therefore be concluded that pre-treatment with D.C.A. prolongs the life of the rat following pyloric or high intestinal obstruction.

Experiment No. 7 :- "Effect of Desoxycorticosterone Acetate upon the Blood Chemistry of the Rat after Pyloric Obstruction."

The work of Selye and Nielsen (176) has shown that pre-treatment with D.C.A. would inhibit the rise in N.P.N. after bilateral nephrectomy. Since pyloric obstruction produces a blood picture similar in many respect to that seen after bilateral nephrectomy, the effect of D.C.A. pre-treatment ought to be quite instructive. All animals were killed thirty hours post-operatively.

T A B L E 8.

| Blood Constituents | Serum Na No. | Serum Na Meq | Blood Cl No. | Blood Cl Meq | Serum Cl No. | Serum Cl Meq | Na/Cl No. | Na/Cl | Serum K No. | Serum K Meq | N.P.N. No. | N.P.N. Mgs | Hb. No. | Hb. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Controls. | 23 | 140 | 18 | 68 | 23 | 90 | 18 | 1.58 | 21 | 4.55 | 17 | 56.5 | 12 | 14.7 |
| D.C.A. | 23 | 141 | 17 | 61 | 23 | 81 | 17 | 1.73 | 18 | 2.81 | 17 | 50.5 | -- | ---- |
| Controls. Pyl. Obs. | 26 | 144 | 12 | 58 | 15 | 85 | 11 | 1.69 | 10 | 7.76 | 13 | 83.4 | 13 | 16.5 |
| D.C.A. Pyl. Obs. | 16 | 147 | 12 | 56 | 16 | 79 | 12 | 1.86 | 10 | 5.48 | 12 | 71.2 | 13 | 15.2 |

The above data have been collected from many different animals at varying times, the number of animals involved for each constituent being noted.

The well-known Potassium-lowering effect of D.C.A. is very well illustrated here. In animals suffering from pyloric obstruction, the serum Potassium rises precipitously, and as has already been mentioned in the review, is considered by some (155,160,161,162) as the main contributing factor to the cause of death. The effect of D.C.A. in prolonging life after obstruction, which has been demonstrated earlier (experiment No. 6), may therefore, at least partly, be ascribed to the lowering of the blood Potassium.

The hypochloremic effect of D.C.A. is also illustrated in the above table. The values for the Hemoglobin show that the hormone at least partly offset the dehydration and loss of blood volume which accompanied pyloric obstruction. The non-protein nitrogen of the blood, however, high after pyloric obstruction, doesn't seem to have been reduced to a very great extent by the pre-treatment with D.C.A.

Experiment No. 8 :- "Effect of Desoxycorticosterone Acetate upon the Blood Chemistry of the Rat after Bilateral Nephrectomy."

After having studied the effects of D.C.A. upon the blood picture after pyloric obstruction, it was decided to observe the changes occurring after total nephrectomy in the animals treated with this steroid.

Twelve female rats, weighing about 200 gms. on the average, were divided into two groups. Group 1 remained untreated in order to act as a control. The animals of Group 2 were each implanted subcutaneously with a 40 mg. pellet of D.C.A. After a two-week period of treatment, all the animals in both experimental groups were bilaterally nephrectomized, the time of operation being noted. Thirty hours post-operatively, the animals were bled to death, the blood being taken for chemical determinations. At this time, all the animals were uremic, and it was therefore expected that any chemical changes occurring in the blood after complete nephrectomy, would manifest themselves in that period of time. The results are presented in Table 9, the number of animals used for each determination being noted.

As can readily be observed, these results do not agree with those of Selye and Nielsen (176) in so far as the lowering of the N.P.N. is concerned. Experimental conditions, it must be recalled, were quite different; smaller animals having been employed and larger doses having been administered, and by injection.

It will be noticed that the effect of Desoxycorticosterone Acetate is especially marked upon the concentration of the serum Potassium, where there is a drop of 4.25 meq. in the D.C.A.-treated, bilaterally nephrectomized animals as compared with the controls. Whereas the hypochloremic effect of D.C.A

is marked in the control groups, there is no such effect apparent in the totally nephrectomixed animals. Whether this absence of the hypochloremic effect is in some way related to the already low blood and serum chlorides seen in nephrectomized rats, or is independent of it, remains to be clarified. It is quite possible that the inability of the animal to excrete chloride in the urine after nephrectomy raises this constituent in the blood.

However, the conclusion seems to be that the effect of D.C.A. in lowering the plasma Potassium is one of the ways in which this salt-active hormone prolongs life in the nephrectomized rat.

Hoff, Smith and Winkler (192) maintained that a fatal rise in Potassium concentration was unlikely in clinical uremia. Nevertheless, the work of Durlacher and Darrow (191) showed that the administration of D.C.A. delays the rise in serum Potassium to toxic levels in the totally nephrectomized rat, and also demonstrated that both D.C.A. as well as the simultaneous ingestion of diets low in Potassium prolonged survival time.

TABLE 9.

| Blood Constituents | Serum Na | | Blood Cl | | Serum Cl | | Na/Cl | | Serum K | | N.P.N. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. | Meq | No. | Meq | No. | Meq | No. | | No. | Meq | No. | Mgs. |
| Controls. | 5 | 143 | 6 | 73 | 6 | 91 | 6 | 1.55 | 6 | 4.59 | 6 | 46.8 |
| D.C.A. | 6 | 140 | 6 | 65 | 5 | 79 | 5 | 1.77 | 6 | 2.60 | 6 | 46.1 |
| Controls. Ky. Exp. t. | 6 | 130 | 6 | 62 | 6 | 75 | 6 | 1.75 | 5 | 10.72 | 2 | 235 |
| D.C.A. Ky. Exp. t. | 5 | 142 | 5 | 65 | 5 | 77 | 5 | 1.85 | 5 | 6.20 | 5 | 232 |

In this connection, the recent report of Rodbard (193) ought to be mentioned. In experiments on the dog, he showed that the administration of D.C.A. for some time before operation, increased the survival time of this animal from an average of 85 hours to an average of 143 hours after double nephrectomy. However, he contends that the D.C.A. did not affect the rate of accumulation of Potassium in the plasma of the bilaterally nephrectomized dogs.

Experiment No. 9 :- "Effect of Desoxycorticosterone Acetate upon the Blood Chemistry of the Rat after Pyloric Obstruction. II. Influence of 1 % NaCl Solution as Drinking Water.

As has previously been mentioned in the review section of the thesis, there seems to be evidence that the administration of Sodium Chloride to animals suffering from pyloric obstruction may be of benefit in prolonging life[159]. Therefore, an experiment was planned which differed from Experiment No. 7 only in that the animals were given 1 % NaCl solution as drinking water, instead of the usual tap water. The results follow in Table 10.

T A B L E 1 0 .

| Blood Constituents. | Serum Na No. | Serum Na Meq | Serum Cl No. | Serum Cl Meq | Na/Cl No. | Na/Cl | Serum K No. | Serum K Meq. |
|---|---|---|---|---|---|---|---|---|
| Controls. | 6 | 143 | 6 | 99 | 6 | 1.44 | 6 | 4.54 |
| D. C. A. | 6 | 143 | 6 | 93 | 6 | 1.54 | 6 | 3.67 |
| Controls. Pyloric Obstruction. | 6 | 147 | 6 | 81 | 6 | 1.82 | 4 | 4.58 |
| D. C. A. Pyloric Obstruction. | 6 | 152 | 6 | 84 | 6 | 1.81 | 4 | 5.02 |

It will be noted that the administration of the saline, although not greatly affecting the blood chemistry prior to pyloric obstruction, does have some effects following the surgical intervention. The serum Potassium values, which were rather high in animals kept on tap water (7.76 in Table 8), are quite within the normal physiological range in the rats receiving saline. In the case of the D.C.A.-treated animals, the serum Potassium is actually higher than in the untreated animals. The chloride values are slightly high before the operation, and rather low following the obstruction. There is not much difference between the treated and untreated animals in this respect.

Gastric Contents.

In the course of several experiments performed and reported here, the gastric contents were taken following autopsy, their volumes determined and their chloride concentration determined. (The chloride concentration is reported as the total number of milliequivalents present in the entire sample.) The gastric chlorides were determined by the method of van Slyke (119), and the volume of gastric contents recorded. These are given in Table 11, below.

TABLE 11.

| Treatment | Controls | D.C.A. |
|---|---|---|
| No. of Animals | 11 | 8 |
| Gastric Volume | 7.3 ±5 | 3.3 ±4 |
| Gastric Chloride | 0.89 ±0.7 | 0.37 ±0.9 |

There seems to be an indication that pre-treatment with D.C.A. tends to inhibit the secretion of water and chloride into the gastric lumen, thereby

conserving the water and chloride of the body against the dehydration and hypochloremia brought about by pyloric or high intestinal obstruction.

## Summary and Conclusions.

It had originally been expected that some kidney damage might occur as a result of the surgical intervention. That this was not observed, we might perhaps explain by the fact that an acute loss of chloride was effected rather than a chronic one, as in clinical cases. The animals in this series of experiments died too soon for renal lesions to develope. However, from what has been presented on the foregoing pages, the following conclusions may be arrived at.

1. Pre-treatment of rats with D.C.A. prolongs life after obstruction.

2. Simultaneous pyloric obstruction and bilateral nephrectomy shortens the survival period as compared to that after either operation alone.

3. Pre-treatment with Desoxycorticosterone Acetate brings about a lowering of the high levels of plasma Potassium after either total nephrectomy or pyloric obstruction.

4. In the rat, the administration of a 1 % NaCl solution as drinking water, prevents the rise in serum Potassium as well as the fall in serum Chloride which occur as a result of pyloric obstruction. Simultaneous treatment with D.C.A. seems to be without further effect upon the blood chemistry.

5. Pre-treatment with D.C.A. inhibits to some extent the accumulation of gastric juice in the stomach after pyloric obstruction. The volume of the stomach contents as well as the total content of chloride are both reduced after treatment with D.C.A.

## Proteins and Amino-Acids.

The early history of attempts to ascertain the effects of diets rich in proteins, is highlighted by a dispute between two schools of thought. One of these groups, led by Newburgh and co-workers, contended that high protein diets were definitely injurious to the kidney, giving rise to such pathological manifestations as tubular dilatation, hyaline casts and albuminuria. In these arguments, they were supported by numerous investigators. On the other hand, the group led by Osborne and Mendel maintained that diets high in protein produced a physiologically normal hypertrophy of the kidney, primarily affecting the renal tubules, without in any way exerting any damaging effect upon the organ as a whole. A discussion of the effects of protein overdosage, therefore, must commence with a short review of the work accomplished by these early workers, and the interpretations offered by them as an explanation of observed phenomena.

