Prostaglandin Synthesis by Glomeruli Isolated from Rats with Glycerol-Induced Acute Renal Failure

JEAN-DANIEL SRAER, LUC MOULONGUET-DOLERIS, FRANCOISE DELARUE, JOSEE SRAER, AND RAYMOND ARDAILLO

SUMMARY In vitro PG synthesis by glomeruli isolated from rats with glycerol-induced acute renal failure (ARF) was measured by radiometric high performance liquid chromatography after incubation with [14C]arachidonic acid and radioimunoassay (RIA). The four PGs, 6-keto-PGF<sub>1α</sub>, TXB<sub>2</sub>, PGF<sub>2α</sub>, and PGE<sub>2</sub> were each synthesized by glomeruli from both control and treated rats but the synthesis rates were greater after glycerol. This increase was not apparent 1 hour after injection but, at 24 hours, all PGs were produced in greater amounts by glomeruli of treated rats. Thus, we studied PGE<sub>2</sub>, PGF<sub>2α</sub>, and TXB<sub>2</sub> synthesis by glomeruli at various time intervals after induction of ARF using direct RIA. PGF<sub>2α</sub>, and TXB<sub>2</sub> synthesis were greater only at 24 hours and only in the presence of arachidonic acid, whereas PGE<sub>2</sub> synthesis was greater at 24 hours, irrespective of arachidonic acid, but at 48 hours only with arachidonic acid. The stimulatory effect of arachidonic acid was always greater in glycerol-treated than in control rats for these three PGs in the later period, whereas a significant decrease for PGE<sub>2</sub> was observed at 1 hour. The late increase in PG synthesis may be due to stimulation of the renin-angiotensin system since it was abolished in rats pretreated for 48 hours with captopril. A late increase in PG synthesis by the papilla of the treated rats also was observed. We conclude that any increase in the glomerular production of vasoconstrictor PGs could contribute to the maintenance of acute renal failure, whereas the early fall in the stimulatory effect of arachidonic acid on PGE<sub>2</sub> synthesis could play a role in its initiation.


GLYCEROL, when injected intramuscularly or subcutaneously in large doses to dehydrated rats, produces acute renal failure (ARF) similar to that observed in patients with crush syndrome (Oken et al., 1966; Ayer et al., 1971; Flamenbaum et al., 1973; Churchill et al., 1977), but the mechanism of the failure remains largely unknown. Whereas renal plasma flow and glomerular filtration rate decrease (Chedru et al., 1972) due to an increased pre-glomerular resistance (Ayer et al., 1971; Chedru et al., 1972), a change in the function of the glomerular cells themselves has not been suggested, although it is now recognized that the glomerular capillary ultrafiltration coefficient may be reduced in various pathophysiological models (Baylis et al., 1977; Bohrer et al., 1977) and with various agents, particularly angiotensin II (Blantz et al., 1976). This effect of angiotensin II is enhanced by substances that inhibit PG synthesis (Baylis and Brenner, 1978), a potentially important finding in relation to our demonstration that isolated glomeruli synthesize various PGs and mainly PGE<sub>2</sub> (Sraer et al., 1979b). Taken together with the findings that indomethacin enhances glycerol-induced ARF in rabbits (Papanicolaou et al., 1975) and that salt loading protects glycerol-injected animals (Torres et al., 1975; Chedru et al., 1972), roles for both PGs and renin-angiotensin seem possible in ARF. The present study was designed to follow PG synthesis by isolated glomeruli during the development, the sustained phase, and the recovery from glycerol-induced ARF in the rat in order to appreciate better whether changes in synthesis play a role in the initiation and the maintenance of this syndrome.

Methods

Preparation of Animals

Male Sprague-Dawley rats weighing 180-200 g were kept without water for 24 hours before the experiment. They were anesthetized lightly with ether. Acute renal failure was induced by the intraperitoneal injection into both hindlimbs of 10 ml/kg body weight of 50% glycerol in 0.28 M glucose (30.5 mmol glycerol/kg). Control rats received the same amount of solvent. The animals were allowed free access to tap water after the injection. At different times after the induction of acute renal failure (0.5, 1, 2, 4, 24, 48, 72, and 192 hours), two treated and two control rats were anesthetized with pentobarbital sodium (5 mg/100 g, ip). A cannula was inserted into the lower aorta, and isotonic cold heparinized saline was perfused after the aorta had

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been clamped above the renal arteries and a renal vein or vena cava opened for drainage. Blanched kidneys were removed and immersed in ice-cold 20 mM Tris-HCl buffer, pH 7.4, containing 125 mM NaCl, 10 mM KCl, 10 mM sodium acetate, and 5 mM glucose. All subsequent steps were carried out in the same buffer at 0–4°C. This experiment was repeated 4–12 times according to the stage of ARF investigated.

