Shift Toward Anaerobic Glycolysis in the Regenerating Rat Kidney

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IN 1924 WARBURG *et al* found increased anaerobic glycolysis in some malignant tissues.[1] Since that time others have reported a relationship between the metabolism of glucose to lactate and malignant change in selected, but not in all, neoplasms.[2-4] The proliferating tissues of several regenerating organs have been investigated in this respect, but no clear relationship has been demonstrated. Regenerating liver has a decreased concentration of aerobic respiratory enzymes, but there is no apparent increase in tissue lactate concentration, and changes in glycogen content are equivocal.[5] Needham reviewed the evidence from many animal forms that the "regressive" or early phase of regeneration is accompanied by a decreased oxygen consumption, but that the "progressive" or later phase is associated with an increase in oxygen consumption.[6]

The production of lactate from glucose involves the coupled production of 2 moles of adenosine triphosphate (ATP) per mole of glucose; the pathway can exist in the absence of mitochondria since the utilization and production of reduced nicotinamide adenine dinucleotide (NADH) are equal. Induction of the anaerobic glycolysis pathway during regeneration would not only increase levels of pyruvate, an important intermediate in the biosynthetic pathways of proteins and lipids, but would also provide ATP in the absence of mitochondria.

The hexose monophosphate shunt (HMP shunt) has been shown to increase in regenerating tissues.[7,8] This pathway is usually evaluated by the activity of the first enzymes in it, the HMP shunt dehydrogenases, which produce reduced nicotinamide adenine dinucleotide phosphate (NADPH) from oxidized nicotinamide adenine dinucleotide ($NADP^+$). The NADPH produced by this pathway, and the triose phosphates which can also be derived from it, are essential for lipogenesis. The pathway also produces ribose, necessary for polyribonucleotide synthesis.

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Supported by Research Grant Am-12064 from the US Public Health Service.

Accepted for Publication May 14, 1970.

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Previous studies in this laboratory have shown that uniform proximal tubular necrosis may be obtained in rats by a single intravenous injection of 0.15 mg mercuric chloride/100 g body weight. Light and electron microscopic studies have shown a few residual cells at 1 day after injection, with subsequent marked cell replication to completely reline the tubules by 5 days.[9] Tritiated thymidine labeling of nuclei, mitotic index counts, and deoxyribonucleotide (DNA) assays have shown a peak of mitosis at approximately 3 days.[10] Renal function returns to normal between 7 and 14 days after the administration of mercuric chloride.[9]

The purpose of this experiment was to study the pathways of anaerobic glycolysis and the HMP shunt in the rat kidney, and to determine whether the activity of either of these energy pathways changes during regeneration of kidney cortex. The uptake of glucose and lactate from blood was studied to indicate the activity of these pathways in the kidney *in situ*.

Methods

Production of Renal Proximal Tubular Necrosis

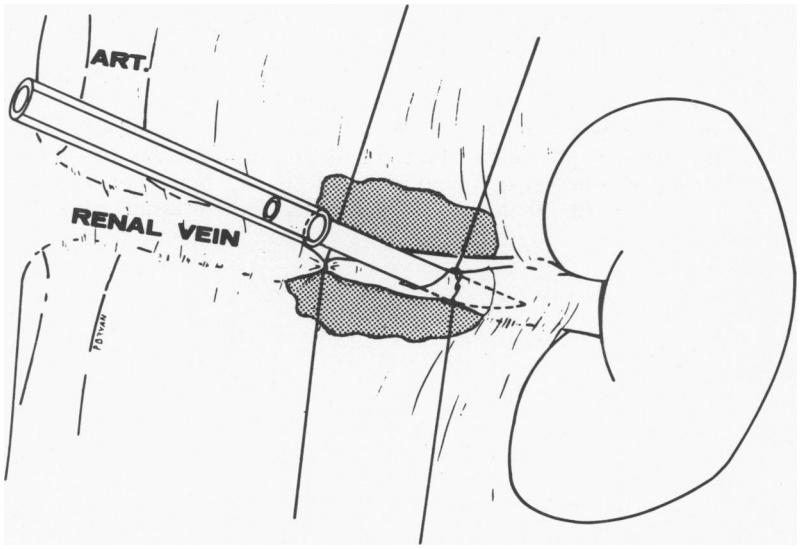
Male Sprague-Dawley rats, weighing from 150 to 200 g, were obtained from the Charles River farms and fed Purina rat chow and unrestricted amounts of tap water. After preliminary experiments using rats for standardization of assays, a total of 48 rats was used for the investigation. Each of 38 was injected with 0.15 mg mercuric chloride/100 g body weight, by tail vein, under pentobarbital anesthesia (0.2 ml of 2% solution of sodium pentobarbital/100 g body weight, injected intraperitoneally). Groups of 2–6 rats were studied at various intervals between 1 and 21 days after the injection of mercuric chloride. An additional group of 10 control rats, anesthetized but not previously injected with mercuric chloride, was similarly studied at appropriate intervals.