In the 1908 edition of his "Practice of Medicine", Sir William Osler stated that "It is quite possible that in persons who habitually eat and drink too much, the work thrown upon this organ (i.e. the kidney) is excessive,and the elaboration of certain materials is so defective that in their excretion from the general circulation, they irritate the kidneys."

This view led Newburgh (194) to investigate the effects of high protein diets upon the structure and function of the kidney. He fed such protein substances as egg white, casein and soya beans to rabbits and obtained lesions

in the kidney, which he considered similar to those seen in Bright's Disease. No controls were included in these experimental studies. To-day, it is a well-known fact that spontaneous pathological changes are by no means of rare occurrence in the rabbit kidney. With rats, in which spontaneous lesions are seldom seen, Polvogt, McCollum and Simmonds (195) observed hyaline casts in the urine on a diet containing 31% to 43% of protein. The aforementioned results as well as many concepts then regarded as suitable explanations of the mechanism of action of these protein diets, are reviewed by Newburgh (196).

Further experimental work by the same investigator resulted in the production of vascular lesions similar to those seen in arteriosclerosis (197,198,199,200). The irritating effect of high protein diets in patients suffering from kidney disorders as well as in normal men, was claimed as well (201). In experiments intended to produce hypertension in rabbits, Nuzum, Osborne and Sansum (202) fed a diet high in protein to rats, but completely lacking in what we now recognize as the vitamins of the B-complex. They obtained a significant rise in blood pressure, measured by the method of von Eweyk (203) and Schmidtmann. This is not very surprising in the light of knowledge which we now possess regarding the rôle of these substances in the prevention of hypertension of nutritional origin. Arteriosclerotic changes in the aorta were observed by the above workers in subsequent studies (204), while evidence of kidney damage was also noted. Further work by Newburgh (205) convinced him that the diet must play a role in the pathogenesis of nephritic conditions, at least in the rabbit. Evans and Risley (206), working with rats, also obtained obtained morphological alterations of a pathologic nature in the kidneys. They admitted, however, that their diets were deficient in both vitamins and minerals.

In England, Drummond, Crowden and Hill (207) fed high protein diets to rats and kittens, including in their rations all the other food factors then (1922) accepted as indispensable to proper nutrition. No kidney lesions were found, and the authors concluded that the pathological changes which had been noted by others (194,208) up to that time, had been brought about by a deficiency in some food factor, necessary for the structural and functional integrity of the kidney, and which had been left out of the dietary regimens employed by these earlier workers. Osborne, Mendel, Park and Darrow (209), in 1923, investigating the effects of excess protein in the diet, observed kidney hypertrophy in rats. However, no inflammatory or degenerative lesions of any kind could be detected. Atrophy of the thymus gland occurred in all the experimental animals, indicating that they had actually been in a state of chronic starvation for the duration of the experiment, the high protein having been nutritionally quite inadequate. Further studies by the same group (210) confirmed these results, as did the work of Reader and Drummond (211), Anderson (212), Addis, MacKay and MacKay (213), Kennedy (214), and Jackson and Riggs (215). MacLean, Smith and Urquhart (216) resolved the apparent contradictions in this field to some extent by their discovery that the addition of plenty of green vegetables to a high protein diet given to rabbits would completely prevent the albuminuria and urinary hyaline casts seen when these supplements were lacking. The work of Miller (217), who kept rats on a high protein intake for six months without getting any pathological changes in the kidney, also supported the Osborne-Mendel point of view, which was that the ingestion of excessive amounts of protein brought about a kidney hypertrophy without at the same time producing renal lesions. Histologically they observed that the diameters of the capillary tufts and tubules were larger than those of control animals, but were still quite normal, functionally.

The same investigator (217) also studied the effect of this type of diet upon the remaining kidney after unilateral nephrectomy. The work of Hinman (218) and of Addis, Myers and Oliver (219) threw further light upon the role which high protein diets may play in augmenting the compensatory hypertrophy which results after one kidney is excised. The best picture which we can obtain of the various factors affecting this phenomenon may be found in the publications of Mofse and Smith (220,221,222,223,224,225,226), who worked in the laboratory of Osborne and Mendel. They stressed the importance of age as a factor in determining the production of lesions in the kidney of an animal which had been sensitized by partial nephrectomy, and was in the process of undergoing compensatory hypertrophy. The lesions which were seen by these investigators were not all similar to those seen in nephritic conditions, but rather resembled the senescent focal lesions in old rats, which occur spontaneously, though relatively rarely. These results were confirmed by Jackson and Moore (227), who obtained lesions in only 4 out of 14 adult rats which had been deprived of one kidney and had been receiving a diet containing as much as 76 % of protein, for as long as fourteen months. Mitchell (228) reviews the results of these extensive investigations up to the year 1928, and presents the many difficulties inherent in this type of nutritional research. He points out the practical impossibility of trying to compare results obtained under such a variety of different experimental conditions. Stress is placed upon the great biological factor of individual variation which is a cause of such great annoyance to the nutritional research worker.

Osborne, Mendel et al. (229) present their results in a manner which is much less confusing and far more accurate than that of other workers. Especially enlightening is their description of the histological changes which occurred as a result of feeding high protein diets to rats. All histological

observations were made in the kidney.

"The lumina of the tubules were considerably dilated," these workers report, "In general the dilatation was conspicuous in the midzone of the kidneys and was more marked in certain parts than in others. Where it existed, however, it was fairly uniform in contrast to the dilatation found in the old control rats which seemed to involve tubules here and there."

Osborne, Mendel and co-workers (229) were thus able, both in this publication and in the following one (230), to distinguish between the physiologically normal hypertrophy and the spontaneous senescent lesions in old rats. A detailed description of these lesions is also given (230).

The work of MacKay, MacKay and Addis presents an outstanding example of what accurate observation, coupled with cautious interpretation, can accomplish in the furthering of scientific achievement. The earliest reports of their work (231) deals with the effect of high protein diets and high urea diets upon the degree of compensatory hypertrophy of the remaining kidney, following unilateral nephrectomy. They find that there is augmentation in kidney size after the ingestion of high protein diets, but no such effect with high urea intake.

Their main contribution to the study of dietary effects upon the kidney consists of a series of papers, collectively entitled "Factors Which Determine Renal Weight". The first three papers (232,233,234) of this series are devoted to the methods employed and to the effects of age and sex upon renal weight in the normal rat. Next they attack the problem of high protein diets (235,236,237,238) and deduce a linear relationship between renal weight and protein intake. They also reject the suggestion that kidney hypertrophy on a high protein diet may be brought about by an increased need for kidney fun-

ction. They sytematically examine the affects of age and sex upon the renal changes resulting from excessive protein intake in the diet in two papers of the series (239,240), and deduce a precise mathematical expression which connects the kidney weight with the protein intake and is not much affected by differences of age or sex.

The same investigators also attempted to substitute urea for the protein of their diets, in order to see whether their formula would hold in this instance. Previous attempts to increase kidney size by the feeding of large amounts of urea had proven unsuccessful (218,229,230). They noted a significant increase in kidney size, but not in accord with their mathematical predictions(241).

We now return to a consideration of the work of Newburgh et al. during this period (1928). In a paper published in that year, Newburgh and Curtis (242) admitted some of their previous shortcomings, and suggested that the ability to produce actual kidney lesions by the feeding of diets high in protein may be affected by the type of protein which is administered, muscle protein being far superior in this respect, to casein. They attributed the difference in nephropathic potency to the amino-acids present in the material, and thereby place emphasis upon the individual amino-acids as possible nephropathic agents. This interpretation is supported by their observation that excessive amounts of urea, though capable of causing renal enlargement (241), never produced lesions. Using dried beef liver as a source of protein, they produced nephropathological changes in the white rat (243). They also presented a case history of a young man, who, after living on a high protein diet for six months, developed significant albuminuria and a twenty-fold increase in the excretion of urinary casts(244).

In Japan, Ishiyama (245) fed soya beans together with vegetable products to rabbits and found enlargement and hyperemia of the renal glomerular tufts as well as an increase of interstitial connective tissue. Krylow (246) in a study of the effects of high protein diets upon the kidney of the frog, noted enlargement of the tufts, degeneration of capillary cells and proliferation of the capsular epithelium. "Cloudy swelling and fatty changes were evident in the epithelium of the tubules,"the author reported.

Blatherwick, Medlar, Connolly and Bradshaw (247) reported the production of typical glomerular nephritis in unilaterally nephrectomized albino rats kept on a diet high in protein, especially in liver protein. At autopsy, the kidneys were very edematous and had an irregular and pitted surface. Microscopically, sclerosis of the glomeruli was in evidence. The convoluted tubules showed marked dilatation and were filled with large hyaline casts. In a more detailed communication, Blatherwick and Medlar (248) maintained that diet alone can produce nephritis, but since they kept their animals on this type of dietary regimen for as long as two years, the spontaneous developement of lesions in old rats cannot be entirely ruled out as a possibility. The same workers found, that in addition , females seemed more refractory to the nephropathic action of these diets. They added some dessicated thyroid to their rations, and found that this served to favor the developement of the lesions. If the experiments of Selye et al. (102) concerning the augmentation of the nephrosclerotic action of an anterior pituitary preparation by the simultaneous admimistration of Thyroxine, these results seem to be quite reasonable. In a detailed study concerning the pathogenesis of nephritis, the same investigators (249) discussed the changes which occurred in the kidneys in those conditions.

They maintained that the initial kidney lesions were focal in character. "Subsequent to the glomerular damage,"they stated,"injury, hyperplasia, and dissolution of the tubular epithelium of the glomerular capsule, loops of Henle and distal convoluted tubules,occur." The principal features of the chronic degenerative nephritis which they observed were enumerated as :- "sclerosis of glomeruli with or without obliteration of the capsular spaces; interstitial fibrosis in the regions of the tubules where the epithelium has been seriously affected: chronic inflammation which may be considerable; and cystic dilatation of the proximal convoluted tubules, which may or may not be extensive."

Horn (250) in his review on the experimentally produced nephropathies, concluded that "Although tubular lesions may occur, the bulk of the experimental evidence seems to indicate that the degenerative alterations following such a regimen (i.e. high protein) are minimal, and that the only anatomic result is a work-hypertrophy of the kidney consequent to the increased excretion of protein."

Bischoff (251) reviewed the work on diets and kidney morphology up to year 1932, and came to the conclusion that "diet may have little to do with the spontaneous kidney and blood vessel changes observed in lower animals and with the cardiovascular renal diseases found in man." In this last statement, he was supported by a great deal of accumulated clinical evidence. Numerous observations, such as those of Mosenthal (252) and Saile (253) concerning the effects of diets high in proteins upon the production of kidney lesions as well as upon the pathogenesis of hypertension, tended to support the general contentions of Bischoff (251). They also indicated the relative harmlessness of these diets to human subjects.

