Renin and Acute Circulatory Renal Failure in the Rabbit


ABSTRACT
Plasma renin concentration (PRC) was measured in 25 rabbits before and 6, 24, or 72 hours after subcutaneous injection of glycerol. Renal failure and tubular necrosis developed in most animals and PRC rose sixfold to a maximum at 24 hours. Small insignificant changes of PRC were present at 6 and 72 hours. None of these changes was observed in a control group of nine animals killed 24 hours after an injection of saline. The amount of renin extractable from single superficial glomeruli and from renal cortical tissue was reduced after injection of glycerol. In a second study of 11 anesthetized rabbits, renal venous PRC increased on average from 151 to 1810 units/liter following a 4-hour period of renal artery occlusion. Arterial PRC did not change significantly during this time, but the kidneys showed histological changes of acute tubular necrosis. These experiments are compatible with the suggestion that renin is involved in the pathogenesis of acute circulatory renal failure.

KEY WORDS
glomerular renin
glycerol-induced renal failure
angiotensin
acute tubular necrosis
renal artery occlusion

Vasoconstriction within the kidney, particularly in the outer layers of the cortex, is held to be important in the pathogenesis of acute renal failure (ARF) (1, 2). Renin is stored mainly in the outer layers of the renal cortex (3, 4) and, like the plasma or blood levels of renin, renin activity and angiotensin are often increased during the early stages of ARF in man (5-10) and may, by their vasoconstrictor effect, play some part in producing the condition (9, 11). However, analysis of results in man is compromised by lack of information on changes in renin in the early stages of ARF (9). In the first group of experiments reported here, therefore, plasma renin concentration was measured in rabbits before and during the development of ARF produced by glycerol.

The second question investigated was the extent to which levels of renin in peripheral plasma reflect those within the kidney under conditions, such as in ARF, in which renal blood flow is reduced. It is known, for example, that renal venous plasma renin concentration is inversely related to renal blood flow (12). In a second series of rabbits, therefore, the renal artery was occluded for a period sufficient to produce ischemic changes within the kidney, and renin was measured in samples of arterial and renal venous plasma taken before, during, and after the occlusion.

A brief account of some of these results has been given recently (13).

Methods
A. ACUTE RENAL FAILURE PRODUCED BY GLYCEROL
Forty-nine rabbits were given the acidifying diet described by Lalich (14) to increase susceptibility to ARF (15), which in preliminary experiments had proved difficult to produce. Within 3 weeks, urine pH had decreased from 6.9 or more to 5.5 or less in all animals. Drinking water was then withheld, and after 24 hours...
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hours a 12-ml sample of blood was taken from the central artery of the ear. The animals were subsequently divided into three groups.

Group 1: Glycerol-Injected Animals.—In 35 rabbits a 50% solution of glycerol in 0.9% NaCl (17 ml/kg) was injected into the loose subcutaneous tissues behind the neck, after the injection site had been infiltrated with lidocaine. After 6, 24, or 72 hours (Table 1) a second blood sample was taken from an ear artery and the animal was killed with pentobarbital. The kidneys were removed rapidly, and transverse sections were cut from their equator for histological examination and for assay of extractable renal renin and glomerular renin.

Group 2: Saline-Injected Controls.—In nine control animals, 0.9% saline (17 ml/kg) instead of glycerol was injected, and 24 hours later the second blood sample was taken and the animal was killed as before.

Group 3: Uninjected controls.—Five animals were killed immediately after the first blood sample had been taken.

Extractable Renal Renin.—This was measured by homogenizing a weighed slice of kidney with 0.15% saline, pH 5.7 phosphate-saline buffer (5 ml of buffer/g of renal tissue). The extract was centrifuged and the supernatant fluid decanted, filtered (Whatman 541), and stored at —20°C. Prior to assay the extract was thawed and further precipitate was removed by centrifugation and filtration. The filtrate was assayed directly (16) against a standard solution of rabbit renin containing 42 units/ml as measured by an enzyme-kinetic technique (17). The renin activity in the renal extract was expressed as follows:

Glomerular renin.—The renin extractable from single glomeruli was measured as described previously (4) in 26 rabbits. Twelve of these were from the glycerol-injected group (six killed after 6 hours and six after 24 hours). Nine were from the control group injected with saline and five from the uninjected controls. Between five and ten glomeruli were dissected from the superficial layers of the renal cortex in each animal and a similar number from the deepest layers. The logarithm of the glomerular renin value was used in statistical analysis and a value of 600 units/glomerulus = 10,000 was arbitrarily assigned to levels greater than this.