Blood Glucose and Lactate Assays

Rats were anesthetized with the above procedure, and a T-shaped incision was made in the anterior abdominal wall. Viscera were laid to the right and wrapped in moist saline gauze. A small horizontal incision was made in the posterior parietal peritoneum, and the left renal vein was separated from the left renal artery by blunt dissection. The peritoneum above the renal pedicle was incised, two ligatures were placed around the renal vein, and the one nearest the vena cava tightened. An 18-gauge needle with attached plastic tubing (Clay-Adams PE 190 polyethylene) was inserted into the renal vein and the second venous ligature tightened around the vein and indwelling catheter (Text-fig 1). The renal vein flow was interrupted for approximately 10 sec. Blood from the catheter was collected for 1 min in a precooled, heparinized, graduated 2-ml cylinder, and the amount recorded. The venous catheter was then removed and the ligature nearest the kidney tightened. A blood sample was quickly drawn from the abdominal aorta. The two kidneys were then removed and placed in beakers of

cold KCl-Tris buffer containing 0.15 M KCl, 0.005 M Tris, 0.005 M EDTA, and 0.005 M $MgCl_2$. Previous studies in this laboratory have determined that the average urine output of rats after mercuric chloride poisoning, under these circumstances, is 10–20 ml/24 hr, or 0.007–0.014 ml/min. Since measurements of renal vein effluent were accurate only to ±0.1 ml, the effluent approximates the renal blood flow (afferent) without correcting for urine production. Similarly, lymph flow may be disregarded, and in this experiment the term renal blood flow is equivalent to renal vein effluent.

Blood glucose was assayed with Glucostat enzymic reagents (Worthington Biochemical Corporation, Cleveland, Ohio) according to the semimicro method described in their accompanying literature. Blood lactate was assayed with lactic dehydrogenase (LDH), NAD^+, and hydrazine, according to the method of Hohorst.[11] A small amount of color developed in mixtures without blood extract, and a blank of this type was run for each assay. Arteriovenous concentration differences of both glucose and lactate were multiplied by renal blood flow to give uptake or production in terms of μM/min.



TEXT-FIG 1. Method of obtaining renal vein samples. An 18-gauge needle with attached plastic tubing was inserted into left renal vein and held in place by a ligature.

Preparation of Homogenates

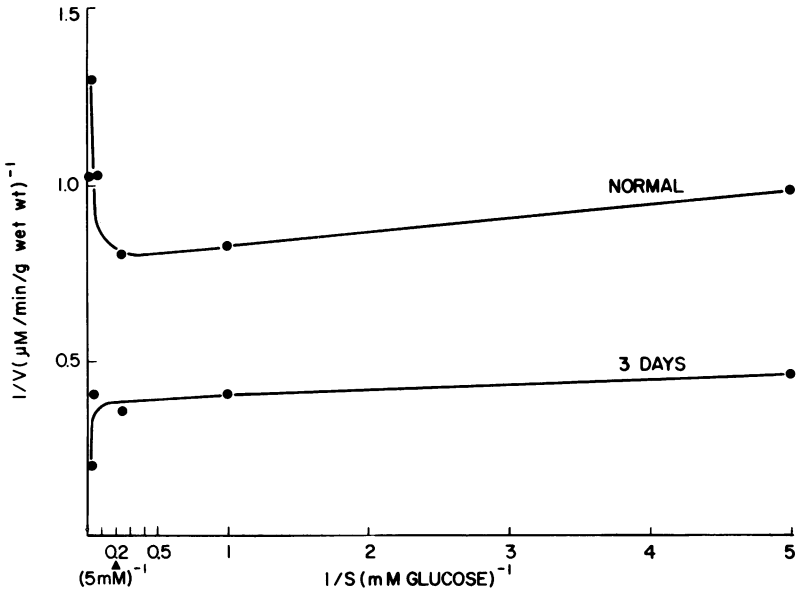
Each kidney was sliced under cold KCl-Tris buffer into six transverse (horizontal) sections. All the following procedures, except the enzyme assays, were performed on ice or in a centrifuge at 2 C. The transverse tissue slices, except the two poles, were dissected at the corticomedullary junction, and the cortex was then bisected into outer and inner parts. Pieces of outer cortex, inner cortex and medulla were combined from both kidneys and weighed. Each portion of the kidney was then homogenized with KCl-Tris buffer (20 times v/w) in a Dounce hand homogenizer. This buffer is similar to that used by DiPietro in the study of glucokinase and hexokinase,[12] and to that used by Dies and Lotspeich to assay the HMP shunt dehydrogenases.[13] The homogenates were centrifuged for 5 min at 1000 × *g*,

and the supernatant decanted in order to clear visible particles and decrease diffraction in the spectrophotometer.

Glucose Phosphorylating Assays

The assay of glucose phosphorylation was performed with exogenous glucose-6-phosphate dehydrogenase (G6PD) (Type X, Sigma Chemical Co), following the method of DiPietro.[12] A volume of 0.02 ml of the homogenate was added to the reaction mixture. The optical density (OD) at 340 mμ was observed at 9 and 15 min after adding the homogenate and placing the cuvet in the spectrophotometer at 37 C. The rate was found to be linear with time up to 30 min. In many homogenates a significant rate was found at 0 mM glucose concentration. This rate was subtracted from those recorded at 0.2–100 mM glucose concentrations, and in no case was this more than 30% of the rate with glucose. Numerous checks were made upon the validity of the assay: the velocity was linear with respect to homogenate concentration at all glucose concentrations; NADPH was formed in approximately 1:1 ratio to glucose-6-phosphate when limited glucose-6-phosphate was added; the activity of homogenates was tested with time and found to increase slightly over 8 hr; 10^{-3} to 10^{-2} M cyanide was added without effect; and mercuric chloride was added without significant effect.