A limited number of rats were treated differently. They were not dehydrated before glycerol administration but for 48 hours were offered either water or water containing 2.5 g/liter captopril (Squibb), an orally active inhibitor of angiotensin I-converting enzyme. Each of these groups was then divided into two subgroups treated either with glycerol in glucose solution or with glucose solution alone. The rats were killed after a further 24 hours, during which time captopril was maintained in the drinking solution.

Preparation of Glomeruli and Papilla

Glomeruli were isolated as previously described (Sraer et al., 1979b). Kidney cortical tissue from two rats was minced to a paste-like consistency and gently pressed through a 106-μm stainless steel sieve. The sieved tissue was suspended in 10 ml buffer, poured onto a 50-μm stainless sieve, and washed with buffer. The tissue recovered on the upper face was then resuspended in 10 ml buffer, passed through a 25-gauge needle, and centrifuged at 120 g for 90 seconds. This operation was repeated three times. The final pellet consisted of decapsulated glomeruli with less than 2% tubular contamination. The glomeruli of treated and control rats were prepared in parallel. Glomeruli isolated in this way can be considered as viable for at least 1 hour for two reasons: They exclude trypan blue stain; their cells grow rapidly after trypan blue exclusion. Incubations were similar to those stated above. Incubation was stopped after 60 minutes by centrifugation at 3000 g for 2 minutes at 10°C. In some experiments, three incubation times (15, 30, and 60 minutes) were studied. Supernatants of each tube were collected and frozen until PG radioimmunoassays were performed. Protein concentration (300–800 μg/ml) was determined according to the method of Lowry et al. (1951).

PGs of the incubation medium were measured by radioimmunoassay either directly or after separation by high performance liquid chromatography (HPLC). When HPLC was used, isolated glomeruli (approximately 2.6 mg protein) were incubated in the presence of 8.9 μg [14C]arachidonic acid (1.6 μCi) under a volume of 1.3 ml. The other conditions of incubation were similar to those stated above. Incubation was stopped after 60 minutes by centrifugation. The supernatant was collected and immediately used for extraction and chromatography analysis.

Prostaglandin Assays

High Performance Liquid Chromatography

PGs resulting from the conversion of [14C]arachidonic acid were extracted and purified by silicic acid chromatography according to the method of Dray et al. (1975), with the following modification: the totality of cyclo-oxygenase end-products were eluted simultaneously by 10 ml of benzene:ethylacetate:methanol (60:40:20, vol/vol). Prior to extraction, tritiated PGs (PGE2, PGF2α, TXB2, and 6-keto-PGF1α) purchased from the Radiochemical Center or New England Nuclear were added as internal standards to the supernatant of the glomerular suspension. The elution product was evaporated at 45°C under a nitrogen stream. The dry residue was dissolved in 120 μl of HPLC eluent (acetonitrile:benzene:acetic acid:water, 210:2:1:790, vol/vol). Five microliters were kept for 14C and 3H counting, and 100 μl were submitted to HPLC. We used the model 5000 Varian liquid chromatograph equipped with a micropack CH 10 column (4 mm id × 30 cm). The flow rate of the eluent was 1.6 ml per min and per tube. The content of each tube was divided into two aliquots. One was immediately used for 14C and 3H counting. The other was lyophilized and kept for PG radioimmunoassay.

PG Radioimmunoassay

RIA was carried out according to the method of Dray et al. (1975) for PGE2 and PGF2α, that of Sors...
et al. (1978) for TXB₂ and that of Dray et al. (1978) for 6-keto-PGF₁₀. The corresponding antiserums were obtained from the Institut Pasteur. Each of them is specific and cross-reacts only negligibly with the other PGs. RIA was performed either directly, using the untreated incubation medium, or after HPLC purification. In most experiments, direct RIA was used. A blank value obtained with the medium incubated in the absence of glomeruli was subtracted.