Material for microscopy was fixed in formal saline followed by corrosive formalin, and sections were stained with hematoxylin-eosin. Plasma renin concentration was measured by an enzyme-kinetic technique (17) and blood urea by autoanalyzer. Samples for urea estimation in rabbits 13–18 (Table 1) were lost.

B. RENAL ARTERY OCCLUSION IN ANESTHETIZED RABBITS

Eleven rabbits (2.7–4.5 kg) were anesthetized with thiopental sodium. The left kidney was exposed through a loin incision and the renal vein on this side catheterized via the left adrenolombar vein. The central artery of the ear was cannulated. Twenty minutes later, 10-ml samples of arterial and renal venous blood were taken. The junction of the inferior vena cava and renal vein was occluded by a polyethylene snare during the collection of these samples to avoid reflux of blood from the inferior vena cava. The left renal artery was then occluded for 4 hours and, during the final 15 minutes of this period, a second pair of blood samples was taken as before.

The arterial clamp was released briefly during the collection of renal venous blood. The clamp was then removed and the wound closed with the venous catheter in position. Twenty hours later the animals were anesthetized again, the kidney was exposed and a third pair of blood samples was taken. The position of the venous catheter was checked and the animal was killed with thiopental sodium. Studies of renal histology and measurement of plasma renin concentration were made as in the experiments with glycerol (section A).

TERMINOLOGY

Acute Tubular Necrosis and Cortical Infarction.—The diagnosis of acute tubular necrosis was based on criteria used previously (9). Cortical infarction was characterized by extensive areas of necrosis affecting tubules, glomeruli, blood vessels and interstitial tissue.

Acute Renal Failure.—Glycerol injection in the rat produced a syndrome similar to acute renal failure in man (18) with rising blood urea, decreasing urine-plasma urea ratio, and reduction of glomerular filtration. We have used the term "acute renal failure" to describe the similar consequences of glycerol injection in the rabbit. We do not imply that acute tubular necrosis is the pathological counterpart of ARF since either condition frequently occurs without the other (19–21).

Results

A. ACUTE RENAL FAILURE PRODUCED BY GLYCEROL

Ten rabbits died shortly after injection of glycerol, and data from these are not considered. Observations in the remaining experimental, saline-injected, and uninjected animals are summarized in Table 1.

Histological changes.—Unequivocal acute tubular necrosis was present in 17 of the 19


**TABLE 1**

Values before and after Injections of Glycerol or Saline

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Plasma renin conc</th>
<th>Renal tissue renin (U/g)</th>
<th>Blood urea</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
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<td>After</td>
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<tr>
<td>1</td>
<td>72</td>
<td>71</td>
<td>152</td>
<td>21</td>
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<td>42</td>
<td>283</td>
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<td>6</td>
<td>100</td>
<td>290</td>
<td>256</td>
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</tbody>
</table>

**MEAN ± SE**

|        | 67 ± 13 | 108 ± 51 | 263 ± 35 | 27 ± 2.5 | 40 ± 1.7† |

**Six Hours after Glycerol**

|         | Mean ± SE | 105 ± 16 | 660 ± 150† | 243 ± 49 | 50 ± 5 | 168 ± 7† |

**Twenty-Four Hours after Glycerol**

|         | Mean ± SE | 54 ± 16 | 163 ± 93 | 181 ± 27 | 47 ± 5.3 | 208 ± 42† |

**Seventy-Two Hours after Glycerol**

|         | Mean ± SE | 41 ± 6.8 | 50 ± 12 | 488 ± 142 | 35 ± 4.8 | 30 ± 4.0 |

**Twenty-Four Hours after Saline**

|         | Mean ± SE | 52 ± 7.2 | 347 ± 146 | 45 ± 3.7 |

Significance (Student’s *t*-test) of the difference of PRC and blood urea following injection of glycerol or saline are indicated for individual groups: *P* < 0.01; †*P* < 0.001. For other differences, *P* > 0.05. ATN = acute tubular necrosis. Ext = extensive changes; Occ = occasional changes.
animals killed 24 hours or more after glycerol (Table 1). It developed in rabbit 22 without marked change in blood urea, and in rabbit 7 urea rose markedly without necrosis. Tubular casts only were seen in the group killed 6 hours after glycerol. Acute tubular necrosis and casts were not found in the two control groups (Table 1).

**Blood urea.**—Blood urea increased after glycerol in all animals in which measurements were made, progressively higher values being found as the time after injection increased. Blood urea did not change significantly in animals injected with saline (Table 1).

