THE PRODUCTION OF CARDIAC AND RENAL LESIONS IN RATS BY A DIET EXTREMELY DEFICIENT IN POTASSIUM *

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In 1937, Schrader, Prickett and Salmon [1] described pathological alterations in the heart, kidneys, pancreas and intestines of rats which had been placed on a diet deficient in potassium. More recently, by restricting the dietary potassium, Thomas, Mylon and Winternitz [2] have confirmed the cardiac lesions reported by Schrader, Prickett and Salmon in rats, though they failed to note any change in the other tissues. They were also able to produce the same myocardial lesions in hogs. However, they concluded that both in the latter animals as well as in rats the development of the lesions was dependent on a diet not only deficient in potassium but in vitamin B_6 as well. Sykes and Alfredson [3] have noted electrocardiographic changes in calves on diets of low potassium content.

The present study deals with the pathological alterations in the tissues of rats placed on a diet adequate in all known respects save in its content of potassium (0.01 per cent). The composition of this diet was as follows: wheat gluten, 4.0; lactalbumin (highly purified), 10.0; gelatin, 4.0; salts, no. 21, 4.6; butter fat (purified), 8.0; dextrose to 100 per cent; viosterol, 15 drops per Kg. of diet; liver concentrate (Lederle), approximately 3.5 gm. of liver per rat per day; thiamin, 20 γ per rat per day, and vitamin E concentrate, 3 mg. per rat per day. The growth, behavior and metabolism of animals placed upon this diet are being reported elsewhere by Orent-Keiles and McCollum.[4]

MATERIALS AND METHODS

Ninety rats of the McCollum strain, about equally divided as to sex, have been examined. Their ages, when placed upon the potassium-low diet, varied from 25 to 28 days. They were killed after being on the diet from 4 to 327 days. Approximately one-half of the animals served as controls and received the potassium-supplemented basal diet either *ad libitum* or in amounts restricted to the intake of the potassium-deficient animals. The latter precaution was taken to exclude, as far as possible, any rôle inanition might have in producing the changes

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observed. Seven of the animals were born from mothers that had been placed on the potassium-low diet 10 days before their birth and had continued on the diet during suckling. They remained on the diet after weaning as long as 67 days of age.

The animals were killed with ether and autopsied immediately. Tissues were placed in Zenker-formaldehyde solution and in a 4 per cent solution of formaldehyde alone. After imbedding in paraffin, they were stained with hematoxylin and eosin. Bones were decalcified in 5 per cent nitric acid. Since it seemed inadvisable to examine all tissues from every animal, a preliminary group of animals was studied thoroughly and then those organs which showed any deviation from the normal controls were studied more minutely in other animals in order to trace the development and course of the changes. The following tissues were studied: heart, lung, liver, spleen, pancreas, adrenal, kidney, esophagus, stomach, intestine, ovary, uterus, vagina, testis, seminal vesicle, prostate, thyroid, parathyroid, thymus, arteries, skin, skeletal muscle, brain, hypophysis, bone and bone marrow. Only the positive gross and microscopical findings will be described. There were marked differences in the gonads and accessory sexual apparatus, thymus and bones of the animals fed *ad libitum* and of the potassium-deficient animals, but when the factor of inanition was ruled out by paired feeding these differences were not present.

Gross and Microscopical Changes

Heart

Grossly, changes were not seen in the heart before the third week. At 15 days, however, with the aid of binocular loupes, tiny, opaque grayish areas could be seen beneath the epicardium of some of the deficient animals. By the sixth week these lesions could be detected with the naked eye and from then on these areas were seen in practically all potassium-deficient animals. The controls showed nothing. The only other gross finding of note was a difference in the weight of the hearts of the deficient animals and their controls fed *ad libitum*. The ratio of heart weight to body weight indicated that on the whole the hearts of the former group were the larger, but interpretation of this is not accurate because we do not know how much of a rôle inanition was playing. However, some light was cast on the assumption that there was a true hypertrophy by the fact that in a group of seven animals (four deficient and three controls) on the paired feeding regimen for 87 days, the deficient animals weighed 38 per cent less than their controls, but their hearts weighed 13 per cent more than those of the latter.