Statistical Analysis
Replicate data were subjected to two-factor (time of sacrifice-treatment by glycerol) analysis of variance. Separate analyses were performed for data obtained under basal conditions, in the presence of arachidonic acid and also for the differences between these two. The replications (4 to 12) corresponded to identical experiments performed on different days. In order to evaluate the effect of the treatment by glycerol at each time after the injection, we performed t-tests using as variance of each group the residual variance calculated from the two-factor analysis of variance. We have similarly evaluated the differences between two selected times when we felt it necessary.

Results

HPLC Profiles of [¹⁴C]PGs Produced by Glomeruli Isolated from Control and Glycerol-Treated Rats

Separation of the metabolites of [¹⁴C]arachidonic acid was performed using the incubation mediums of glomeruli isolated from control and glycerol-treated rats sacrificed at 1 and 24 hours. The results are shown in Figure 1. The data obtained from control rats sacrificed at one hour have been omitted since they were strictly identical to those obtained with control rats killed at 24 hours. The same profile was observed in each case. Four PGs, PGE₂, PGF₂₀, TXB₂, and 6-keto-PGF₁₀, were clearly identified since, for each of them, the [¹⁴C] peak coincided both with the peak of the corresponding [³H] standard and with that of PG concentration measured by specific radioimmunoassay. Coincidence was perfect in the latter case, whereas there was a short delay between the elution times of [¹⁴C] and [³H] PGs. Other peaks localized between 6-keto-PGF₁₀ and TXB₂ or eluted initially before 6-keto-PGF₁₀ were also present but could not be identified. A clear difference appeared between the heights of the peaks obtained under the three different conditions. The [¹⁴C] peaks of PGE₂, PGF₂₀, TXB₂, and to a lesser degree, of 6-keto-PGF₁₀, were markedly greater with glomeruli obtained from glycerol-treated rats killed at 24 hours than with the two other preparations (Fig. 1). There was, however, no clear difference between the heights of the unidentified peaks. These results were confirmed by the

![Figure 1 Radiometric HPLC of the incubation mediums of glomeruli isolated from control rats (killed 24 hours after solvent administration) and glycerol-treated rats (killed either 1 hour or 24 hours after glycerol administration). The continuous lines correspond to the [¹⁴C]-labeled products resulting from [³H]arachidonic acid conversion. The dotted lines correspond to the [³H]-labeled PG standards. The shaded areas correspond to the elution tubes containing the PG indicated above as demonstrated by specific radioimmunoassay.](image)

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Production Rates (ng/mg per hr) of 6-Keto PGF₁₀, TXB₂, PGF₂₀, and PGE₂ by Glomeruli Isolated from Control and Glycerol-Treated Rats Killed at 1 and 24 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td><strong>Glycerol-treated rats (1 hr)</strong></td>
</tr>
<tr>
<td>6-Keto PGF₁₀</td>
<td>0.54 ± 0.08</td>
</tr>
<tr>
<td>TXB₂</td>
<td>6.0 ± 1.94</td>
</tr>
<tr>
<td>PGF₂₀</td>
<td>3.14 ± 0.71</td>
</tr>
<tr>
<td>PGE₂</td>
<td>4.52 ± 0.28</td>
</tr>
</tbody>
</table>

PGs were estimated by radioimmunoassay after HPLC purification. Results are means ± SEM from six (control rats) or four (treated rats) individual values. Rank sum test was used to compare the means (glycerol-treated rats vs. control rats).

* P < 0.05; †P < 0.01.
Figure 2. PGE\(_2\) synthesis by glomeruli (direct RIA) isolated from control and glycerol-treated rats that were killed at different times (0-4 hours below and 24-192 hours above) after administration of the solvent or of the drug. On the left are shown the results obtained under basal conditions and on the right those obtained in the presence of arachidonic acid (5 μg/ml).

The height of each rectangle represents the mean, and the vertical bar the SEM of the individual results (4-12 according to the time of sacrifice). The results obtained with and without arachidonic acid were analyzed separately according to a two-factor (glycerol-treated vs. control at eight times of sacrifice) analysis of variance with a variable number (4-12) of repetitions.

*P < 0.05; **P < 0.01; ***P < 0.001.

Note the differences in scale between the right and the left parts.