Preliminary assays of homogenates with 0, 0.2, 1, 5, 20, 50 and 100 mM glucose showed a linear 1/V vs 1/S plot for concentrations between 0.2 and 5 mM, but marked deviations between the 5 and 100 mM rates (see Text-fig 2). Thereafter, most assays for glucose phosphorylating activity in homogenates of experimental rat kidneys were performed at 0, 5, and 100 mM glucose. Some homogenates were assayed at 0, 0.2, 1, 5, 20, 50 and 100 mM. Results of these assays were expressed



TEXT-FIG 2. Typical plots of 1/V vs 1/S for glucose phosphorylating activity in homogenates of inner cortex from control rat and experimental rat (3 days after mercuric chloride injection). Changes in 5 and 100 mM rates are evident, but K_m of linear part of plots is not changed.

as μM of NADPH formed/min/g wet weight, and the assumption made that each mole of glucose-6-phosphate formed resulted in 1 mole of NADPH. The grams wet weight of tissue per assay were calculated from the fraction of homogenate used, disregarding the small fraction removed as residue in the centrifugation.

HMP Shunt Dehydrogenase Assays

Assay of HMP shunt dehydrogenase activity was performed according to the method of Glock and McLean, except 10 mM rather than 2 mM glucose-6-phosphate was used.[14] No significant rate was found in the absence of glucose-6-phosphate. Rates of change of OD were linear with respect to homogenate concentration. Rates were expressed as μM NADPH produced/min/g wet weight, as in the phosphorylation assays.

Statistical Analysis

The results of all uptake studies and enzyme assasy were expressed as ±1 standard deviation. Student's t tests were performed on all differences between normal and regenerating kidney values, and one-sided P values were calculated from these t values.

Results

Gross Renal Changes and Renal Blood Flow

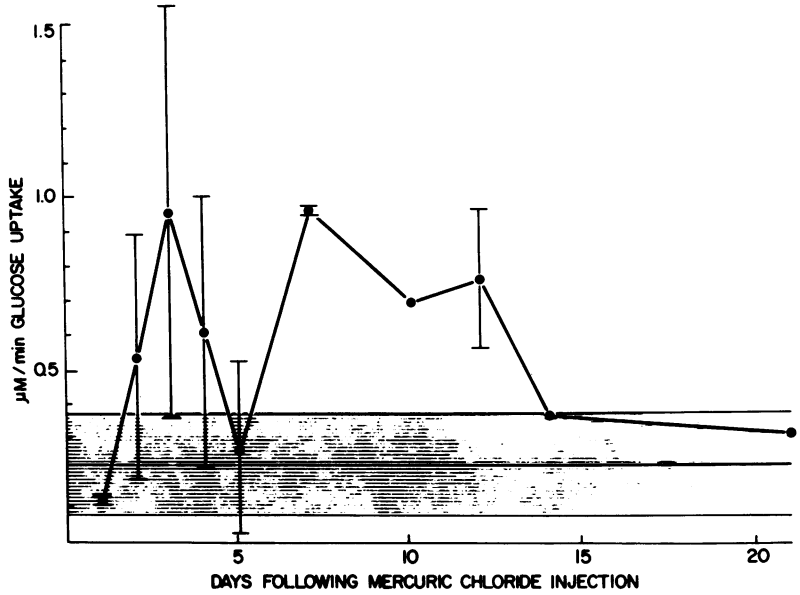
Five percent of the animals injected with mercuric chloride died during the 3 weeks of follow-up and were excluded from the study. At laparotomy, the kidneys of the remaining experimental rats appeared slightly pale, mottled, and swollen during the first 5 days. On section, a well-demarcated area of necrosis was visible in the inner cortex during the first 5 days. Subsequently, the kidney returned to a normal gross appearance.

The renal blood flow of the uninjected control rats was variable: 0.91 ± 0.43 ml/min. The renal blood flow of rats after mercuric chloride injection was not significantly different from normal: 1.07 ± 0.49 ml/min. There was a slight increase during the 3 weeks after mercuric chloride injection: 0.03 ml/min/day when analyzed by least squares ($r = 0.37$).

Blood Glucose Assays

Kidneys of control rats had a glucose uptake of 0.22 ± 0.14 μM/min. This rate did not change during the 3-week experimental interval. The average arterial glucose concentration was 5.71 μM/ml; the average renal uptake was 4% of the afferent glucose.

Significant changes in glucose uptake occurred during regeneration in the rats receiving mercuric chloride (Text-fig 3). Glucose uptake at 1 day was at lower limits of normal, at 2 days was above control levels,



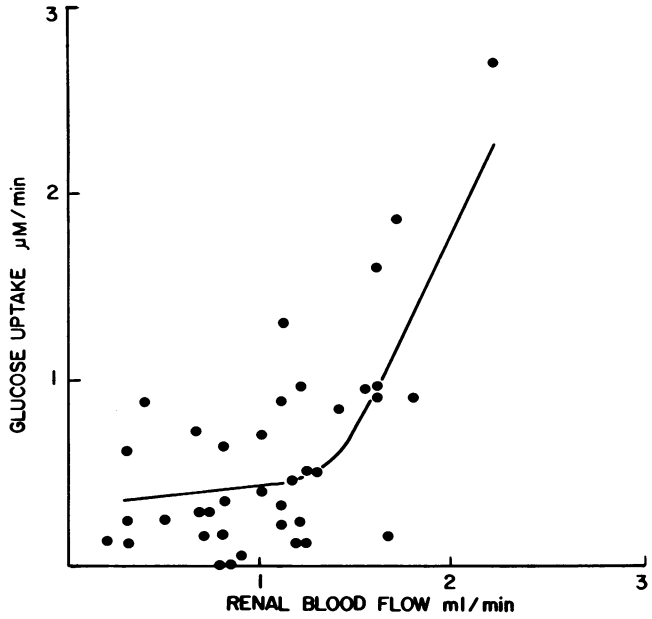
TEXT-FIG 3. Glucose uptake by left kidney *in situ*. Biphasic nature of uptake is noted. Shaded area indicates control levels ± 1 SD.

