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Concerning the amount and distribution of stainable lipoid material in renal epithelium in normal and acutely nephropathic animals, with observations on the functional response of the kidney.

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The following observations are based upon a study of twenty-six dogs. The animals were under two years old. The dogs were placed in metabolism cages and studied for eight days prior to any experimental interference. During this period, as well as during the period of the experiments, the animals were given 250 c.c. of water twice a day by stomach tube. The diet consisted of scraps of bread and cooked meat. The urine was collected once a day and examined qualitatively for albumin and glucose. The phenolsulphonephthalein test for renal function was made every other day according to the technique of Rowntree and Geraghty. Daily determinations of the alkali reserve of the blood (R.p.H.) were made by the method of Marriott. Blood-urea determinations were made by the method of Marshall as modified by Van Slyke and Cullen.

At the end of the eight-day period allowed for normal observations, seven of the animals were killed without the use of an anesthetic and used as control experiments. The remaining nineteen animals were given one subcutaneous injection of 6 mgs. of uranium nitrate per kilogram. Following the use of uranium, observations similar to those previously outlined were continued. The animals that were given uranium were killed without the use of an anesthetic 6 hours, 12 hours, 24 hours and 48 hours following the commencement of the intoxication. At the termination of the observations on both the normal control animals and the acutely nephropathic animals, kidney tissue was at once obtained for microscopic study. Sections of tissue extending through each lateral half of both kidneys were placed in isotonic salt solution

and without any fixation were frozen, sections made, and stained for lipoid material by Herxheimer's Scharlach R. method. Such sections were counterstained with Mayer's Haemalum. Other tissue from both kidneys was fixed with formaline, Zenker's fluid, and in corrosive-acetic, imbedded in either paraffin or celloidin, and used for a general histological study.

NORMAL CONTROL ANIMALS.

During the eight days of observation, urine formation by the seven normal control animals has varied from a minimum output of 385 c.c. to a maximum output of 621 c.c. The urine was free from both albumin and glucose. The elimination of phenolsulphonephthalein by the respective animals in a two-hour period varied from 65 per cent. to 80 per cent. The blood urea varied from 12 to 18 mgs. per 100 c.c. of blood. The reserve alkali of the blood was normal and gave readings between 8.0 to 8.1.

When such animals were killed without the use of an anesthetic and kidney tissue studied for the amount and distribution of stainable lipoid material by the use of Scharlach R., the following observations were made. The endothelium of the glomerular capillaries and other vascular tissue of the kidney failed to show the presence of stainable lipoid. The convoluted tubule epithelium in young animals such as have been used in this study does not show the presence of stainable lipoid with Scharlach R. In old normal animals as has been previously noted,[1] stainable lipoid may appear in the epithelium of this portion of the tubule in the form of fine dust-like particles. All of the normal control animals show stainable lipoid in both the descending and ascending limbs of Henle's loops. In this portion of the tubule such material appears as small particles or fused droplets.

The study of the normal control group of animals indicates that stainable lipoid as demonstrated by Scharlach R. with Herxheimer's technique of staining is confined to the epithelial cells of the loops of Henle. Lipoid material in this location has no harmful effect on the functional capacity of the kidney and does not interfere with that function of the kidney which is concerned with maintaining a normal acid-base equilibrium of the blood.

[1] MacNider, Wm. deB., *Jour. Pharm. and Exp. Therap.*, 1921, xvii, 289.

ACUTELY NEPHROPATHIC ANIMALS.

The experiments on the animals acutely nephropathic from uranium were terminated as follows. Four animals were killed six hours after the administration of uranium, five at the end of twelve hours, five at the end of twenty-four hours and the remaining five animals at the end of forty-eight hours.

The four animals killed six hours following the administration of uranium showed no change in the normal functional response of the kidney. During this period the animal of Experiment 4 formed 129 c.c. of urine. The urine was free from both albumin and glucose. Casts were not present. The elimination of phenolsulphonephthalein during the last two hours of the intoxication was 75 per cent. as compared with the output of 72 per cent. of the dye prior to the use of uranium. The blood urea remained unchanged from the normal reading of 18 mg. per 100 c.c. of blood. The reserve alkali of the blood was unaffected and remained at the normal reading of 8.1.

A study of the kidneys of the four animals killed at this early period of the intoxication shows the glomeruli to be normal. Stainable lipoid is not present in the endothelium of the capillaries. The stainable lipoid in the cells of the loops of Henle, especially in the cells of the ascending limb of the loop, shows an increase in amount when compared with the amount of such material that can be demonstrated in this location in the normal control animals. The lipoid is in the form of granules and well-defined droplets. The convoluted tubule epithelium failed to show stainable lipoid with Scharlach R. at this early stage of the intoxication. These cells appear normal.

The five animals killed at the end of twelve hours following the use of uranium have all shown some change from the normal in the functional response of the kidney. In the urine from three of the animals both albumin and glucose were present. The urine of the two remaining animals contained a trace of glucose but no albumin. The results obtained in Experiment 7 are characteristic for this group. During the twelve-hour period of the experiment the animal formed 322 c.c. of urine. Heavy traces of both albumin and glucose were present. The elimination of phenolsulphonephthalein was reduced from the normal of 70 per cent. to 55

per cent. Blood urea was unchanged. The reserve alkali of the blood was reduced from 8.05 to 7.95.