### Table 2. Stimulatory Effect of Arachidonic Acid on PG Production by Glomeruli Isolated from Control (C) and Glycerol-Treated (G) Rats (ng/mg per hr).

<table>
<thead>
<tr>
<th>Time of sacrifice (hr)</th>
<th>PGE(_2) C</th>
<th>G</th>
<th>PEF(_2\alpha) C</th>
<th>G</th>
<th>TXB(_2) C</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>3.98 ± 0.34</td>
<td>3.68 ± 0.95</td>
<td>1.36 ± 0.36</td>
<td>1.02 ± 0.33</td>
<td>0.266 ± 0.22</td>
<td>0.027 ± 0.14</td>
</tr>
<tr>
<td>1</td>
<td>3.49 ± 0.59</td>
<td>2.40 ± 0.37*</td>
<td>1.10 ± 0.44</td>
<td>0.651 ± 0.20</td>
<td>0.43 ± 0.13</td>
<td>0.37 ± 0.21</td>
</tr>
<tr>
<td>2</td>
<td>2.37 ± 0.38</td>
<td>1.81 ± 0.32</td>
<td>2.33 ± 0.46</td>
<td>1.85 ± 0.42</td>
<td>0.010 ± 0.003</td>
<td>5.09 ± 1.701</td>
</tr>
<tr>
<td>4</td>
<td>3.23 ± 0.58</td>
<td>2.99 ± 0.46</td>
<td>2.74 ± 0.48</td>
<td>1.75 ± 0.42</td>
<td>0.085 ± 0.087</td>
<td>0.85 ± 0.33</td>
</tr>
<tr>
<td>24</td>
<td>2.57 ± 0.63</td>
<td>12.02 ± 1.23†</td>
<td>0.70 ± 0.22</td>
<td>2.84 ± 0.091</td>
<td>0.010 ± 0.003</td>
<td>5.09 ± 1.701</td>
</tr>
<tr>
<td>48</td>
<td>3.48 ± 0.39</td>
<td>6.74 ± 1.37†</td>
<td>1.44 ± 0.39</td>
<td>1.26 ± 0.48</td>
<td>0.085 ± 0.087</td>
<td>0.85 ± 0.33</td>
</tr>
<tr>
<td>72</td>
<td>3.71 ± 0.81</td>
<td>5.42 ± 0.94*</td>
<td>1.64 ± 0.36</td>
<td>1.75 ± 0.42</td>
<td>0.788 ± 0.34</td>
<td>2.51 ± 1.2</td>
</tr>
<tr>
<td>192</td>
<td>4.71 ± 1.7</td>
<td>4.24 ± 0.80</td>
<td>1.50 ± 0.16</td>
<td>1.85 ± 0.42</td>
<td>0.464 ± 0.11</td>
<td>0.97 ± 0.28</td>
</tr>
</tbody>
</table>

Results represent the means ± SEM of individual differences between PG productions obtained in parallel with and without arachidonic acid. Two-factor (time of sacrifice, treatment by glycerol) analysis of variance with replication was used to analyze the data obtained for each PG. A test between the two groups was subsequently performed at each time using as variance of each group the residual variance calculated from the two-factor analysis of variance.

*P < 0.05; †P < 0.001

Quantitative data obtained by RIA after HPLC separation (Table 1), showing that the production rate of PGs by glomeruli from glycerol-treated rats killed at 24 hours was much greater (2- to 4-fold) than that by glomeruli from the corresponding control rats. No modification of PG synthesis by the glomeruli could be observed 1 hour after glycerol administration (Table 1). These initial results prompted us to analyze more precisely the changes with time of PG synthesis by glomeruli isolated from control and treated rats.

**PGE\(_2\), PGF\(_2\alpha\), and TXB\(_2\) Synthesis by Glomeruli from Control and Glycerol-Treated Rats Killed at Different Times after Administration of the Drug**