McCollum et al. (254), in the Fifth Edition of their text, "The Newer Knowledge of Nutrition", admitted that "the relation of dietary protein level to kidney lesions remains a mystery." Cuthbertson (255), in his review, published in 1940, has no definite conclusions to offer regarding the effects of high protein intake upon the kidney.

Mention should be made of the study of Thomas (256) regarding the health conditions of the Eskimos in Greenland, a group of people which subsist almost entirely on meat. He concluded that "the Greenland Eskimo, on a carnivorous diet, exhibits no increased tendency to vascular and renal disturbances." These conclusions have been amply confirmed by Rabinowitch and co-workers (257,258,259) who also studied health and nutritional conditions among the Eskimo tribes. They noted an elevated blood level of non-protein nitrogen, but failed to find evidence of kidney function impairment, which usually is the cause of such an elevation of the N.P.N. They attributed this rise to the high protein diet consumed by these primitive people.

Lieb (260), and McClellan and Dubois (261) reported on a study of two men, Arctic explorers, who had spent much of their time with the Eskimos and who volunteered to live for one year on a diet consisting only of meat. No renal damage was incurred and roentgengrams failed to show any signs of kidney enlargement. Tolstoi (262) found no increase in the non-protein nitrogen of the blood of these subjects, while all the function tests were indicative of normal renal function. Seven years later, Lieb (263) examined one of these "human guinea pigs" and found him to be completely normal and healthy.

In 1939, Hamilton (264) confirmed the direct relationship between kidney weight and amount of protein eaten, a relationship which had first been studied by MacKay, MacKay and Addis (235,236,237,238).

Vitamins and Protein Excess.

Present day research in the physiological functions of the vitamins has resulted in the acceptance of the general principle that these essential food factors serve mainly as co-enzymes in the many enzymatic reactions going on in the living organism. Thus, to cite one representative case, it has been found that Thiamine functions as the co-enzyme for Carboxylase, necessary for the decarboxylation of Pyruvic Acid, which is such an important intermediate substance in many biochemical reactions. It has been established then, that Thiamine is very important for the proper metabolism of carbohydrate substances, most of which must pass through the Pyruvic Acid stage before being ultimately metabolized. An increased intake of carbohydrate substances, then, necessitates an increased utilization of Thiamine, which diminishes the amount of this substance present in the body. Unless more of this vitamin is ingested, a deficiency may result.

In 1926, Tscherkes (265) showed that the developement of avitaminosis B was aggravated by a high protein intake. In England, Reader and Drummond (266) and Hassan and Drummond (267) demonstrated that the addition of yeast extract, containing what we now know as the Vitamin B-complex, would prevent the hypertrophy of the kidney to a large extent, which occurred on a high protein diet. "This hypertrophy, " they stated, "is only marked when the composition of the diet is such that the animal does not grow at a normal rate." This linking up of protein with vitamins marked an important step forward. Henceforth, investigators in nutritional work were obliged to include ample vitamins with the increased protein in their diets.

Hartwell had already demonstrated quite conclusively that a relationship existed between the amount of Vitamin B and the protein of the diet of the

lactating rat, when she performed several experiments upon the effect of vitamin supplements upon the renal lesions produced by high protein diets (268). Using Edestin as a source of protein, she showed conclusively that the addition of a comparatively large amount of yeast would completely prevent the appearance of kidney damage.

It is very interesting to note at this point the type of kidney lesions seen by Hartwell (268). Using 40 gram rats, she reported the following observations :-

"After a week, several of the rats became weak and were obviously ill, 5 of them died on the 13th day, having shown previous loss of weight. The remaining 7 were killed and a post-mortem examination showed only one to normal, while the other 6 had kidneys of a deep purple color and gorged with blood." One is very much tempted, in view of the age of the animals employed and the diet used, to conclude that this was in reality a manifestation of Choline deficiency. The beneficial effects of the vitamin supplements in this case, given as yeast, were a result of the Choline content of the yeast, rather than any specific effect of a B-vitamin present in the supplement.

In this discussion of the beneficial effects of yeast and other sources of members of the B-complex upon the integrity of the kidneys on a diet high in protein, we must recall the work of MacLean, Smith and Urquhart (216), mentioned previously, who found that the addition of green vegetables to the meat diet on which the experimental animals, were kept, would prevent any renal damage.

On the other hand, Francis, Smith and Morse (269) entirely failed to prevent the renal hypertrophy on high protein diets in both normal and unilaterally nephrectomized rats, by the administration of yeast supplements. Longwell,

Hill and Lewis (270), however, were able to prevent the renal enlargement brought about by the feeding of excess protein and Cystine (see below) , with Vitamin B supplements.

MacKay (271) studied the effects of amino nitrogen administered as Glycine and Glutamic Acid, and found practically the same influence of these substances upon the kidneys as that of a typical protein such as casein. Urea nitrogen, however, was found to be much less effective in increasing renal weight. MacKay and MacKay (272) also found that the feeding of the protein, Gelatin produced a much larger kidney than the feeding of any other protein, although both egg and blood albumin also produced larger kidneys than did casein. The experiments of Jackson, Sommer and Rose (273) may be mentioned here. They found that when Gelatin is fed as the sole source of protein, severe renal damage is incurred. Hemorrhages were seen, while some of the kidneys remained soft, pale and pitted. Amino-acids had been added to the Gelatin used in these experiments in order to render this protein adequate with respect to its content of "essential amino-acids". As the lipotropic substance, Methionine, had as yet not been isolated and consequently, had not as yet been listed as an essential amino-acid, and since Gelatin is entirely lacking in this substance, the evidence seems to point to another case of Choline deficiency, in which an absence of lipotropic factors was involved.

To return to a discussion of the work of MacKay and MacKay (272), however, we find a paper by Wilson (274), who confirmed the kidney enlarging effect of Gelatin, although he differed from both MacKay and MacKay (272) and Jackson et al. (273) in regarding this hypertrophy as a physiologically normal process, rather than a pathological enlargement, occasioned by kidney damage.

Low Protein Diets.

Jackson (295) reviewed the literature up to 1924, on the effects of underfeeding upon the weights of the various organs of the body. We cannot consider all the factors which may enter into the changes produced by inanition. We must therefore limit ourselves to the specific effects of protein deficiancy upon the kidney.

Winters; Smith and Mendel (3) fed diets low in Casein and diets in which Gliadin was the sole protein used, to rats. Since Gliadin is peculiar in that it is completely deficient in the essential amino-acid, Lysine, the diet was really a Lysine-deficient diet. An increase in kidney weight occurred on this ration, although the low protein (low casein) produced no detectable renal changes. Limson and Johnson (276) carried on extensive studies on the effects of low protein diets upon the size of various bodily organs. The kidneys showed no statistically-significant changes in size and weight on this type of diet. It may be concluded, keeping in mind the paucity of evidence, that low protein diets exert no special effects upon the renal parenchyma.

Amino-acids.

As in the field of mineral nutrition, investigators first worked out dietary regimens which either excluded proteins altogether, or contained substances of protein nature in very high concentration. It is within recent years that attention has begun to be paid to the individual "building blocks" of proteins, the amino-acids. The effects of overdosage and deficiency of various amino-acids have been studied. As a result, some data are available regarding the effects of these substances upon the kidney.

After protein has been ingested in the diet, it is broken up in the gastro-intestinal tract into its constituents, the amino-acids. Only these

substances, and not the proteins as such, are absorbed into the bloodstream. In fact, no matter what diversity of proteins we may eat, they are eventually broken down to a maximum of about two dozen different chemical compounds. As these circulate in the blood, they are utilized by the various organs for purposes of growth, that is, for the synthesis of new protein material, and for the many other purposes to which amino-acids are put by the organism.

The classical work of Schönheimer (277) has revolutionized our concepts of protein metabolism. Schönheimer envisages a circulating "pool" of amino-acids, into which the body pours the amino-acids produced by the continuous breakdown of body protein, as well as those resulting from the ingestion of dietary protein. In this "pool", all amino-acids, no matter from what sources they may have originated, become interchangeable. Therefore, we must admit that any effects which high protein diets may have upon the kidney cannot be due to the protein per se, but must be the result of the individual amino-acids constitute that particular protein. An overdosage of protein of a specific type will therefore cause an excess of certain amino-acids in certain definite proportions to be introduced into the circulating blood. The body may attempt to utilize these substances, or to break them down by the ordinary detoxification and metabolic pathways open to it. Large quantities of intermediary products may be formed. These may be the cause of various toxic manifestations throughout the body. A case in point is the production of the pressor base, Tyramine, formed by the detoxification of the amino-acid Tyrosine, and known to produce Hypertension in the renal arterioles, a fact which will be discussed later in this review.

We may also exhaust the supply of vitamins in the body by overdosing with amino-acids, that is, proteins. Facts are coming to light which implicate

various members of the B-complex with the enzymatic reactions undergone by some amino-acids in the course of their catabolism. An example is the relationship between the vitamin Pyridoxine and the amino-acid, Tryptophane(278). Thus diets which are very high in this substance, that is, protein diets, may produce secondary deficiencies of this substance. The whole question is one of great complexity, and it is certainly not the intention of the author of thos thesis to discuss it in all its numerous implications and ramifications. Sufficeth to say that a clear correlation has been established between the data obtained in this field of nutritional investigation, and those obtained in pure biochemistry or enzymology. A recent and quite comprehensive review covering the metabolism of the amino-acids has been published by Sahyun (279).

Newburgh and Marsh (280) were among the first to study the effects of individual amino-acids, administered in comparatively large doses, upon the kidneys. Dogs and rabbits were injected with various amino-acids. It was found that Alanine, Leucine, Glycine, Phenylalanine and Glutamic Acid were completely harmless at doses as high as 2 gms. per kg. of body weight. Arginine and Aspartic Acid appeared to be slightly nephrotoxic. Lysine produced kidney changes in which there was vacuolar degeneration of the epithelial cells lining the straight portions of the convoluted tubules. Histidine produced a moderate amount of cloudy swelling, and scattered casts were visible. Tyrosine proved to be quite nephrotoxic, the microscopic appearance of the kidney being featured by swollen and granular epithelial lining of the tubules, collections of irregular epithelial cells in the Bowman's space, and other changes of a degenerative nature. Tryptophane produced a widespread necrosis of the renal tubules, while Cystine produced some calcification in sections of the tubules in which the epithelial lining had disappeared.

The nutritional study of Sullivan, Hess and Sebrell (281) and the pathological analyses of the animals used in this study, by Lillie (282), are quite illuminating and should be discussed further. Various amino-acids were given to rats in varying percentages of the total diet, and the kidneys of the animals examined at autopsy.