Microscopically, the earliest change to be noted was a loss of striation of individual muscle fibers. This was seen either in isolated fibers or in groups of fibers after the animals had been on the potassium-low diet for 8 days. The hyaline fibers next lost their affinity for eosin and took on a grayish yellow color. After this they disappeared altogether, leaving an empty membrane. No fatty change could be demonstrated with the Scharlach R stain. Coincident with the change in the myofibrils was one which was found in the nuclei. As the fibers were undergoing necrosis the nuclei began to shrink and very soon underwent karyolysis or karyorrhexis. Associated with these changes there was an emigration of leukocytes into such areas (Fig. 1). In the early stages there were polymorphonuclear leukocytes while later mononuclear phagocytes predominated. There was a tendency for the earlier lesions to be more numerous beneath the epicardium and endocardium but necrotic fibers were found also in the center of the ventricular walls. No reaction was ever seen on the surface of the epicardium or endocardium. Lesions were scanty in the auricular musculature. Mural thrombi were not found in the ventricular chambers. Aside from the leukocytic infiltration in the zones of necrosis, no other cellular response was found. There were no perivascular accumulations of leukocytes, nor any changes in the vessels themselves.

From the eighth to the fifteenth day these lesions increased both in size and in number, so that at 2 weeks numerous large areas, covering as much as two low-power microscopical fields in greatest diameter, could be found (Fig. 2). In some animals the process took on the appearance of a diffuse myocarditis with widely scattered necrotic fibers surrounded by leukocytes. Other areas suggested the lesions that are encountered in human diphtheria. In the ensuing weeks the involvement became more extensive so that by the sixth week the necrotic foci were extremely numerous. By this time, too, there was an increasing proliferation of connective tissue at the sites where earlier lesions had been present. These scarred regions consisted of single fibers or groups of intact muscle fibers separated by connective tissue cells and bundles of collagen (Fig. 3). Scattered about, too, were mononuclear leukocytes. It was interesting to note old scarred lesions close to muscle fibers which were undergoing the necrotic changes as described. From the sixth week to the end of the period of observation in the 47th week the scarring became more extensive, while in contrast there were fewer fresh lesions. However, it must be noted that lesions indicating recent myocardial damage were found in animals on the deficient diet as long as they were observed (327 days).

When the animals which had been born to rats on the deficient diet

were examined, the same myocardial lesions were seen. At 4 weeks of age there were fresh necroses together with scars, and these same changes were found in animals examined in the sixth, eighth and tenth weeks after birth. Because of the report [5] of cardiovascular lesions in calves due to magnesium deficiency, a group of animals was given magnesium, in excess of that in the basal diet. This supplement had no effect on the development of the lesions.

In summary, in the myocardium of all animals which had been on the potassium-deficient diet 8 days or longer there occurred necrosis of the muscle fibers. Such areas then became infiltrated with leukocytes and healing took place with a proliferation of connective tissue. Similar lesions were not found in the control animals on the same diet plus an adequate potassium intake.

Kidneys

Grossly, the most prominent change in the kidneys was an increase in size of those of the potassium-deficient animals. Most marked from the sixth week on, the difference was most impressive in the paired feeding group where, although the deficient animals weighed less than their restricted controls, the kidneys of the former group weighed more than those of the latter animals. This was not only true of the wet weight but of the dry weight as well. The surfaces were finely pitted and they were paler than the corresponding control organs.

Microscopically, the first change to be noted appeared after the animals had been on the potassium-deficient diet for 8 days. In the cytoplasm of the cells of the convoluted tubules small vacuoles appeared between the basement membrane and the nucleus. When frozen sections were stained with Scharlach R, these droplets were found to be lipid in nature (Fig. 4). When examined through the polarizing microscope they were not doubly refractive. By the 14th day the lipid had increased so that most of the cells of the convoluted tubules contained it. However, by this time other changes were present. Necrotic epithelial cells as well as swollen cells with poorly staining nuclei could be found. These cells became detached from the basement membrane and lay as pink hyaline masses in the lumina of the tubules. Coagulated pink-staining material was found in other lumina. There were also dilated tubules lined by flattened epithelium, which had apparently regenerated (Fig. 5). A few multinucleated tubular epithelial cells were found. In one kidney at 15 days a few "calcified" epithelial cells were found. During this early period no changes could be detected in the glomeruli or blood vessels.

By the sixth week there were many dilated tubules lined by flattened

epithelium (Figs. 6 and 7). At this time an additional feature was added to the picture. In a few tubules there appeared a blue-staining, granular material. One or two such casts could be found in the tubules in each section and as time went on they became more numerous. A few calcified epithelial cells were noted also. The only other finding of importance was an increase in size of the individual epithelial cells when compared with the same cells from control animals on the paired feeding regime. At the twelfth week and from then on to the end of the experiment the dilatation became more marked. The amount of "calcified" material did not increase greatly or reach the degree reported in magnesium deficiency [6] and following parathormone administration.[7]

In summary, the kidneys showed necrosis of the tubular epithelium, dilatation of the tubules and later deposition of "calcified" material in the lumina of some of the tubules.