**Renin.**—Mean plasma renin concentration (PRC) increased sixfold in animals killed 24 hours after glycerol, and 9 of 11 animals in this group showed a distinct increase (Table 1). Smaller and less consistent increases of renin were present at 6 and 72 hours. Taken together, the results suggest that renin increased to a peak at some time between 6 and 72 hours after glycerol, and thereafter decreased toward the control values. In contrast, PRC did not change significantly in the group of control animals injected with saline (Table 1). In the period before injection, PRC was lower in the

<table>
<thead>
<tr>
<th>GLOMERULAR RENIN UNITS X 10^-6</th>
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<tbody>
<tr>
<td>CONTROLS</td>
</tr>
<tr>
<td>A UNINJECTED</td>
</tr>
<tr>
<td>B SALINE</td>
</tr>
<tr>
<td>C 6 HOURS</td>
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<td>D 24 HOURS</td>
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**FIGURE 1**

Renin values of single glomeruli from the superficial cortex (open circles) and deep cortex (solid circles) in control and glycerol-injected animals. The mean ± 2 SE is shown for each group.

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Changes of arterial and renal venous plasma renin concentration (mean ± se) during and after a 4-hour period of renal artery occlusion in 11 rabbits.

only when control group B was compared with animals injected 6 hours previously ($t = 2.40, P < 0.02$).

The main changes produced by glycerol were therefore acute renal failure, acute tubular necrosis, an increase of plasma renin concentration and a decrease in the amount of renin extracted both from kidney slices and from individual glomeruli.

B. RENAL ARTERY OCCLUSION

Structural changes.—Kidneys whose renal artery had been occluded were swollen and considerably heavier than untouched kidneys (mean weight 15.4 g as compared with 9.1 g; $t = 5.10, P < 0.001$). Areas of pallor were visible on the cut surface of the cortex in all clamped kidneys except one. Satisfactory specimens for histology were obtained in 10 of the 11 animals, and acute tubular necrosis was found in all of these, with the addition of cortical infarction in 8. Untouched kidneys appeared normal macroscopically, and in no instance was tubular necrosis or cortical infarction found on subsequent microscopic examination.

Plasma Renin Concentration.—Renal venous PRC increased in all animals during the renal artery occlusion, the increase ranging from 1.14 to 34-fold with a mean increase of 12-fold (Fig. 2). Lower values were found 20 hours after the clamp had been released ($t = 2.29, P < 0.05$). Arterial PRC, by contrast, increased only slightly and insignificantly during the occlusion (Fig. 2; $t = 0.814, P > 0.05$), and again lower values were found after 20 hours ($t = 4.38, P < 0.001$). In the period before occlusion, mean arterial PRC was indistinguishable from renal venous PRC (151 and 150 units/liter respectively, Fig. 2). In four animals AV differences at the time were greater than +25%. This suggests that more renin was entering the kidney in the arterial blood of these animals than was leaving in the renal vein, the difference being too large to be attributed to methodological variation (12). We have encountered a similar phenomenon in the dog (12).

The main changes produced by renal artery occlusion in these experiments were therefore a large increase in renal venous plasma renin concentration and tubular necrosis together, in some animals, with cortical infarction.

Discussion

Circulating levels of renin, renin activity, and angiotensin are usually increased during the early stages of acute renal failure in man, lower values being found as the disease progresses (5, 7–10). This pattern was apparent also in the present study, in which ARF was produced in rabbits by glycerol (Table 1). It was not clear from the earlier work whether the increase in PRC was present before renal failure developed (9). The experiments described here have shown that in rabbits with ARF produced by glycerol PRC increased as the disease developed, the peak of renin preceding the peak of blood urea.
This rise in renin could be explained in three ways: (1) renin and ARF might be provoked independently of one another by a common stimulus; (2) renal failure might increase renin or, (3) renin might in some way produce ARF.

**Increased Renin and Acute Renal Failure: a Common Stimulus.**—An association between increased renin and ARF would arise if both had a common cause. Hemorrhage, sodium depletion, and surgery, for example, are capable both of provoking ARF (22-24) and of stimulating renin (25-28). These states were often present among patients in our earlier study, and it is possible that they might have stimulated renin independently of renal failure (9). This is unlikely to have produced the rise in PRC in glycerol-injected animals in the present study, however, as PRC did not increase in the control groups subjected to a similar degree of hemorrhage, sodium depletion, and surgery. We cannot, on the other hand, exclude the possibility that glycerol or hemolysis stimulated renin and independently provoked ARF.