The animals receiving Glycine were normal at all levels of intake. 20 % Lysine produced a parenchymatous and fatty degeneration of the renal convoluted tubules. An acute nephrotic condition was in evidence. 20 % Tryptophane produced a moderate parenchymatous degenration of the renal tubules. The distal convoluted tubules showed many hyaline casts in their lumina. Cystine, in concentrations from 5 to 20 % produced parenchymatous and fatty degeneration of the tubules. The cytoplasm of the tubular epithelium was acidophilic, opaque in appearance, often vacuolated, with opaque rounded acidophil masses next to, and in the lumen. 20 % Tyrosine produced a minor grade of parenchymatous degeneration of the kidney. The addition of 5 % Cystine to 5 % Tyrosine caused the animals to survive much longer. No renal changes whatsoever were observed in those rats receiving 5 % Cystine together with 10 % Tyrosine. The Cystine somehow seemed to inhibit the toxic action of the Tyrosine to a considerable degree. 20 % Glutamic Acid produced hyaline casts in the renal tubules, but not any fatty degeneration. 25 % of the tripeptide, Glutathione, produced a marked swelling of the tubules, so that the lumina were almost completely closed up.

Cystine.

Blum (283) injected approximately 1 gram of Cystine per kg. of body weight in a dog. Death resulted a short time later and severe hemorrhagic nephritis was seen at autopsy. Smaller doses produced no effects; Wohlgemuth (284) did not observe any toxic manifestations after the feeding of Cystine to rabbits.

The work of Newburgh and Marsh (280) has already been cited and need not be repeated again. Lewis (285) fed small amounts of Cystine to rabbits and caused serious renal damage. The kidneys of these animals showed evidences of acute congestion, cloudy swelling of the convoluted tubules and necrosis of the collecting tubules. The study was carried out on four rabbits, two of which showed signs of pyogenic infection. The results are therefore not very conclusive. Addis, MacKay and MacKay (213) failed to find any pathological alterations in the renal parenchyme of rats kept on a 1% Cystine diet. On the other hand, Lignac (286) injected mice with a 1 gram of Cystine over a period of three weeks and observed that the kidneys of the animals were enlarged and showed cloudy swelling. Curtis and Newburgh (287) found that, whereas low concentrations of the amino-acid (below 0.5 % of the diet) were quite harmless, concentrations of over 5 % of the diet, which also contained 8 % of casein, definitely acted in a nephrotoxic manner, producing a diffuse hemorrhagic necrosis of the renal parenchyme. Cox, Smythe and Fishback (288) kept young rats weighing less than 60 gms. on a synthetic diet, completely deficient in the members of the B-complex, and containing from 0.3 to 0.9 % of added Cystine. A large increase in the N.P.N. was observed to take place very suddenly, indicating renal damage. At autopsy, the kidneys were severely affected, hemorrhages having occurred. Several of the rats which had survived a little longer, showed what the authors (288) termed white spots, but which seem to fit in very well with the "frosted kidney" of the recovery period of Choline deficiency. Furthermore, since the authors also stated that rats of 80-90 gms. were not susceptible to the "nephrotoxic" action of Cystine, there seems no doubt that a Choline deficiency was present here with all its characteristic manifestations. Since it is now known that the addition of Cystine to a low Choline diet will aggravate the deficiency (see section on Choline farther on), we may conclude that the Cystine acted here in a capacity of a

secondary stimulus rather than the primary nephrotoxic agents which the authors would have us believe. Cox and Hudson (289) then systematically proceeded to find the means of preventing these lesions. Turning to the experiments of Hartwell (268), in which she prevented the lesions caused by a high protein diet (in reality produced by a Low Choline diet) by adding yeast extract (containing Choline) to the diet, they proceeded to do the same, with similar success, and for identical physiological reasons. MacKay and MacKay (290), not satisified with attributing the inability of 80 gram rats to react to Cystine(288), postulated that a higher dose, in proportion to the greater body weight, was to chance, necessary in order to produce kidney hemorrhages in the older rats. The action of yeast extract in preventing the nephrosis brought about by the inclusion of excess amounts of Cystine in the diet, was confirmed by Longwell, Hill and Lewis (270). They stated that "Cystine was not nephrotoxic to these animals in the sense of causing degenerative lesions in the kidney."

All this on so-called "Cystine Nephrosis" may therefore be quite satisfactorily attributed to a Choline deficiency already present and aggravated by the Cystine to such an extent as to be quite dramatic in appearance in so far as the kidney hemorrhages are concerned. György and Goldblatt (291) supported this contention by their statement that "the renal lesions and fat infiltration of the liver, observed in rats kept on a Vitamin B-free diet, supplemented with Thiamine, Riboflavin and Vitamin $B_6$ (Pyridoxine) are indistinguishable from those hitherto attributed to Cystine intoxication." Experiments by Earle and Victor (292,293), in which rats were kept on a 10 % Cystine diet, showed no kidney lesions at autopsy. One hemorrhagic kidney, of the Choline deficiency type, was seen at autopsy, among the animals receiving 5 % Cystine in their diet. These diets (292,293) were adequate with respect to their Choline content, which explains the almost complete absence of hemorrhagic renal lesions.

dl-Methionine.

The feeding on an excess of this essential amino-acid was carried out by Earle, Smull and Victor (294). They fed a diet containing from 10 to 12.4 % of dl-Methionine to rats. At autopsy, dilatation of the convoluted and collecting tubules of the kidney was the most common change seen in the kidney, and occurred in half of the animals of the experimental group. In one case, several mitotic nuclei were found among the vacuolated epithelial cells. The renal glomeruli remained unaltered. Three out of the twenty-six rats receiving the amino-acid, showed atrophic kidneys.

A deficiency of Methionine, when coupled with a simultaneous deficiency of other lipotropic factors, such as Choline, will give rise to fatty livers, and to the kidney hemorrhages seen in Choline deficiency. The subject is discussed in great detail later on in this thesis.

Tyrosine.

In 1912, Abderhalden (295) reported that 150 gms. of Tyrosine could be ingested safely in the course of a single day. Medes (296) confirmed this observation that Tyrosine was relatively non-toxic to human beings. Lillie's pathological reports (282) concerning Tyrosine can be quoted here as follows :- "20 % Tyrosine caused a minor grade of parenchymatous degeneration of the kidney".

Martin (297) fed diets containing from 2.5 to 20 % of Tyrosine to young rats. Hypertension was observed in these animals after several days. The authors ascribed this phenomenon to the decarboxylation of large quantities of Tyrosine in the kidney, which led to the formation of the pressor base, Tyramine, in larger amounts than the body could conveniently detoxify. Hence the hypertens

A detailed pathological study was carried out by Hueper and Martin (298), and among the notable changes which had taken place were "hyaline nephrotic changes. Focal necrotizing lesions, involving the cortex as well as the medulla, often affecting wedge-shaped zones. The mildest reactions of this type consisted in accumulations of leukocytes in the tubular lumens, with moderate leukocytic infiltration in the surrounding tissue, and cystic dilatation of tubules, with atrophic metachromatic, dark-staining cells in the corresponding cortical areas." They go on further to state that "In more advanced lesions there were central hyaline necroses surrounded by leukocytes and fibroblasts. The pink-stained necrotic centres consisted of a peculiarly filamentous matter arranged in radiating fashion." In addition to these tubular changes, there was thickening of the arterioles of the kidney.

The authors (297) considered the lesions of the renal parenchyme and pelvis and those of the vascular system of the kidney of quite distinct origin. A biochemical study (299) reported a hyperglycemia following the administration of large amounts of Tyrosine. It was also noted that the simultaneous administration of Ascorbic Acid seemed to alleviate the severity of the condition. The work of Sealock (300,301), who demonstrated the the existence of an interrelationship between Vitamin C and the metabolism of Tyrosine, is mentioned in an effort to find an acceptable explanation for the beneficial effect of the antiscorbutic vitamin in this case. A recent review (302) discusses the whole topic of Tyrosine overdosage.

Serine.

Here, as in the case of Tyrosine and other amino-acids, there seems to be a close relationship between vitamins and amino-acids. In 1942, Fishman and Artom (303) observed an interesting and unexpected phenomenon in the course ~~of experiments~~ designed to study the relationship of the effects of

diets on the phospholipid content of various tissues. Working with animals kept on a synthetic diet adequate in most essential nutritional constituents, he administered 100 mgs. of dl-Serine per day, by stomach tube, to male and female rats, weighing around 100 gms. The animals soon ceased to grow and lost weight. After three to seven days of this treatment, death occurred, mainly among the male animals. Animals kept on a standard stock diet and receiving the amino-acid, seemed relatively unaffected. At autopsy, a severe tubular damage in the kidneys of the rats which had succumbed as a result of the treatment was seen.

Fishman and Artom ( 304) reported furthermore, that the addition of Choline, Cystine and Glycine, together with an injection of a mixture of most of the vitamins of the B-complex, would protect the animals against the injury due to Serine overdosage, to the same extent as the stock diet had done. Pyridoxine seemed to be particularly effective in this respect. In a further paper, they offered more details of this protective action(305). The route of administration seemed to be of particular importance (306), as the inclusion of this substance in the diet had no effect whatever. The organism must be flooded with this amino-acid very suddenly, if its toxic action is to be made manifest.

Fishman and Govier (307) recently carried out a series of biochemical studies on the co-carboxylase content of the kidney as influenced by the over-dosage with Serine. On a stock diet, after two doses of the amino-acid, the cocarboxylase of the kidney fell from 50 γ per gm. to only 34 γ per gm. The authors therefore concluded as follows, regarding the mechanism of action :- "The first dose of Serine leads to profound necrotizing lesions in the kidney of rats of either the stock diet or the Vitamin-B deficient diet. At this

time there occurs a drop in co-carboxylase and a reduction in its further breakdown. Processes of repair are very extensive and almost completed by the fourth day in animals on the stock diet, and hence we see a return to normal of the tissue co-carboxylase and the rate of its disappearance on anaerobic incubation. In the animals on the Vitamin B-deficient diet, however, the kidneys never return to their former healthy state and the necrotic tissue is replaced by Calcium deposits. Hence we find no return to normal co-carboxylase levels or to a normal rate of its disappearance in incubation."

The relation of kidney co-carboxylase to the pathological changes in the kidney tubules, is very puzzling, yet very interesting, for it poses a number of questions regarding the mechanism of injury to the kidney produced by a diversity of agents. A study of the levels of various enzymes and co-enzymes found in large quantities in the renal parenchyme before and after treatment with these nephrotoxic agents, seems to be indicated. Another puzzling phenomenon is the fact that Pyridoxine is the member of the B-complex which seems to be of particular benefit to the Serine-overdosed animal (304, 305). Since co-carboxylase is Diphosphothiamine, chemically, we should expect that Thiamine ought to be very helpful during Serine intoxication, but this has not been demonstrated to be the case.