and at 3 days reached a peak ($P < 0.01$). The level decreased to control levels at 5 days, then rose again to a second peak between 7 and 12 days ($P < 0.0005$ for this period). Uptake was normal by 21 days. Arterial glucose concentrations during the 3-week period similar to that of the control group showed no changes (5.71 ± 0.71 μM/ml). There were no peaks in renal blood flow during the 3-week experimental interval. However, renal blood flow was variable (see above), and there was a correlation between glucose uptake and renal blood flow, at higher flow rates (Text-fig 4).

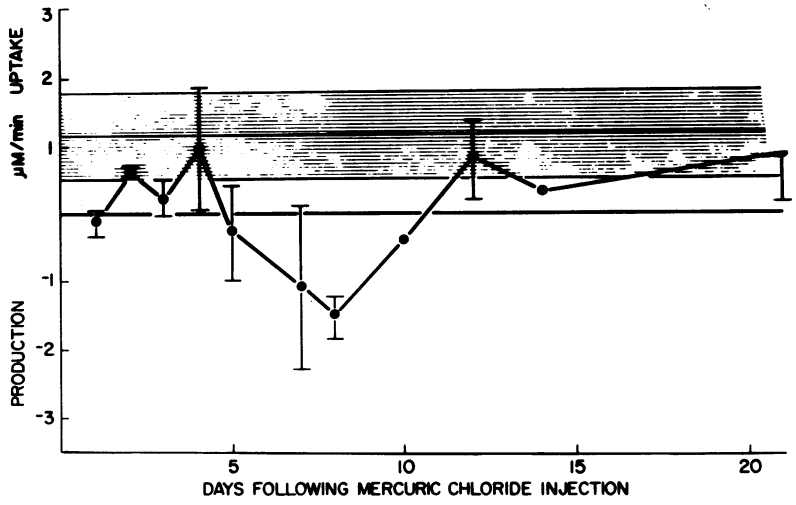
Blood Lactate Assays

The kidneys of control rats had a lactate uptake of 1.14 ± 0.63 μM/min. There was no trend in this value during the 3 weeks. The average arterial lactate concentration of the control rats was 1.63 ± 0.92 μM/ml. The average afferent lactate was 1.9 μM/min, and thus the average percent uptake of afferent lactate was 60%.

Lactate uptake in the regenerating kidneys decreased to near zero on the first day after injection ($P < 0.025$) but was generally within normal limits during Days 2–4 (Text-fig 5). During Days 5 through 10, the kidney produced lactate rather than extracted it, with a peak production of 1.5 ± 0.28 μM/min appearing at 8 days ($P < 0.0005$).



TEXT-FIG 4. Glucose uptake vs renal blood flow for the left kidney *in situ*. Elevated renal blood flow occurred randomly during the 21 days after injection.

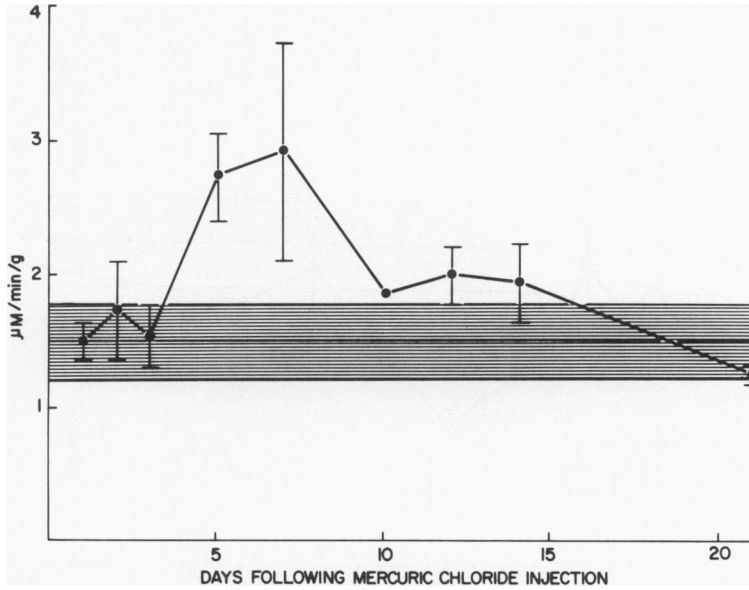


TEXT-FIG 5. Lactate uptake and production by the kidney *in situ*. Shaded area represents control levels of uptake ± 1 SD. Shift from uptake to production begins 5 days after injection.

Levels of uptake were normal at 12–21 days. During the 3-week interval, arterial lactate levels were varied but averaged normal (1.20 ± 0.74 μM/ml). There were slightly diminished levels on Day 1. A plot of lactate uptake versus renal blood flow showed no obvious trend ($r = 0.045$). Lactate uptake also did not correlate with arterial lactate concentration ($r = 0.05$).