**Increase of Renin Provoked by Acute Renal Failure.**—Renal failure could raise PRC either by increasing renin release or by reducing the clearance of renin from blood. That the latter might contribute is suggested by the longer survival of injected renin in nephrectomized animals (29). However, in the glycerol experiments blood urea continued to increase as renin decreased (Table 1), and blood urea was unrelated to concurrent PRC ($r = +0.20$, $P > 0.05$). Taken with the reduction of glomerular and extractable renal renin which occurred at this time, a more likely explanation of the rise in PRC is that renin was discharged from the kidney into the blood. Whereas a reduction of renin synthesis could also contribute to the renin-depleted state of kidney tissue, it could not account for the increased renin content of blood.

The experiments described here do not exclude the possibility that reduction of renal blood flow is the cause of the increased renin.

**Acute Renal Failure Produced by Renin.**—For a variety of reasons we (9) and others (30, 31) consider it likely that renin is involved in some way in the pathological sequence of events leading to the circulatory form of acute renal failure, although there are clearly some circumstances (32) in which very large increases in PRC do not produce renal failure.

Oliver et al. (33) have argued that “toxic” ARF is an entity distinct from the circulatory type such as results from glycerol. This is borne out by subsequent micropuncture studies (34). In contrast to glycerol-induced ARF, it has also been shown that PRC is not significantly increased in rabbits with the “toxic” type of acute renal failure which follows injection of cephaloridine (35).

Evidence favoring the view that renin is involved in the circulatory type of acute renal failure is summarized in an earlier paper (9). Since this report it has been shown that infusion of angiotensin in a high dose in the rabbit is capable of producing ARF and acute tubular necrosis (36).

It is known that saline loading and denervation of the kidney reduce its renin content (37-39) and protect against acute renal failure (30, 31, 40). As shown here, glycerol reduces the amount of renin extractable from glomeruli and from renal tissue, and this may be relevant to the recent observation that a first injection of glycerol protects against the ARF produced by a second injection (41).

The idea that renin might produce ARF is not new. Histological studies of the juxtaglomerular apparatus in the crush syndrome and eclampsia led Goormaghtigh (11, 42, 43) to suggest that a high concentration of angiotensin generated in the region of the juxtaglomerular apparatus by renin might reduce renal blood flow and precipitate renal failure. Although high renin levels in peripheral blood in ARF are compatible with this, information on the renin content of blood within the kidney is more relevant.

The object of the second group of experiments described here was to determine the extent to which renin concentration of plasma within the kidney could differ from that in the
peripheral circulation at a time when renal blood flow was grossly reduced. Occlusion of the renal artery led to a large increase in renal venous PRC with only minor changes in arterial PRC. The increase in renal venous PRC was almost certainly an underestimate, since, to collect sufficient venous blood, it was necessary to release the arterial occlusion momentarily (see Methods) and thus arterial blood of relatively low renin content would have entered the kidney, diluting renin-rich renal blood. If, as seems likely, the concentration of renin normally present in blood is sufficient to produce vasoconstriction and thereby influence blood pressure (44, 45), and if the conversion of angiotensin I to the vasoactive angiotensin II can take place within the kidney (9) the large increase in renal venous renin seen in these experiments could well have produced vasoconstriction within the kidney. That it can produce vasoconstriction outside the kidney is suggested by the observation that arterial and renal venous renin rise transiently together with blood pressure after removal of a clamp from the renal artery (12, 46, 47).

In conclusion, a large increase in PRC occurs at an early stage of ARF following an injection of glycerol in rabbits. This is probably produced in part at least by release of renin from the kidney, since renal and glomerular renin are reduced as the level in the peripheral plasma rises. These changes are associated usually with ARF and acute tubular necrosis. The experiments suggest that ARF is not itself the cause of the increase in renin since the relation between renin and blood urea was not close. Other points suggested by the study are that increased PRC is not entirely the result of a susceptible state existing before ARF occurs, and that the rise of renin is not a consequence of hemorrhage, sodium depletion, or surgery. Renal artery clamping experiments further showed that the renin content of blood within the kidney may rise when the renal circulation is markedly reduced. For reasons discussed, this increase might be sufficient to produce vasoconstriction within the kidney, although we cannot exclude the opposite possibility that vasoconstriction and reduction of renal blood flow stimulate renin release. As discussed earlier (9), both processes may be at work as a vicious-circle mechanism in the circulatory type of ARF. The histological changes could have resulted from the 4-hour period of clamping or from vasoconstriction persisting after removal of the clamp or from both processes.

References


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