Very recently indeed, Artom, Fishman and Morehead (308) repeated their experiments on the effects of Serine injury, using the natural l-Serine isomer as well as the racemic dl-Serine, which they had used in all their previous experiments (303,304,305,306,307). They found that the l-isomer was completely harmless, whereas the nephrotoxic action of the dl-isomer was once more confirmed. The toxic action of dl-Serine on the kidney seems therefore to be due entirely because of the presence of the unnatural d-isomer. This discovery reduces the

possible physiological significance of this phenomenon and renders the complete solution of the problem one of pharmacological rather than physiological interest. It is, however, quite interesting to speculate whether this nephrotoxic action of the unnatural isomer of Serine, is specific for this amino-acid, or is a general, property of all d-isomers of amino-acids.

Several months ago, the report of the pathological findings in the kidneys of Serine-intoxicated rats was published (309). "Bluish intracytoplasmic" granules were seen, which later served as nuclei for the deposition of Calcium. The continued administration of Serine did not augment the severity of the lesions which had been produced by the first dose of the substance.

## Summary and Conclusions.

As can readily be observed from the foregoing review, the effects of proteins and amino-acids upon the kidney are by no means as yet clearly understood. No theories have been advanced to interpret the observed phenomena, and it is a hazardous undertaking to attempt to draw some conclusions from the mass of conflicting and contradictory reports of experimental work or clinical observations by diverse groups of research workers, working under different experimental conditions. A few salient points, however, may perhaps be considered on the basis of fundamental importance to an understanding of the mechanism involved in these actions.

1. The feeding of excess amounts of protein tends to produce a kidney hypertrophy, which may be of the nature of a work-hypertrophy.

2. Some amino-acids, present in proteins, exert a specific pharmacological action upon the kidney when given in excess. This action may be due either to the acid itself or to some intermediary metabolite.

3. High protein diets, if unaccompanied by an increase supplement of B-complex vitamins, will produce secondary B-avitaminoses, by exhausting the existing stores of these substances in the body, in the course of their metabolism.

4. Proteins of animal origin, such as liver or muscle protein, seem to be much more nephropathic than proteins of vegetable origin or substances such as casein. This may be due to either of two factors or to both. The peculiar amino-acid composition of the nephropathic protein may be the cause of its kidney-damaging, or the renal organ may be affected by minute quantities of pharmacologically-potent substances, not of protein structure, but present in animal proteins.

5. The sulfur-containing amino-acids, Methionine and Cystine, play a very important role in the prevention and production of dietary kidney lesions.

Further work in this field is demanded in order to clarify the precise relationships between the amino-acids which act upon renal tissue to prdduce various pathological changes and the effects of vitamin deficiencies upon this same vital organ. A vast amount of experimental work in biochemistry is now being done on the reactions involved in the catabolism of the amino-acids, and as new facts emerge, a theory capable of explaining the renal alterations of structure in the light of these new discoveries, will certainly be forthcoming.

## Fats and Fatty Acids.

There are very few papers in the literature dealing with the effects of dietary excesses or deficiencies of fat. Diets high in their content of fat are extremely unpalatable. There are a few papers dealing with the effects of deficiencies of fatty substances upon the kidney.

Burr and Burr (310) placed new-born rats upon a diet adequate in its content of proteins, carbohydrates, minerals and vitamins (those then known), but completely lacking in fats. After more than two months on this regimen, the experimental animals developed a characteristic syndrome and eventually died. At autopsy, the kidneys were found to be abnormal. In a second paper, the same authors (311) laid more emphasis on the kidney lesions. The cause of death was attributed to them. The addition of an excess of protein to the fat-free diet resulted in an aggravation of the kidney lesions and the resulting hematuria. Unfortunately, no report of the pathological observations can be found in the literature. At about the same time (1929), McAmis, Anderson and Mendel (312) carrying out similar experiments on fat-free diets, found kidneys which were mottled and which presented many surface indentations upon macroscopic examination. The renal pelves of several of these animals contained calculi.

Burr and Burr (311) further demonstrated that the syndrome produced by the feeding of fat-free diets could be completely cured by the administration of Linoleic Acid. This was confirmed by the work of Evans and Lepkovsky (313,314).

Further work by Burr et al. (315) resulted in the discovery of several more compounds which could prevent the damage produced by a fat-free diet. Both Linoleic and Linolenic Acids were found to be active in this respect, as well as many naturally-occurring fats and oils. It was also observed that the Iodine numbers of the curative substances were unusually high, which is to say, that these substances were highly unsaturated, containing numerous double bonds in the molecule. Thus, the degree of unsaturation seemed to bear some relation to the beneficial effects in animals on a fat-free diet. Oleic Acid, with only one double bond to the molecule, was of no benefit at all, while Linoleic Acid with its two double bonds and Linolenic Acid with its three double bonds, representing an increasing level of unsaturation of the same basic compound, were both highly active in preventing the renal changes seen in animals deprived of fat.

Evans and Lepkovsky ( 316) contributed greatly to the clarification of the mechanism whereby the deficiency symptoms were both produced and prevented. They showed that the rat could not synthesize highly unsaturated fatty acids. Since these highly unsaturated acids were necessary for growth and maintenance, and had to be derived from the food, they were considered as a new vitamin, and called Vitamin F, a term which has since been discarded. In order to be nutcalled Vitamin F, a term which has since been discarded. In order to be nutritionally adequate, the diet must therefore contain some of these "essential fatty acids", as they are now called. The recent work of Schönheimer (277) has demonstrated that whereas the body can form fatty acids containing one double bond to the molecule, it cannot form those more highly saturated. They must be derived from the diet, if pathological manifestations of deficiency are to be avoided.

## Carbohydrates.

The effects of diets rich or poor in their content of carbohydrates, upon the kidney, are not very striking and hence not very much has been reported on this subject in the medical literature. One of the main difficulties encountered in such a study, is the unescapable fact that any diet low in carbohydrate will not necessarily cause a deficiency of this foodstuff alone, since the body is capable of converting protein into carbohydrate in the liver. On the other hand, a diet low in carbohydrate is not missed very much by the body. Diets rich in carbohydrate must at the same time be poor in protein, thereby producing the symptoms of protein deficiency at the same time.

In 1916, Carlson, Hektoen and LeCount (317) fed rats on a diet containing only about 2 % of Glucose and noted no particular changes anywhere in the body. Cori (318) fed his rats upon an 80 % Glucose diet without detecting any renal changes. This was confirmed by the experiments of Evans and Burr (319,320), Burr and Burr (310) and Evans and Lepkovsky (313).

A more detailed study was carried out by Jackson (321). Newly-weaned rats were placed on a diet containing 80 % commercial Glucose for a period of more than six months. The animals were then killed, various organs examined and weighed. The kidneys appeared to weigh 10 % more than those of the control group in which the carbohydrate content consisted of 45 % of Starch. No report of the pathological findings is available in the literature.

## Vitamins.

Much emphasis has been placed upon the importance of vitamins in human and in animal nutrition. Pathological studies of vitamin overdosages and deficiencies have been accumulated in the literature. There is good reason to believe that with the possible exception of Vitamin D, overdosages with the various vitamins will not cause any toxic actions or undesirable effects. The pathological findings in the deficiencies of vitamins have recently been reviewed by Wolbach and Bessey (322). First of all, we shall deal with two of the fat-soluble vitamins, Vitamin A and Vitamin D, after which the review will be devoted to a discussion of the effects of Vitamin C and to the members of the B-complex.

### Vitamin A.

The most recent review which covers the chemical and physiological properties of this substance is that of Heilbron, Jones and Bacharach (323). Since the effects of Vitamin A overdosage are little understood and information concerning its possible influence upon the kidney is mainly confined to unavailable continental literature, only the effects of Vitamin A deficiency upon the kidney will be dealt with here.

The effects of Vitamin A deficiency upon the organism in general may be defined in one short sentence. The main action of this vitamin is the maintenance of the surface epithelium of the body. When this essential substance

is lacking, the epithelial structures lining the body cavities and organs undergo a degeneration, often characterized by a widespread keratinization of the many epithelial surfaces, such as the mucous membrane, the lining of the genito-urinary tract, etc. The new epithelium which is formed as a result of this keratinization is quite identical with the epidermis. A great deal of the ability to protect the organism against bacterial invasion is thereby lost, so that infection accompanies Vitamin A deficiency in a great number of instances.

The specific effects of a deficiency in Vitamin A upon the renal epithelium was first noticed by Osborne, Mendel and Ferry (324), who found renal calculi in rats kept upon a diet which was deficient in this vitamin as well as in other essential food factors. The effect of Vitamin A deficiency in bringing about this condition was further studied by Fujimaki (325) in Japan. McCarrison (326,327,328,329,330,331,332,333) in India, carried out a great number of investigations upon the etiology of renal calculi. This subject interested him because of the very high incidence of renal calculi in certain localities in India. He found that calculi could be found in the bladders of rats kept upon diets deficient in Vitamin A and high in the phosphate content. Among the factors which tended to aggravate the condition was a high Calcium intake. The importance of the Ca/P ratio in this connection was emphasized by Ranganathan (334). Further work by McCarrison (335,336) showed that Vitamin A deficiency was only one of the many equally important factors bringing about the formation of renal calculi. McCarrison (337,338,339) reviewed his numerous contributions to our knowledge of the etiology of renal calculi, and discussed the role of Vitamin A deficiency in the experimental production of this clinical condition. At about the same time, van Leersum (340,341) carried out several experiments upon the production of Urolithiasis by means of Vitamin A

deficiency. He found extensive deposits of Calcium in the lumina of renal tubules as well as in the ureters and bladder, and came to the conclusion that they had been produced by the impregnation of epithelial cells with Calcium. The experiments of Higgins (342) demonstrated that keratinization of the genito-urinary epithelium preceded the formation of the calculi, and also indicated the possible involvement of infectious processes in this phenomenon. Alkaline urine, which is a common condition in A-avitaminosis, was held responsible for the formation of renal calculi by Bliss, Livermore and Prather (343). These authors review the various theories current at the time regarding the etiology of this condition and came to the conclusion that an imbalance of Calcium and Phosphorus metabolism must be the main precipitating agent, and that both Vitamins A and D must somehow be involved. Further work by Higgins (344,345) seemed to strengthen his contention that infection was involved. Keyser (346) discussed some clinical aspects of the subject, while Blackfan and Wolbach (347) reported the findings of a detailed pathological study of infants suffering from this condition. Metaplasia of the epithelium of the renal pelvis was a constant phenomenon and was interpreted as a precursor of calcification and formation of renal calculi which might become dislodged from the renal epithelium and might subsequently appear in the ureters, the bladder, ar be voided with the urine. In India, Wilson and Mookerjee (348) discussed local factors, such as dietary variations, which were held largely responsible for the high sectional incidence in some states of that country. A very fine experimental and clinical review upon the whole subject is that of Gray (349). A discussion on the effects of diet in the etiology of renal calculi is given by Hou (350).