Glucose Phosphorylation

Homogenates of inner cortex from control animals phosphorylated glucose at a rate of 1.31 ± 0.35 μM/min/g at 5 mM and 1.38 ± 0.29 at 100 mM. Outer cortex homogenates phosphorylated 1.69 ± 0.49 μM/min/g at 5 mM and 1.50 ± 0.14 at 100 mM. The medullary homogenates phosphorylated 2.16 ± 1.23 μM/min/g at 5 mM and 1.91 ± 1.15 at 100 mM. K_m values were determined on some of these homogenates by analyzing assays performed between 0.2 and 5.0 mM glucose, since this part of the 1/V vs 1/S plot was linear (Text-fig 2). The K_m for the outer cortex was 0.06 ± 0.02 mM (four homogenates); the K_m for the inner cortex was 0.13 ± 0.08 mM (six homogenates), and the K_m for the medulla was 0.05 mM (three homogenates). These

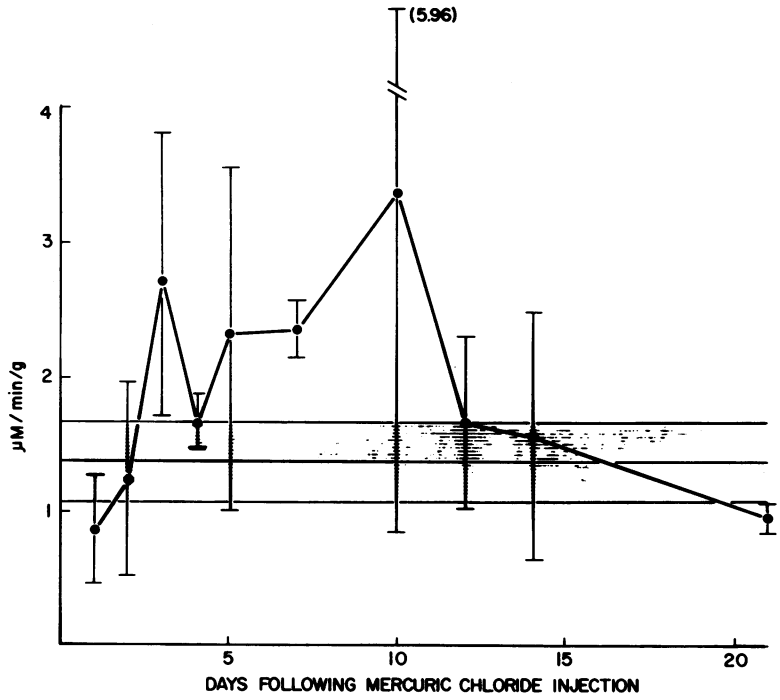


TEXT-FIG 6. Glucose phosphorylating activity in homogenates of outer cortex, assayed at 100 mM glucose concentration. There is significant increase during later regeneration, but not during early regeneration. Shaded area represents control values ± 1 SD.

K_m values are statistically equivalent for the three portions of kidney.

Glucose phosphorylating activity increased significantly in homogenates of inner and outer cortex during regeneration, but not in homogenates of medulla (Text-fig 6–8). When assayed at 100 mM, glucose phosphorylating activity was elevated to approximately twice normal between Days 5–12 in both the inner and outer cortex ($P < 0.0005$ for both). However, a separate earlier peak occurred in the activity of the inner cortex. After a decrease to approximately one-half normal on Day 1 ($P < 0.01$), there was a continual increase to twice normal on Day 3 ($P < 0.01$). There was then a decrease to normal by Day 4. This early peak paralleled the early peak in glucose uptake (Text-fig 3).

Changes in glucose phosphorylating activity measured at 5 mM were similar to those described above for the 100-mM assays, but levels were elevated only 50% above normal during the periods when levels were elevated approximately 100% by the 100-mM assays. P values were still significant: $P < 0.0025$ for inner cortex, 3 days; $P <$

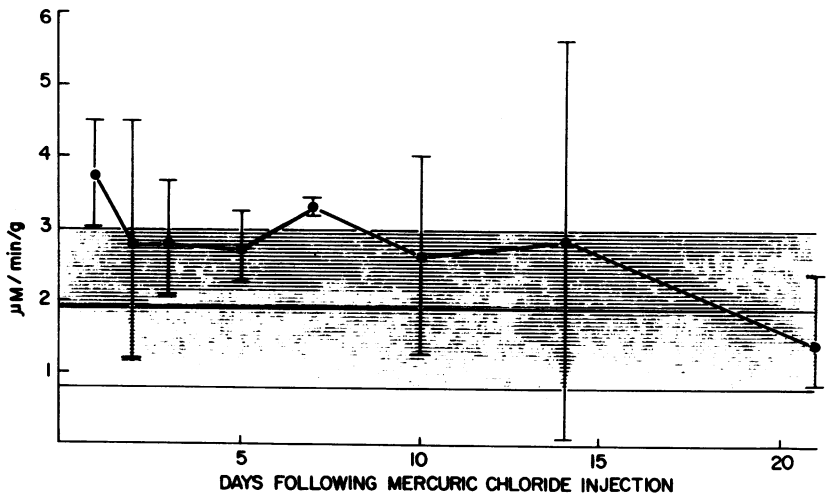


TEXT-FIG 7. Glucose phosphorylating activity in homogenates of inner cortex assayed at 100 mM glucose. Biphasic peak is apparent. Shaded area represents control values ± 1 SD.

0.0025 for outer cortex, 5–7 days; $P < 0.0025$ for inner cortex, 5–12 days. There were no changes from normal levels in assays of homogenates of medulla.