Discussion

By feeding rats a diet extremely low in potassium but adequate in all other known constituents, necrosis of the cardiac musculature and damage to the renal tubular epithelium have been produced. The other tissues appeared normal. These myocardial lesions are apparently exactly similar to those observed in rats by Schrader, Prickett and Salmon [1] and in both rats and hogs by Thomas, Mylon and Winternitz.[2] However, although the cardiac lesions are alike, there are discrepancies noted in the other organs in these three studies. Schrader, *et al.* also described alterations in the kidneys similar to those here described and, in addition, lesions in the pancreas and intestine. They failed to eliminate the factor of partial inanition. Thomas and his associates noted lesions in the heart only. The compositions of the diets used in these three studies differ. McCollum, Orent-Keiles and Day[8] have already criticized the diet employed by Schrader, Prickett and Salmon as being inadequate in other dietary essentials aside from potassium. The diets used by Thomas, Mylon and Winternitz were deficient in factors of the vitamin B complex other than thiamin, riboflavin and B_6. In addition, their diet I contained an excess of cod liver oil and was deficient in vitamin E. These workers concluded that potassium was not the only factor in producing the lesions but that a deficiency of vitamin B_6 was also necessary. These differences in the diets employed may be sufficient to explain the discrepancies in the lesions produced in these experiments.

Since the classic studies of Ringer,[9] in 1882, the importance of potassium in the normal physiology of the heart has been well recognized.

The exact mechanism for the production of cardiac lesions by diets low in potassium is quite obscure. Unfortunately, at this time, we do not know how much the blood-potassium level was affected in the rats on our potassium-low diet. The potassium content of the heart muscle, however, was 35 per cent lower than that of the controls. When one attempts to apply these experimental findings to disease in man associated with low blood potassium there is very little available information. In familial periodic paralysis there is a low blood potassium during attacks and at such time electrocardiographic changes have been described.[10] Another possibility which comes to mind is the lowering of serum potassium in patients with Addison's disease to whom an excessive amount of desoxycorticosterone acetate had been administered. Here, cardiac failure has been described,[11] though it may be due to some factor other than lowering of the serum potassium. To our knowledge no histological studies on such hearts have been reported.

It is worth while pointing out that the cardiac musculature was the only type affected. The striated myofibrils as well as the involuntary fibers were not damaged, even though the potassium content of the voluntary muscle was lower than that of the myocardium.

The changes in the kidneys are fairly characteristic of the so-called "necrotizing nephroses" which can be produced by various chemical poisons, such as salts of mercury or uranium or bacterial poisons as well. The exact mechanism for the renal changes in this experiment is obscure. It was hoped that metabolic studies might help in explaining the pathogenesis of the lesions. In the deficient animals the potassium excretion was very low during the first 3 weeks and after this period practically none of this cation was found in the urine. There was a diminished excretion of magnesium from the first to the seventh week in the potassium-deficient animals as well as a diminution in the urinary output of phosphate as compared with the normal controls. These data are difficult to interpret. Renal lesions have been reported in rats on a magnesium-deficient diet, though the various observers do not agree as to the exact renal changes. Greenberg, Lucia and Tufts [6] have described extensive calcium deposition in the renal tubules with atrophy of the tubular epithelium. We have recently been able to examine the kidneys of three rats that had been placed on a magnesium-deficient diet for 35 days. In these there was necrosis of the epithelium of the convoluted tubules. Calcified material was found in only one of the animals. The change was much more severe than we observed in the potassium-deficient animals, nor was tubular dilatation prominent. McKay and Oliver [12] have observed lesions in rats which bear a striking similarity to those we have observed by feeding an excess of inorganic

phosphate. In our animals, as previously noted, there was a diminution in the output of phosphate. The exact nature of the mechanism involved in the renal tubular necrosis in the potassium-deficient animals is obscure.

Summary

By the utilization of a diet adequate in all known respects except for an extremely low content of potassium (0.01 per cent) lesions have been produced in the heart and kidneys of rats restricted to this diet. In the heart, necrosis of the myocardial fibers followed by scarring has been described. Necrosis of the renal tubular epithelium with regeneration and tubular dilatation was also observed. The exact mechanism for the production of these lesions has not been elucidated.

Note: The technical work was performed by Miriam C. Reed and the photomicrographs were made by Milton Kougl.

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DESCRIPTION OF PLATES

PLATE 6

FIG. 1. Fresh lesion in the myocardium of a potassium-deficient animal showing leukocytic infiltration about necrotic muscle fibers. × 400.