Vitamin D seemed to aggravate the condition, according to these workers (349,350), as did a reduction in the intake of Calcium and Phosphorus. They

also subscribed to the belief (342) that the keratinization of the pelvic epithelium facilitates the formation of the calculi.

Oppenheimer and Pollack (351) failed in an attempt to dissolve renal calculi by the ingestion of a high Vitamin A diet. A detailed description of the pathological changes occurring in the urinary tract in Vitamin A deficiency in guinea pigs was given by Steiner, Zuger and Kramer (352). Hyperplasia, followed by metaplasia and finally by atrophy of the pelvic and ureteral mucosa occurred. Pieces of desquamated epithelium served as nuclei for the defelopement of calculi consisting largely of Calcium Carbonate.

The review of Randall (353) sums up the evidence for the role of hypovitaminosis A in the etiology of renal calculi. He points out that a deficiency of Vitamin A is usually accompanied by two other factors, that of a consistently alkaline urine and that of a disturbance in the balance between Calcium and Phosphorus accompanied by a phosphaturia. Infection may also set in because of the inability of the keratinized epithelium of the urinary tract to exertk its characteristic protective action.

Vitamin D.

Here, in ddrect contrast to the action of Vitamin A, the toxic and harmful manifestations of the vitamin, in so far as the renal organ is concernéd, are due to an overdosage of the vitamin, rather than to a deficiency. The role of Vitamin D in the prevention of rickets is too well-known to be discussed in any great detail in this review. The effects of hypervitaminosis D are much more important for the purpose of this study, and will therefore be discussed in some detail.

The early workers in this field were the German investigators, who succeeded in producing kidney lesions in many different animals with an excess of Vitamin D. Kreitmair and Moll (354) were among the first to observe calcification of the renal arteries as a result of a high dose of the vitamin. Pfannenstiel (355) reviewed the early work and discussed some of the basic principles which had been enunciated. Further work by Kreitmair and Hintzelmann (356) demonstrated the widespread occurrence of calcification in the membrana propria and around the convoluted tubules of the kidney. The glomeruli were also affected, and Calcium deposits occurred in the lumina of some of the tubules. The animals used in the course of these investigations were rabbits, rats, mice and cats. Reyher and Walkhoff (357) reported the occurrence of what they termed a "toxic nephrosis" in mice and rats which were fed on irradiated foodstuffs. Rabl (358) discussed the pathological changes occurring in mice after large doses of Viosterol ( a form of Vitamin D ). Calcification of the basal membranes and epithelium of the renal tubules was noted. The simultaneous administration of Calcium Carbonate aggravated the condition as did also the ingestion of Sodium Phosphate. Holtz and Brand (359), Hückel and Wenzel (360) and Billig (361) all commented upon the calcification of the renal arteries and arterioles. Occasional deposits of Calcium were seen in the glomerular spaces. Kraus (362) noted diffuse glomerular enlargement and cellular hyperplasia in the course of his experiments upon the production of Glomerulohephritis.

In America, Klein (363) as well as Smith and Elvove (364) reported upon the occurrence of Calcium casts in the tubules of the kidneys of rabbits receiving massive doses of irradiated Ergosterol. Spies and Glover (365) published a detailed pathological report upon findings in the kidneys of rabbits receiving large doses of the anti-rachitic vitamin. The chief changes seen

were sclerosis and hyalinization of the walls of the renal blood vessels. The basement membrane of the tubules and the glomerular capsules were also sclerosed and accompanied by extensive subepithelial deposits of hyaline material. There was abundant deposition of Calcium in these localities as well. Pronounced atrophy of the tubular epithelium was also observed. Large amounts of albumin appeared in the urine of these animals and the blood non-protein nitrogen showed a marked increase.

Ham and Portuondo (366) believed that Vitamin D acted upon the serum Calcium in much the same manner as did the hormone of the Parathyroid glands. Calcification, according to this interpretation , was caused by the precipitation of serum Calcium upon the kidney epithelial lining. Light, Miller and Frey (367) noted severe kidney calcification on a high Vitamin diet. Reed, Dillman, Thacker and Klein (368) discussed the effects of a high Vitamin D intake in dogs, and showed that the kidney was the hardest hit of any of the organs.

The work of Duguid et al. deserves the mention of a few of their findings at this point in the review. Duguid, Duggan and Gough (369) first studied the effects of the Calcium level of the diet upon the incidence and severity of the hypervitaminosis D kidney lesions, and found that they were aggravated by a high intake of Calcium, which was to be expected. In a further study (370), they distinguished between two types of kidney lesions. First there was the vascular lesions, affecting the arterioles of the kidney and related to the general systemic effect of Vitamin D overdosage on the vascular system(271). Then they distinguished the calcareous cast formation in the tubules as brought about by a change in the solubility of the blood Calcium, which tended to precipitate out in the kidneys. Renal lesions were described in greater detail in a further paper (372).

Duguid (373) reviewed his work in the course of a clinical seminar on chronic nephritis. He compared the lesions which he had observed to those of MacKay and Oliver (73) on a high Phosphate diet without the addition of Vitamin DΔ Cardiac hypertrophy was seen by Duguid (374) in some animals, probably as a result of the renal involvement. Morgan and Samisch (375), investigating the biochemical changes occurring after the administration of Viosterol, demonstrated an increase in water and ash in the kidney. The ash consisted chiefly of phosphate. Goormaghtigh (376) presented a detailed review of the histological changes in the kidney after treatment with Calciferol. They also reported upon the results of their own experiments regarding the action of this substance in the dog, and concluded that "in the dog's kidney Calciferol, causes arteriolonecrosis, with or without nephritis, depending on the dose employed". Their interpretation of the pathogenesis of these changes was similar to that of Duguid, Duggan and Gough (370), the vascular lesions being ascribed to a primary effect of overdosage with the vitamin, and those of the tubules to the secondary hypercalcemic effect exerted by large doses of Calciferol. Chown, Lee, Teal and Currie (377) discussed the experimental production of nephritis in rats by means of this vitamin and attributed the effect to the deposition of Calcium in the renal tubules.

The actual mechanism of action of Vitamin D overdosage is unknown. Why the vascular system should be particularly affected by large amounts of this vitamin is as yet, a complete mystery.

The similarity of these types of kidney lesions and those seen in Magnesium deficiency as far as etiology is concerned, is rather unexpected. In both conditions, the kidney is first lesioned, then calcified. The relationship between the Calcium level of the blood, influenced by Vitamin D, and the Magnesium concentration, has not been elucidated.

Vitamin C.

There is only one paper in the literature dealing with the effects of Vitamin C deficiency on the kidney. Russell and Callaway (378) produced scurvy in guinea pigs by feeding them a ration deficient in this vitamin. Trypan blue was injected into the animals during the last few days before they were sacrificed, in order to determine possible alterations in tissue structure during Ascorbic Acid deprivation. It was found that the dye was heavily deposited in the proximal convoluted tubules of the kidney, although no morphological changes were visible as such. The authors concluded that some pathological change must have occurred in the kidney. More detailed information on this subject is lacking and further experimental work is indicated.

Vitamin B-complex.

Many of the renal changes brought about a deficiency in the members of this group of vitamins, have already been discussed under the heading "Proteins and Vitamins", and will therefore not be dealt with here.

Calder (379) studied the effects of nutritional deficiencies upon the production of hypertension in rats, and came to the conclusion that most of the members of the B-complex were necessary for the prevention of the hypertensive state which occurred on a B-deficient dietary regimen. He also showed that Thiamine was not involved in the prevention of this condition and therefore limited his work to a study of the heat-stable Vitamin $B_2$-complex upon the elavation of the blood-pressure. We may recall that Follis (27) had demonstrated the fact that while Thiamine was concerned with the maintenance of the integrity of the cardiac musculature, it had no specific effects on the kidney.

A second paper by Calder (380) which discussed the renal pathology associated with nutritional hypertension, confirmed the belief that only members of the Vitamin $B_2$-complex were effective in preventing the abnormal rise in blood pressure which accompanied the feeding of deficient diets to rats. Nutritional hypertension was accompanied by changes in the renal parenchyme resembling those seen in advanced Nephrosclerosis, but of a lesser degree of severity. They differed slightly from the changes in essential hypertension as seen clinically, mainly by virtue of the occurrence of areas of ischemic atrophy in the renal medulla, a change which is not seen in human beings. The afferent arterioles and interlobular arteries underwent degenerative changes, with hyaline deposition occurring in the subendothelial layer encroaching upon the lumen in the larger blood vessels. Necrosis of the epithelial lining of the uriniferous tubules was also observed. The glomeruli were affected, to some extent, being reduced in size and the capillary basement membrane being somewhat thickehed. Hemorrhages in the subcapsular and cortical regions were also seen in several animals.

Pantothenic Acid.

The chemistry and physiology of this recent addition to the Vitamin B-complex has been reviewed by Williams (381). We are concerned here with interesting manifestations of Pantothenic Acid deficiency as they affect the kidney and adrenals.

Daft and Sebrell (382) working with deficient diets, observed adrenal necrosis and hemorrhage in the rat, a phenomenon which had first been noted by György et al. (383) several years earlier. The description of the changes in the adrenal was published by Nelson (384), and stressed the occurrence of congestion, hemorrhage, atrophy, necrosis, scarring, fibrosis, and fat depletion

in the cortex. Further experiments by Daft et al. (385) demonstrated that the administration of Pantothenic Acid completley prevented these lesions. Ashburn (386) discussed the effect of this member of the B-complex upon the histopathology of the rat. The curative effect of Pantothenic Acid was confirmed by the work of Mills et al. (387) and by Salmon and Engel (388).

The presence of kidney damage in the Pantothenic Acid deficient rat was first revealed by Supplee et al. (389). The abnormal kidneys were enlarged, mottled and necrotic. The heart was also greatly enlarged as a secondary phenomenon. Since dehydration of the tissue was generally evident, they determined to see whether adrenal function had been in any way impaired, and resorted to the "self-selection" method of Richter (390). Two groups of animals, one to act as controls, and the other, deficient in Pantothenic Acid, were given access both to fresh water and to 3% saline. It was found that the deficient group consumed twice as much saline as did the normal animals.

McKibbin, Black and Elvehjem (391) and Schaefer et al. (392) investigated the effects of Pnatothenic Acid deficiency in the dog. The kidneys of these animals seemed very similar to those seen in Choline deficiency ( see below),,being of a dark-red colour. Macroscopic evidences of hemorrhagic degeneration were present in both the cortex and medulla. It is therefore quite possible that a deficiency of Choline was also present. However, it should be mentioned that no kidney hemorrhages have as yet been reported in Choline deficiency in the dog.

The effect of Pantothenic Acid upon the adrenals has been mentioned in order to link up the actions of this substance upon both the kidney and the suprarenal glands. The tissue dehydration may also be involved in the patho-

genesis of the lesions. Much more experimental work is required before an interpretation of these phenomena can be attempted.