Plots of 1/V vs 1/S for homogenates of regenerating kidney were consistently linear between 5 and 0.2 M, as were plots of normal kidneys (Text-fig 2). K_m values derived from this part of the plot showed no change from control values and no consistent change during the 21-day experimental period; outer cortex, 0.09 ± 0.05 mM; inner cortex, 0.11 ± 0.08 mM; and medulla 0.07 ± 0.06 mM.

An explanation was sought for the significant differences between 5 mM and 100 mM rates of cortical homogenates of both normal and regenerating kidneys. Several solutions were found to cause a specific increase of the 100 mM rate over the 5 mM rate. The addition of 0.02 ml of boiled extract of outer cortex to the assays of inner cortex increased the 100 mM rate but not the 5 mM rate. This type extract produced no effect upon assays of medullary homogenates. The addition of 10^{-4} M $NaHPO_4$ to the assay of inner cortex homogenates increased the 100 mM rate more than the 5 mM but had no effect upon assays of medulla homogenates. Addition of 10^{-5} M N-acetyl glucosamine in the assay of inner cortex caused increases of the 100 mM rate but not the 5 mM rate, and caused decreases of rates in medullary homogenates.

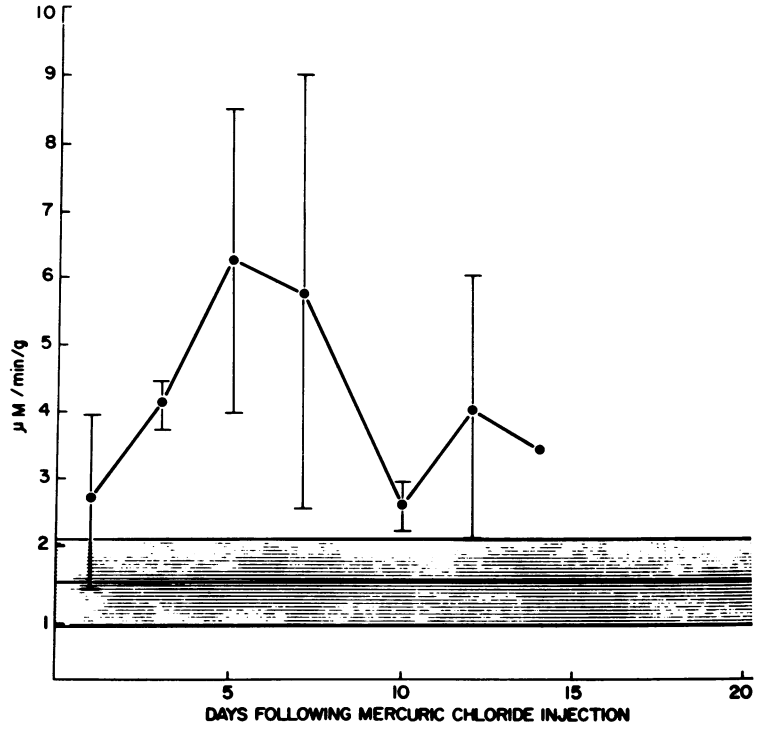


TEXT-FIG 8. Glucose phosphorylating activity in homogenates of medulla assayed at 100 mM glucose. No significant increase is noticed. Shaded area represents control values ± 1 SD.

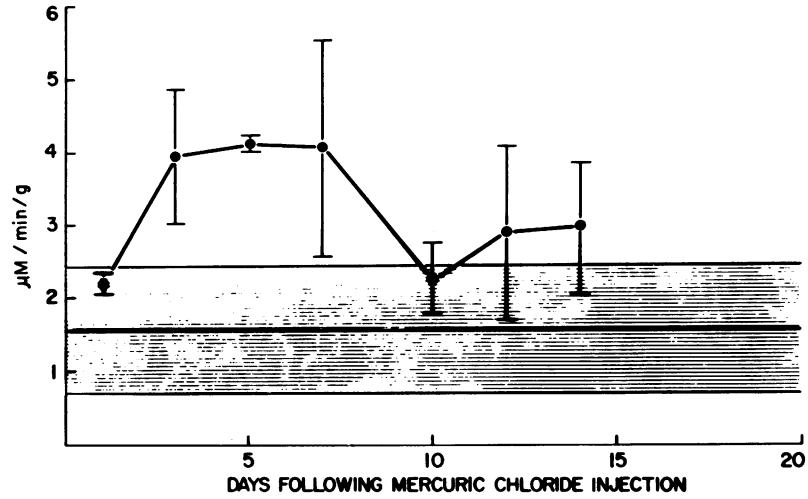
HMP Shunt Dehydrogenase Assays

HMP shunt dehydrogenase activity in homogenates of outer cortex from control kidneys was 1.50 ± 0.66 μM/min/g weight. Homogenates of inner cortex had an activity of 1.59 ± 0.84 μM/min/g, and the medulla, 1.8 ± 0.78. There was no trend seen during the 3-week experimental interval.

During regeneration, the HMP shunt dehydrogenases increased to four times normal in homogenates of outer cortex ($P < 0.005$) with a peak at 5 days (Text-fig 9). The dehydrogenases increased 2.5 times in homogenates of inner cortex ($P < 0.0005$) (Text-fig 10). Increases were less significant in the medulla (Text-fig 11). The HMP shunt dehydrogenase increase of the inner and outer cortex precedes the early increase of glucose phosphorylation by the inner cortex and of glucose uptake by the kidney *in situ*.



TEXT-FIG 9. HMP shunt dehydrogenase activity in homogenates of outer cortex. Increases are significant between 1 and 14 days. Shaded area represents control values ± 1 SD.