FIG. 2. More extensive lesion in the myocardium of a potassium-deficient rat showing diffuse mononuclear infiltration after 15 days on the diet. × 100.

FIG. 3. Photomicrograph showing scarring in the myocardium of a potassium-deficient animal after being on the diet 87 days. × 100.

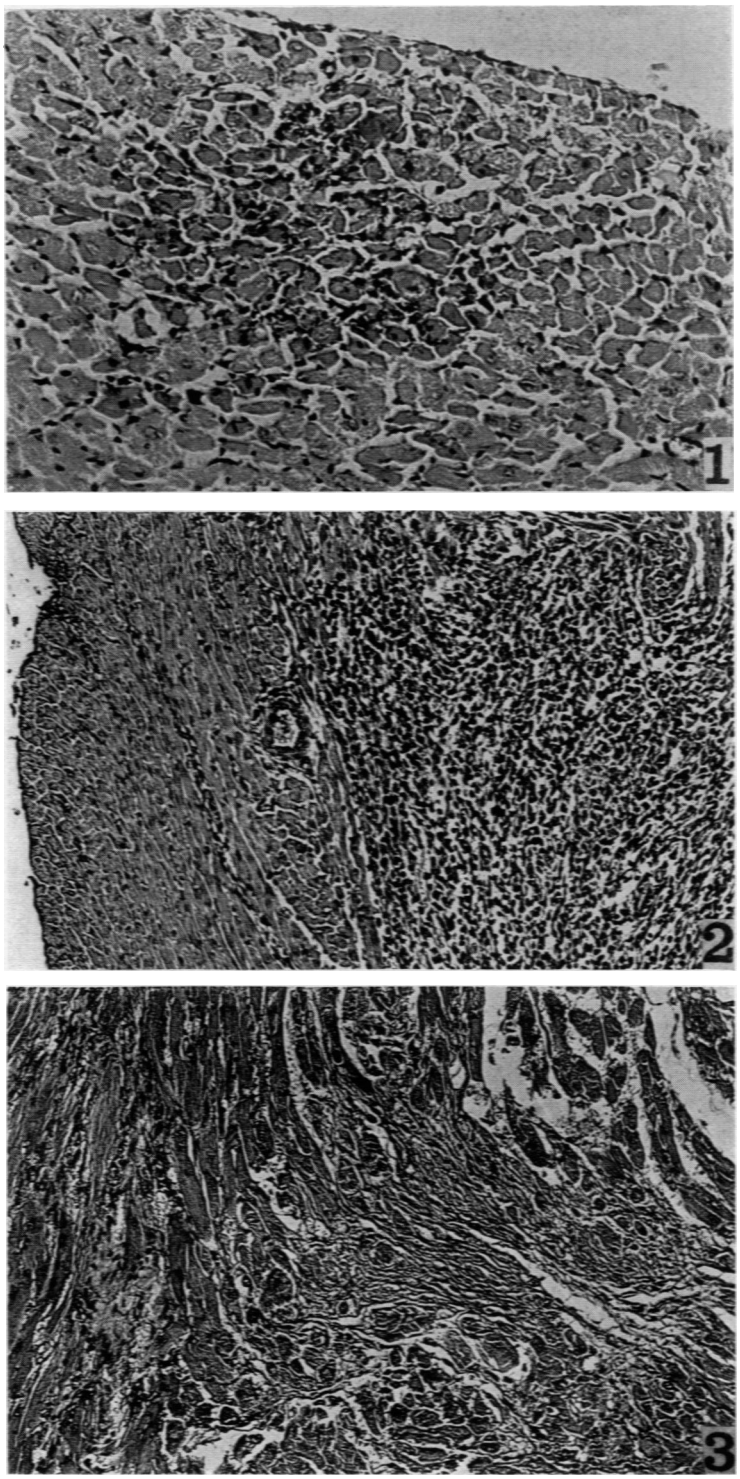
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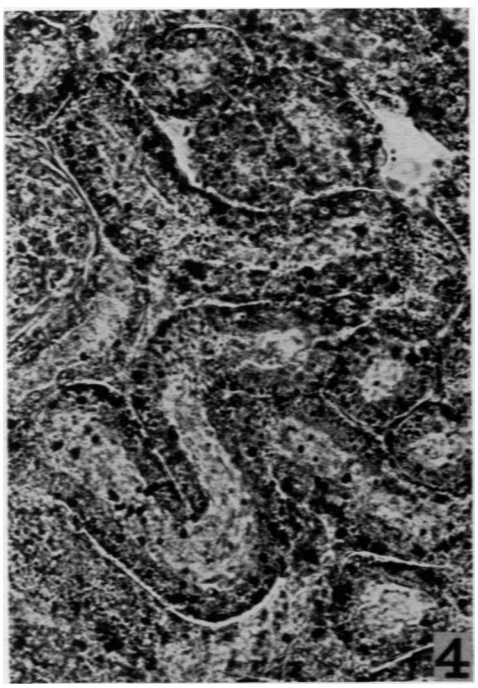
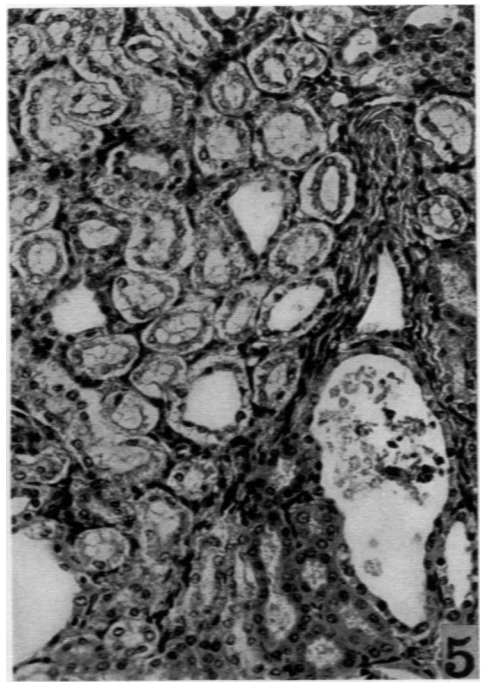
PLATE 7