Miscellaneous.

Other vitamins, such as Thiamine, Riboflavin, Pyridoxine, Vitamin E, etc. do not seem to exert any specific effects upon kidney size or structure.

Choline.

It is perhaps doubtful whether Choline should be included among the vitamins of the B-complex, but for the purposes of this review, we shall consider this physiologically-important substance as a vitamin, in the sense of its being a substance necessary for the proper functioning of the organism and derived from the ingested food.

Among the numerous reviews dealing with the role of Choline in physiological processes, the most recent one is that of McHenry and Patterson (393). The article by Best and Lucas (394) is quite detailed and furnishes much valuable information about the chemical, as well as about the physiological properties of this substance. Griffith, who has done most of the pioneer work in this field, reviewed his work in detail (395,396,397). Other reviews which may prove of interest are those of Frame (398), Morgan (399), Best and Ridout (400) and Best (401). As it is quite impossible to enter into a discussion of the various aspects of Choline deficiency and upon all the important physiological effects upon the animal, it is hoped that the above-mentioned reviews will serve as an introduction to a discussion of the effects of Choline deficiency upon the kidneys.

The pathological changes in the kidney observed by Hartwell (268), Cox and Hudson (289), Cox et al. (288), and many other earlier workers, though ascribed by them to a high protein intake or high Cystine content of the diet, were in reality due to a deficiency in Choline, which was manifest only among the young animals. Griffith (402) first reported the occurrence of hemorrhagic kidneys in experiments designed to study the effects of Low-Choline diets in young male rats. Enlargement of the spleen and atrophy of the thymus gland were accompanying symptoms. In the next publication, Griffith and Wade (403) showed that the amino-acid, Methionine, exerted the same protective action as did Choline itself. A more detailed description of these findings was published by Griffith and Wade (404,405) in which it was clearly demonstrated that the increase in kidney weight may serve as an accurate criterion of the severity of the Choline deficiency. The effect of altering the protein content of the diet was also discussed, a high protein intake (casein, in this instance) preventing the deficiency symptoms. This was attributed to the Methionine content. Addition of the "nephrotoxic" acid, Cystine, the steroid substance, Cholesterol, and other fatty substances were shown to aggravate the hemorrhagic lesions in the kidney (406).

Next, the effects of age, weight and sex of the animal upon the incidence and intensity of the renal changes were studied (407). Kidney damage could be produced in rats only during a specific period in the animal's life, the third week, post-natally, being the most favorable to the developement of these lesions. The deficiency symptoms were found to be more severe in the male than in the female, thus pointing to a possible sex difference (see experimental section). The influence of the supply of "labile Methyl Groups" (408) was next discussed by Griffith and Mulford (409). They found that supplements of Creatine, though decreasing the severity of the condition somewhat, did not entirely abolish it.

Further research yielded results (410,411,412) which showed that Choline deficiency was aggravated by the inclusion in the diet of the proteins Lactalbumin, Fibrin, Edestin, Gelatin, and of the tripeptide, Glutathione. The high Cystine/Methionine ratio of these substances was probably the cause of this "anti-lipotropic" action. Betaine was found to prevent the lesions while the sulfur-containing acids, Taurine and Homocystine, were without effect. A rise in the blood N.P.N. was observed during the critical phase between the sixth and tenth experimental day. Restriction of the food-intake prevented the occurrence of the hemorrhagic lesions by lowering the general metabolic level to such a degree that the animals practically ceased to grow. The relationship between the quantities of Cystine and Methionine in the diet and the amount of Choline required to prevent pathological signs of lipotropic deficiency were also investigated by Griffith and Mulford (413), who found that the addition of Cystine beyond a certain amount to a Choline-free diet, could no longer aggravate the severity of the lesions.

Christensen and Griffith (414) reported upon the involution and regeneration of the thymus in Choline deficiency. Christensen (415,416) reported the pathological changes in Choline deficiency in great detail. The kidneys were greatly enlarged, with a characteristic dark purplish-red appearance. Blotches of varying size were present throughout the surface of the organ. In animals which were less severely affected, dark areas were visible in many parts of the renal surface. In animals which had partly recovered at the time of killing, the so-called 'frosted kidneys' were visible. Yellowish-white areas were present on the kidney surface, while the kidney itself appeared very pale, in contrast to its former dark purple. Microscopically, there were two main types of lesions, those primarily affecting the renal vascular system, and those involving the kidney tubules. The vascular changes consisted mainly in congestion

of the cortical capillaries and capsular blood vessels, followed in many cases by hemorrhages to the peripheral zone of the cortex. Most tubules were full of casts and globules of hyaline material, while necrosis involving whole groups of convoluted tubules was observed. In the most severely affected kidneys, calcification of the degenerated peripheral tubules occurred. A short resume of these pathological findings is available (417).

György and Goldblatt (219) discussed the etiology of liver and kidney damage in Choline-deficient animals and demonstrated that the inclusion of Pyridoxine in the diet aggravated the lesions. They also presented a comprehensive report on the pathological findings in the kidney, which were essentially similar to those seen and described by Christensen (415,416). Earle and Victor (220) in experiments designed to produce experimental cirrhosis of the liver, by the prolonged feeding of Cystine to rats, confirmed the presence of renal hemorrhages. Further work by György and Goldblatt (418) served to connect the occurrence and production of renal lesions with those encountered in the livers of animals on the same diets. Engel and Salmon (419) greatly simplified the procedure employed for the production of Choline deficiency by dietary means, by using the rare protein, Arachin, derived from peanut meal, as the sole source of protein in his diet. Arachin is a protein which is completely lacking in the lipotropic amino-acid, Methionine, and is thus able to produce kidney hemorrhages even in the presence of comparatively large amounts of Choline in the diet. Hemorrhages in the myocardium, adrenal cortex and lungs were observed in addition to these in the kidney cortex. The relations between Choline and the other members of the B-complex were investigated by Engel (420), who also found that Choline was necessary for the proper nutrition of the rat (421). Jacobi and Baumann (422) added the carcinogenic substance, Butter Yellow (N,N-dimethyl p-aminoazobenzene), to the list of substances capable of preventing

the symptoms of Choline deficiency in the rat. The lipotropic action of this dyestuff was interpreted on the basis of the labile methyl groups which it possesses and which may act as a donor for some Choline-precursor in the body. Jacobi and Baumann (423) also carried out measurements of the Choline content of the kidneys during a period of Choline deprivation, in order to arrive at some mechanism of action which would satisfactorily account for the observed phenomena. They mistakenly attributed the lipotropic action of Choline to the presence of labile methyl groups.

It was not until the work of Patterson and McHenry (424,425,426,427) was completed that the accepted interpretation of the mechanism of action of Choline in preventing renal hemorrhages was made known. Working with radioactive Phosphorus as a 'tracer', they first demonstrated that Choline deprivation decreased the turnover of phospholipids in the kidney and liver. Since the normal turnover of this physiologically-important group of substances is greatest in younger animals at the time of most rapid growth, it was to be expected that a deficiency in Choline, an essential constituent of the phospholipid molecule, would then exert its most marked and acute effect. This theory therefore offered a plausible explanation for the occurrence of renal hemorrhages in the rat only at a specific period in its life, at about the third to fourth week post-natally. The renal lesions, therefore, are due to a decreased turnover of the phospholipid content of the kidney which is produced by a lack of Choline for the synthesis of these substances. From the work of Sinclair (428), we know that one of the main physiological functions of the phospholipid group of compounds is to act as 'carriers' of fatty substances in the blood, transporting these materials from the liver to the fat depots for storage. In the absence of sufficient quantities of these fat-carriers, the liver fat cannot

be removed, and the hepatic parenchyme becomes clogged with fat, resulting in a "fatty liver". For some as yet unknown reason, the need for phospholipid in the growing kidney is greater than that of other organs, so that the kidney is 'hit' first of all in the event of phospholipid deficiency. The above interpretation is reviewed concisely in a recent journal (429).

In agreement with this theoretical argument is the work of Wachstein (430), who measured the Renal Phosphatase, both acid and alkaline, after a period of Choline-deprivation. He found that both types of Phosphatase had been greatly decreased in concentration in the kidney. Since these enzymes are known to play a very important role in the metabolism of phospholipids, this observation seems to fit into the picture. The recent study of the lipids of the kidney cortex by Popják (431), demonstrated that the renal cortex had a higher phospholipid concentration than the medulla. This fact may perhaps explain why the renal hemorrhages characteristic of Choline deficiency are encountered almost exclusively in the renal cortex. Honorato and Vadillo (432) found no change in the arterial blood pressure of rats kept on a Low-Choline diet.

Jervis (433) described the occurrence of brain hemorrhages in rats deprived of Choline, while Bellows and Chinn (434) described in some detail the intraocular lesions which they observed in rats kept on a Choline-deficient diet.

Fouts (435) produced cirrhosis of the liver in dogs by the feeding of a Low-Choline diet, but did not observe any kidney involvement. McKibbin, Thayer and Stare (436) and McKibbin et al. (437) carried on extensive experiments on the effects of Choline deficiency in the dog, but did not mention the

occurrence of kidney lesions in their publications. Dutra and McKibbin (438) reported upon the pathological changes in Choline-deficient dogs. Although fatty livers were seen and thymus atrophy occurred, the kidneys remained quite normal in all respects. We may therefore conclude that the appearance of renal hemorrhagic lesions in this state is quite species-specific, and insofar as we now to-day, peculiar to the rat. A review of the work done on the effects of Low-Choline diets on dogs is available (439).

Among recent work published in this field, the experiments of Foâ, Foâ and Field (440) are deserving of mention. They found that inclusion of the B-complex vitamin, Nicotinic Acid, would aggravate the kidney lesions produced by Choline deficiency. Stetten and Salcedo (441), studying the effects of varying the chain-lengths of fatty acids administered to rats kept on fat-free Choline-free diets upon the liver fat, found that only the ethyl esters of Caprylic and Lauric Acid were effective in producing the renal hemorrhages. Pathological findings (442) indicated the presence of severe myocarditis in the rats receiving the Ethyl Laurate. It was also found that the administration of the lipotropic substances, Choline or Methionine, completely prevented the heart lesions, which were often so severe as to prove fatal. The addition of the anti-lipotropic amino-acid, Cystine, to this diet, aggravated the cardiac lesions. It is still too early to speculate regarding the mechanism of production of the myocarditis, but it is quite possible that they were secondary to the renal involvement.

Miscellaneous.

Excesses and deficiencies of Thiamine, Riboflavin, Nicotinic Acid, Pyridoxine, Biotin, Vitamin E, Vitamin K and other dietary essential constituents, do not exert any specific effects upon the kidney, or have not been tested in this respect. A consideration of these substances is therefore not included here.

Conclusions.