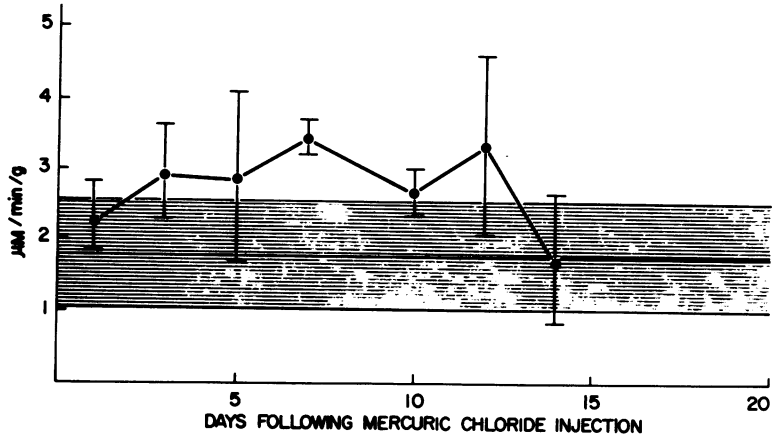


TEXT-FIG 10. HMP shunt dehydrogenase activity in homogenates of inner cortex. Increases are significant by Day 3. Shaded area represents control values ± 1 SD.

Discussion

Control Kidneys

The small glucose uptake demonstrated here for the normal rat kidney *in situ* is in accordance with studies of rat kidney slices and of dog kidney *in situ*.[15,16] It is possible that this slight net uptake is actually the sum of gluconeogenesis by the cortex and glucose uptake by the medulla, since previous studies have demonstrated the high gluconeo-



TEXT-FIG 11. HMP shunt dehydrogenase activity in homogenates of medulla. Shaded area represents control values ± 1 SD. Increases are barely significant.

genic ability of the cortex.[17] The high lactate uptake of kidney is in keeping with gluconeogenesis and high oxidation rate of the cortex.[18]

The glucose phosphorylating activities of the cortex in this study agree well with those of Grossbard and Schimke[19] and Shonk and Boxer.[20] The demonstrated K for activity of cortex and medulla is similar to values for hexokinase of rat kidney.[19]

Early Regenerative Phase

The early peak in glucose uptake is paralleled by changes of glucose phosphorylation in the homogenates of inner cortex, as assayed at either 5 or 100 mM glucose. During this time period, there are no changes in glucose phosphorylating activity in homogenates of the outer cortex or medulla. There is increased HMP shunt dehydrogenase activity in the inner and outer cortex 1 day before the rise of glucose uptake.

Glucose uptake by the kidney *in situ* increased approximately 0.75 μM/min during the first 3 days. The increase in glucose phosphorylating activity of the inner cortex during the first days was 1.3 μM/min/g. Since the average weight of inner cortex of each kidney was approximately 0.4 g, the total increase in glucose phosphorylating activity was 1.3×0.4, or 0.52 μM/min. This increase nearly accounts for the increase of glucose uptake during early regeneration. The HMP shunt dehydrogenase activity increased approximately 2.5 μM/min/g wet weight during this time, in both inner and outer cortex. If this increase was due to increases in $NADP^+$ reduction by both dehydrogenases in the shunt, the increase in rate of flow through the shunt would actually be 1.25 μM/min/g wet weight. This increase is comparable to the increase in levels of glucose phosphorylation in the inner cortex. Thus, a significant portion of the increase in glucose uptake and phosphorylation could be accounted for by increased passage of glucose-6-phosphate through the HMP shunt. However, the glycolytic pathway may also be active at this time, even though lactate is not produced by the kidney *in situ*.

It has been shown by light and electron microscopic studies that the major necrosis and regeneration after mercuric chloride injection occurs in the third portion of the proximal tubules.[9] The peak of cellular proliferation in the proximal tubules occurs at 3–4 days, as indicated by mitotic indices, labeling of nuclei by tritiated thymidine, incorporation of labeled precursors into DNA and RNA, and rate of extractable DNA increase.[10] By these measurements, rapid cellular proliferation has ceased by Day 7. Since increases in glucose phosphorylating and HMP shunt dehydrogenase activity occur within the first 3 days,

and since increases are limited to the cortex, where the necrosis and regeneration occurs, it is likely that the increases in these activities are related to increases in numbers of proximal tubule cells during this time. Since these enzyme activities rise above control levels at Day 3, when cellular replacement of the cortex is incomplete, and then decrease to normal before replacement is complete, it is likely that there are specific increases in these activities in each proximal tubular cell, above the normal levels. It may thus be concluded that these enzymes are in pathways which are of importance to the regenerating cells.

Later Regenerative Phase

A second peak in glucose uptake by the kidney *in situ* occurs between 7 and 12 days, to levels approximately four times normal. This is preceded by a second peak in glucose phosphorylating activity of both inner and outer cortex homogenates, between 5 and 12 days. During this time, lactate is produced in large amounts by the kidney *in situ*, rather than absorbed in large amounts as in the control animals.

The increase in glucose phosphorylating activity was approximately 1.5 μM/min/g above control levels in both the inner and outer cortex. Since the average weight of cortex from one kidney was 0.9 g, the total increase in glucose phosphorylating activity was 0.9×1.5 or 1.4 μM/min. This more than accounts for the increased glucose uptake by the kidney *in situ* during this time, 0.75 μM/min. The maximum production of lactate by the kidney *in situ* during later regeneration was approximately 1.5 μM/min. This production is greater in molar quantities that the increase in glucose uptake at this time, 0.75 μM/min, or the total glucose uptake at this time, 1.0 μM/min. In the Embden-Myerhof pathway, up to 2 moles of pyruvate may be produced from each mole of glucose utilized, and this could explain the molar excess of lactate production. Another explanation is that pyruvate was being produced from nonglucose sources, such as amino acids. Gluconeogenesis by nonregenerating parts of the kidney could also be partially masking increased glucose uptake by the regenerating proximal tubular cells.