Frozen sections from the kidneys of this group of animals when stained with Scharlach R. show an increase in the amount of stainable lipoid in the cells of the loops of Henle and, furthermore, at this stage of the uranium intoxication, stainable lipoid in the form of dust-like particles appears in the convoluted tubule epithelium. The granules are more marked in the periphery of the cells than in the area immediately around the nuclei. Other than these changes in the stainable lipoid content of the tubular epithelium the kidney tissue appears normal. A study of the course of the intoxication to this point shows the first evidence of an injury to the kidney from uranium to consist of inducing such a disturbance in the cells of the loops of Henle that an increase over the normal of stainable lipoid can be demonstrated in these cells. Such a change in the stainable lipoid content of these cells is not associated with any functional disturbance on the part of the kidney. At a later period in the intoxication, after twelve hours, lipoid material stainable with Scharlach R. appears in the convoluted tubule epithelium. With this evidence of injury to these cells the elimination of phenolsulphonephhtalein is reduced; there is a beginning depletion in the alkali reserve of the blood, and albumin and glucose or glucose alone appear in the urine. The total output of urine in such animals is apparently unaffected.

Five animals were killed at the end of the twenty-four-hour period of the intoxication. The formation of urine at this stage of the experiments shows an increase over the normal daily output for the respective animals. The urine from all of the animals shows albumin and glucose. Granular casts are present. The elimination of phenolsulphonephthalein is further reduced. There is no retention of blood urea. The reserve alkali of the blood was depleted in all of the animals.

Experiment 10 is representative of the group. The average daily output of urine for this animal before the commencement of the intoxication was 410 c.c. The urine increased to 618 c.c. on the first day following the use of uranium. The urine contained 1.8 gm. of albumin per liter and 1.1 per cent. glucose. The elimination of phenolsulphonephthalein was reduced from the

normal of 75 per cent. to 30 per cent. There was no retention of blood urea. The reserve alkali of the blood was reduced from 8.05 to 7.9.

Frozen sections from the kidneys of the animals at this stage of the intoxication after staining with Scharlach R. show very little change in the amount of stainable lipoid in the cells of the loops of Henle. Such material is abundant and in the form of droplets and fused masses. There is a marked increase in the amount of stainable lipoid in the cells of the convoluted tubules. In this portion of the tubule the small particles that have been described as appearing in this location at an earlier period of the intoxication have fused so as to form small droplets which are numerous. In addition to this change in the convoluted tubule epithelium, these cells show marked cloudy swelling and a commencing vacuolation. The capillaries of the glomeruli are engorged with blood. They fail to show the presence of stainable lipoid or other evidence of injury.

The remaining five animals were killed at the end of forty-eight hours of the intoxication. In two of the animals the formation of urine was in excess of the normal daily output. In three of the animals there was a reduction in urine formation. Urine from all of the animals has shown an increase in albumin and glucose over that observed at the end of twenty-four hours of the intoxication. The elimination of phenosulphonephthalein has shown a progressive decrease. Only two of the animals show a retention of blood urea. The reserve alkali of the blood shows a progessive depletion. The results obtained in Experiment 16 are representative of the group. The formation of urine was reduced from the average normal daily output of 421 c.c. to 248 c.c. The urine contained 4.7 gm. of albumin per liter and 2.08 per cent. glucose. The elimination of phenolsulphonephthalein was reduced from the normal output of 68 per cent. to 10 per cent. Blood urea had increased from 14 to 42 mg. per 100 c.c. of blood. The reserve alkali of the blood was reduced from 8.1 to 7.85.

Frozen sections from the kidneys of these animals when stained with Scharlach R. show little if any increase in the amount of stainable lipoid material in the cells of the loops of Henle or in the convoluted tubule epithelium. Other changes of degeneration in

these cells that have been preceded by the appearance of stainable lipoid have become more marked. The cells show an advanced swelling, which frequently obliterates the lumen of the tubules. Vacuolation and necrosis are well advanced in many of the cells, especially in those of the convoluted tubules. The glomerular vessels are engorged with blood. Occasionally a slight exudate is seen in the subcapsular space. The endothelium of the capillaries has failed to show stainable lipoid.

CONCLUSIONS.

1. Lipoid material stainable with Scharlach R. is constantly found in the cells of the loops of Henle in normal dogs. The presence of such material in this location is not indicative of a pathological kidney. The functional capacity of such a kidney is normal.

2. When animals are given one subcutaneous injection of 6 mg. of uranium nitrate per kilogram, the earliest evidence of injury to the kidney consists of an increase in the amount of stainable lipoid in the cells of the loops of Henle. At this stage of the intoxication there is no evidence of a functional disturbance on the part of the kidney and no change takes place in the acid-base equilibrium of the blood.

3. At a later stage of such an intoxication (12 hours) stainable lipoid material appears in the convoluted tubule epithelium. The vascular tissue of the kidney is uninjured. Associated with such a disturbance in the metabolism of these cells that leads to the appearance of stainable lipoid in the cell there occurs a reduction in the elimination of phenolsulphonephthalein, a depletion in the alkali reserve of the blood and the appearance of albumin and glucose, or glucose alone in the urine.

4. Following this initial injury to the tubular epithelium changes of a more distinctly degenerative type appear in these cells and the functional capacity of the kidney is more severely impaired.