1. A deficiency in Vitamin A is conducive to the formation of renal calculi. The epithelium of the pelvis of the kidney undergoes desquamation and keratinization; the desquamated epithelial scales acting as nuclei for the formation of renal calculi, which may eventually find their way into the bladder and the urine.

2. An overdosage of Vitamin D produces two types of lesions in the kidney. First there are those which affect the vascular system as a whole, and are especially found in the renal blood vessels. Secondly, there are the tubular lesions, in which calcification occurs as a result of the precipitation of the excess blood Calcium produced by the hypercalcemic effect of this substance, in the kidney parenchyme.

3. A deficiency in the heat-stable Vitamin $B_2$-complex produces renal lesions accompanied by nutritional hypertension.

4. A deficiency in Pantothenic Acid produces adrenal hemorrhages, and kidney lesions.

5. A deficiency in Choline produces very dramatic renal hemorrhagic lesions, accompanied by fatty livers, thymus atrophy and enlargement of the spleen. These changes can only be elicited during a specific period in the life of the animals.

6. The lesions of young rats, suffering from Choline-deprivation, are produced by a deficiency of Phospholipids, resulting from the absence of sufficient Choline for their continued synthesis by the body.

## EXPERIMENTAL SECTION.

### Introduction.

The production of Choline deficiency symptoms in the rat is a problem of some difficulty because of the great number of variables encountered, which may influence the incidence and severity of the condition. It is for this purpose of studying the effects of varying somewhat conditions influencing the production of the renal hemorrhages seen in Choline-deficient rats, that the present series of experiments have been undertaken.

Since Griffith and Wade (404,405) showed that the increase in kidney weight served as a good criterion of the severity of the Choline deficiency symptoms in animals kept on a Low-Choline diet, the kidneys of the animals in these experiments, have been weighed after fixation in Suza Fixative and in 10 % Formalin. The results of the microscopic investigation of the degree of renal damage present, were expressed by a scale of plus signs, ranging from "0" to "+++". The percentage intensity was calculated by adding up the total number of these plus signs and then dividing by the number of animals in the experimental group..The resulting figure was then expressed as a percentage of the maximum theoretical number of possible plus signs. The degree of incidence was similarly expressed as a percentage of the total number of animals in that particular group, except that in this case, the animals showing any type of renal lesions,of any intensity whatever, were included in the totals.

Experimental Diets.

The experimental Low-Choline Diet was also employed for the Normal-Choline, ration, except for the addition of 300 mgs. of Choline Chloride per 100 gms. of diet. The composition of the basal diet is as follows :-

| | | | |
|---|---|---|---|
| Sucrose | 65 gms. | Cod Liver Oil | 2 gms. |
| Casein (Vitamin-free) | 18 " | Cystine | 1 " |
| Vegetable Oil | 10 " | α- Tocopherol (Synkavit) | 1 mg. |
| U.S.P. Salt Mixture No. 2 | 4 " | Nicotinic Acid | 1 " |

Each animal also received a daily subcutaneous injection of the B-complex vitamins in aqueous solution. Each injection contained the following ingredients:-

| | | | |
|---|---|---|---|
| Thiamine Hydrochloride | 25 γ | Pyridoxine | 25 γ |
| Riboflavin | 25 γ | Calcium Pantothenate | 125 γ |

The above ingredients were thoroughly mixed, and fed to the animals, which were also given tap-water for drinking.

Experiment No. 10 :- "Effect of Strain Difference upon the Renal Lesions Produced by Choline Deficiency in Young Rats."

Engel (443) and Copeland (444) have demonstrated that the Choline requirements of the rat varies to a great extent with the strain of the animals involved. Genetic mechanisms are doubtlessly involved.

In order to study the effects of varying the strain, both albino and hooded rats have been employed in this experiment. One group of each strain received Low-Choline diets, while two other groups acted as controls, one for each strain, and received the Normal-Choline ration. Male animals were used,

After ten days, the animals were killed, their kidneys fixed in Suza and in 10 % Formalin, weighed and examined histologically.

T A B L E 1 2 .

| Treatment | Normal Choline Albino. | Low Choline Albino | Normal Choline Hooded | Low Choline Hooded |
|---|---|---|---|---|
| No. of Animals. | 6 | 10 | 10 | 10 |
| Average Final Body Weight. | 57 (53-61) | 38 (25-52) | 43 (32-59) | 34 (29-38) |
| Average Kidney Weight. (2). | 0.618 (0.584-0.675) | 0.594 (0.494-0.771) | 0.450 (0.371-0.539) | 0.812 (0.604-0.938) |
| Av. Ky. Wt. as % Av. Body Wt. | 1.09 (1.04-1.15) | 1.72 (0.86-2.69) | 1.10 (0.98-1.26) | 2.40 (1.73-2.83) |
| % Incidence. | 0 | 80 | 0 | 90 |
| % Intensity. | 0 | 73 | 0 | 77 |

The greater sensitivity of the hooded rats is at once apparent, though it must be admitted that there is some overlap between the kidney weights of the two groups. In the course of further experiments, hooded rats were used.

Experiment No. 11 :- "Effect of Sex upon the Renal Lesions Produced by Choline Deficiency in Young Rats."

Griffith (407) stated that female rats were more resistant to the kidney damaging effects of Choline deficiency. Since a possible sex hormone mechanism may be involved here, it was determined to repeat this experiment on hooded rats, instead of the albinos used by Griffith (407), and to include a group of castrate males in addition to the other two groups of normal males and females.

The experiment was allowed to run for 10 days, after which the animals were killed and their kidneys subjected to the same procedure as in the foregoing experiment.

T A B L E 1 3 .

| Treatment | Normal Males | Castrate Males | Normal Females |
|---|---|---|---|
| No. of Animals | 10 | 9 | 12 |
| Average Final Body Weight. | 28 (22-35) | 28 (23-33) | 29 (21-34) |
| Average Kidney Weight. (2). | 0.747 (0.654-0.854) | 0.676 (0.543-0.792) | 0.729 (0.579-0.857) |
| Av. Ky. Wt. as % Av. Body Wt. | 2.67 (2.20-3.39) | 2.41 (1.75-2.97) | 2.51 (1.81-3.25) |
| % Incidence. | 100 | 90 | 90 |
| % Intensity. | 100 | 83 | 83 |

No sex difference as far as renal weight or percentages of incidence or intensity can be found, as can readily be seen from the data presented in Table 13. It may therefore well be that in hooded rats, no sex difference in this respect, exists, whereas in the case of albinos, such a sex difference may well be present..

Experiment No. 12 :- "Effect of Unilateral Nephrectomy upon the Renal Lesions Produced by Choline Deficiency in Young Rats."

After unilateral nephrectomy, when the remaining kidney is undergoing compensatory hypertrophy, it should prove enlightening to determine what occurs

to the kidney lesions of Choline deficiency during this period. Two groups of male hooded rats were employed. One group remained intact and served as controls for the other group, which was unilaterally nephrectomized immediately before being placed on the Low-Choline diet. The animals were killed after eight days and the kidneys taken and dealt with as in previous experiments. In comparing the information given in Table 14, it should be remembered that the kidney weights for the unilaterally nephrectomized animals represent one kidney as compared to the combined weights of both kidneys of the controls.

T A B L E 14.

| Treatment | Controls. | Unilaterally Nephrectomized. |
|---|---|---|
| No. of Animals. | 8 | 19 |
| Average Final Body Weight. | 30 (26-36) | 28 (19-38) |
| Average Kidney Weight. (2 and 1) | 0.686 (0.595-0.898) | 0.422 (0.304-0.562) |
| Av. Ky. Wt. as % Body Wt. (2 & 1) | 2.38 (1.98-2.66) | 1.83 (1.27-2.60) |
| % Incidence. | 100 | 58 |
| % Intensity. | 87 | 34 |

It can be seen that the incidence as well as the intensity of the renal lesions is significantly reduced by unilateral nephrectomy. During the eight days which this experiment lasted, the remaining kidney of the unilaterally nephrectomized group was as yet unable to undergo the maximum amount of compensatory hypertrophy possible, so that no comparison of kidney weights can be made.

Expefiment No. 13 :- "Effect of an Anterior Lobe Preparation upon the Renal Lesions Produced by Choline Deficiency in Young Rats."

Selye (101,102) has demonstrated the renotropic action of an anterior lobe extract. The present experiment was undertaken with the purpose of determining what effect this preparation would have upon young Choline-deficient rats in the short space of seven days, which was the length of the experimental period. Absolute controls consisting of intact animals constituted the first group. Since testoids exert a renotropic effect, the animals of the other two groups in this experiment were castrate males. Group 2 acted as a direct control upon the third group which received 0.2 ccs. of L.A.P. (Lyophilized Anterior Pituitary) twice daily by subcutaneous injection, and was injected with 0.2 ccs. of Casein (Vitamin-free) in aqueous suspension, twice daily, subcutaneously. This suspension was made up to contain 25 mgs. of Casein per cc., so as to equal the amount of pituitary substance administered.

T A B L E 15.

| Treatment | Controls | Castrate Males Casein | Castrate Males. L. A. P. |
|---|---|---|---|
| No. of Animals. | 8 | 10 | 12 |
| Average Final Body Weight. | 28 (22-35) | 27 (23-33) | 27 (22-34) |
| Average Kidney Weight. (2). | 0.641 (0.529-0.873) | 0.599 (0.483-0.744) | 0.702 (0.454-0.931) |
| Av. Ky. Wt. as % Av. Body Wt. | 2.33 (1.65-2.74) | 2.22 (1.51-2.89) | 2.52 (1.99-3.05) |
| % Incidence. | 100 | 80 | 92 |
| % Intensity. | 87 | 77 | 83 |

As can be seen from Table 15, the renotropic action of L.A.P. manifested itself somewhat in the increased kidney size of the animals of the third group. No alleviation of renal hemorrhagic lesions, however, occurred. It is unfortunate that the experiment could not last longer, because of the mortality of rats on a Low-Choline diet when they enter the "critical period" of Choline deficiency at about the sixth to tenth days after being placed upon the Low-Choline diet. No final conclusions as to the action of this hormonal extract upon hemorrhagic kidneys, can therefore be made.

Mention should be made of the almost universal occurrence of fatty livers upon this diet, in the experimental animals, which were not influenced to any significant degree by any of the treatments undergone in these experiments.

## Conclusions.

1. Hooded rats are more sensitive to the kidney damaging action of Choline deficiency in young rats, than are albino rats.

2. Sex does not have any influence on the sensitivity of hooded rats to the production of kidney lesions by Low-Choline diets.

3. Unilateral nephrectomy alleviates to some extent, the severity of the renal lesions in Choline deficiency in the remaining kidney.

4. The administration of a renotropic agent, lyophilized anterior pituitary extract, in this instance, does not seem to exert a very marked effect upon young Choline-deprived castrate male rats, of the hooded variety. The weight of the kidneys of these animals is somewhat augmented.

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