The increases in glucose uptake and lactate production in the kidney *in situ* and in glucose phosphorylating activity in cortical homogenates suggest that there is a shift toward anaerobic glycolysis in the cortex during later regeneration. Those cells that are most likely to be changing at this time are those of the newly relined proximal tubules. The fact that the second peak of glucose phosphorylating activity occurs in both the inner and outer cortex may mean that the induction of this

enzyme activity occurs in the entire proximal tubule, including the proximal portions in the outer cortex, which were shown in previous studies to have less necrosis. The HMP shunt dehydrogenases remain elevated in the inner and outer cortex throughout the early and late phases of regeneration. If it is assumed that the flow through of this pathway is unchanged, the peak of glucose phosphorylation that occurs during later regeneration is likely a reflection of induction of the anaerobic glycolysis pathway. The fact that peaks of glucose uptake and lactate production parallel the peak in phosphorylation lends support to this conclusion.

One may postulate several stimuli for the induction of anaerobic glycolysis in the regenerating proximal tubule. The first is depletion of products of the pathway; the pathway may simply fill the void left in ATP production by decreased numbers of functioning mitochondria, or it may be stimulated by low ATP levels in locales of protein and fatty acid synthesis in the cell. The second possible stimulus is an increase in substrates and cofactors of the pathway. In this respect, the interstitial fluid and filtrate of the regenerating proximal tubule would likely be different in substrates from those of the normal nephron. The third possibility is that the pathway is induced by a primary stimulus in the nucleus for production of enzymes of the pathway.

Previous studies in this laboratory have shown that renal function returns to normal between 5 and 9 days after mercuric chloride injection.[9] Electron microscopy of the proximal tubular cells between 7 and 14 days has revealed decreasing nuclear to cytoplasmic ratios, developing microvillar brush borders, and increasing numbers of mitochondria, basilar unfolding, vesicles, and endoplasmic reticulum. At this time, between 5 and 14 days, a second peak occurs in the rate of tritiated uridine incorporation into RNA, similar in magnitude to the one between 1 and 5 days.[10] The peaks of RNA labeling closely resemble the peaks of glucose phosphorylation and glucose uptake in this study. All of the above processes would require large energy expenditures and availability of the intermediates of glycolysis, and are likely related to the increased anaerobic glycolysis at the time. The persistence of the HMP shunt would be important for the increased formation of RNA.

Completion of Regeneration

Within 14–21 days after the administration of mercuric chloride, the levels of glucose phosphorylation, HMP shunt dehydrogenases, glucose uptake and lactate production have returned to control levels. By light

and electron microscopy the tubular cells also appear normal by 21 days, at which time tubular function is also normal.[9,10]

Nature of Changes in Glucose Phosphorylating Activity

There are indications that the increase in glucose phosphorylating activity of cortical homogenates of regenerating kidney is due both to changes in amounts of a low K_m enzyme and to changes in activation of this enzyme. Increases in the levels of activity measured at 5 mM, very near the intercept of the straight portion of the 1/V vs 1/S plot, indicate an increased amount of a low K_m enzyme (Text-fig 2). The increases in activity measured at 100 mM are much greater than the increases of 5 mM activity, and cannot be explained on the basis of increases of a low K_m enzyme. Since such specific increases at 100 mM could be duplicated by the addition of phosphate ion, boiled extracts of outer cortex, or N-acetyl glucosamine to assays, the increases seen at 100 mM are likely due to effects of nonprotein molecules working in conjunction with high glucose levels to activate phosphorylation. However, the increases could also be due to artifactual increases in glucose-6-phosphate or NADPH production, also caused by the nonprotein substances and glucose. Regardless of the significance of these activation effects, the assays of glucose phosphorylation at 5 mM show increases above normal in amount of low K_m enzyme. During early regeneration these increases occur at a time when cell replication is incomplete and thus likely represent increases in enzyme concentration per proximal tubular cell.

Summary

Acute renal tubular necrosis was induced in rats by a single intravenous injection of mercuric chloride. Renal vein and arterial blood samples were analyzed for glucose and lactate during the ensuing 21 days. Homogenates of cortex and medulla from the kidneys of these animals and control rats were assayed for glucose phosphorylating and hexose monophosphate shunt dehydrogenase activities. A biphasic increase in glucose uptake occurred, with peaks at 3 and 7–12 days after the injection of mercuric chloride. During the first peak of glucose uptake, the control level of lactate uptake remained unchanged; however, during the second peak, lactate was produced rather than removed from the blood. During each of the peaks of glucose uptake, parallel increases in glucose phosphorylating activity were found in homogenates of cortex, as assayed at several different glucose concentrations. There were minimal changes of this activity in the medulla. Increases in

hexose monophosphate shunt dehydrogenase activity were found in homogenates of cortex from 2 to 14 days after mercuric chloride injection, but there were minimal changes in the medulla.

It is concluded that regeneration of kidney cortex is associated with the induction of anaerobic glycolysis and the hexose monophosphate shunt, and that these changes involve the induction of specific enzymes.

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