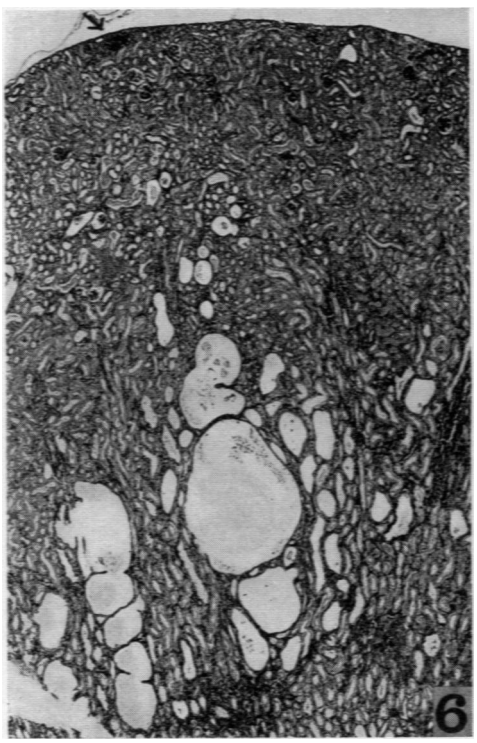
FIG. 4. Frozen section of kidney from a rat on the potassium-deficient diet for 13 days, showing lipid droplets in tubular epithelium (stained with Scharlach R). × 200.

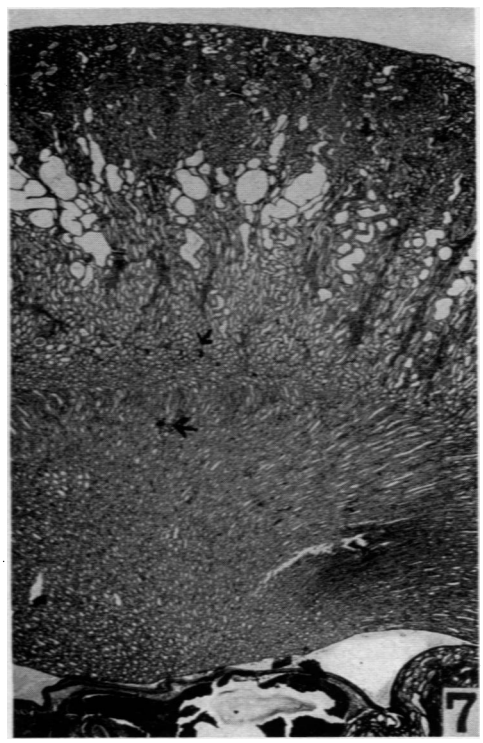
FIG. 5. Photomicrograph showing vacuolated tubular epithelial cells and a dilated tubule containing necrotic cells and débris, from a rat on the potassium-deficient diet for 15 days. × 200.

FIGS. 6 and 7. Medium and low power views of kidney from a rat on the potassium-deficient diet for 6 weeks showing dilatation of the tubules. Arrows point to small collections of calcified material in the tubules. × 90 and × 15.









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Lesions with Potassium Deficiency