In vitro PGE\(_2\) production was studied by direct RIA both under basal conditions and in the presence of 5 μg/ml arachidonic acid (Fig. 2). Under basal conditions, there was no difference between control and treated rats at the early times (0.5-4 hours) after administration of glycerol, whereas PGE\(_2\) synthesis by the glomeruli from treated rats was increased at 24 hours (P < 0.05). This difference disappeared at later times. Similarly, when incubation was performed with arachidonic acid, no difference between the preparations from both groups was observed early but PGE\(_2\) synthesis by the glomeruli from treated rats was greater at 24 (P < 0.001) and 48 (P < 0.01) hours. The stimulatory effect of arachidonic acid estimated as the difference between PG production obtained under basal conditions and in the presence of this precursor was also tested. Stimulation was smaller at 1 hour (P < 0.05) and greater at 24 (P < 0.001), 48 (P < 0.001), and 72 (P < 0.05) hours in glycerol-treated rats than in control rats (Table 2). There was also an influence of time on PGE\(_2\) production by the
glomeruli from both groups of rats; production fell at 2 hours whatever the origin of the glomerular preparation studied and increased again at 4 hours. Differences between production at 2 hours and production at either 1 or 4 hours were significant (P < 0.01) under basal conditions as well as in the presence of arachidonic acid.

PGF\(_2\alpha\) and TXB\(_2\) syntheses were studied for a limited number of periods after sacrifice (0.5 and 4 hours were omitted). PGF\(_2\alpha\) synthesis by the glomeruli from both groups was similar under basal conditions, whatever the times of sacrifice (Fig. 3). In the presence of arachidonic acid, PGF\(_2\alpha\) synthesis by the glomeruli from treated rats was greater at 24 hours (P < 0.001). A similar pattern was observed for TXB\(_2\) synthesis (Fig. 4). There was an increase in TXB\(_2\) synthesis by the glomeruli from treated rats only at 24 hours and in the presence of arachidonic acid (P < 0.001). For both PGs, the stimulatory effect of arachidonic acid was greater at 24 hours in glycerol-treated rats (P < 0.001). It is noteworthy that, contrary to what was observed for PGE\(_2\) and PGF\(_2\alpha\), there was no stimulation of TXB\(_2\) synthesis by arachidonic acid when glomeruli from control rats were studied. Stimulation was observed only with glomeruli from glycerol-treated rats.

These experiments confirmed and extended the data obtained after HPLC purification. The main new observation was the early decrease of the stimulatory effect of arachidonic acid on PGE\(_2\) production at 1 hour.

**Effect of Pretreatment by Captopril on PGE\(_2\) Synthesis by Glomeruli Isolated from Control and Glycerol-Treated Rats Killed at 24 Hours**

In the absence of pretreatment by captopril, the results obtained confirmed those already observed. PGE\(_2\) synthesis by glomeruli isolated from glycerol-
TABLE 3  Effects of Treatment by Captopril on PGE₂ Production (ng/mg per hr) by Glomeruli Isolated from Glycerol-Treated Rats Killed at 24 Hours

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Treatment</th>
<th>Control rats</th>
<th>Glycerol control</th>
<th>Control rats</th>
<th>Glycerol treated rats</th>
<th>Glycerol control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>Without captopril</td>
<td>1.344 ± 0.137</td>
<td>0.91</td>
<td>1.296 ± 0.040</td>
<td>0.83</td>
<td>1.234 ± 0.038</td>
</tr>
<tr>
<td></td>
<td>Glycerol-treated rats</td>
<td>2.157 ± 0.062</td>
<td>1.88</td>
<td>2.196 ± 0.040</td>
<td>1.88</td>
<td>2.134 ± 0.038</td>
</tr>
<tr>
<td></td>
<td>With captopril</td>
<td>1.057 ± 0.311</td>
<td>0.91</td>
<td>1.034 ± 0.377</td>
<td>0.83</td>
<td>1.061 ± 0.438</td>
</tr>
<tr>
<td></td>
<td>Glycerol-treated rats</td>
<td>1.314 ± 0.560</td>
<td>1.88</td>
<td>1.346 ± 0.438</td>
<td>1.88</td>
<td>1.361 ± 0.438</td>
</tr>
<tr>
<td></td>
<td>Glycerol control</td>
<td>1.541 ± 0.681</td>
<td>1.88</td>
<td>1.577 ± 0.819</td>
<td>1.88</td>
<td>1.603 ± 1.042</td>
</tr>
</tbody>
</table>

Results are means ± SEM from three individual values. Three-factor (time of incubation, treatment by captopril, treatment by glycerol) analysis of variance with replication was used to analyze separately the data obtained under basal conditions and in the presence of 5 μg/ml arachidonic acid. There was a highly significant interaction (P < 0.01) between the factors “captopril” and “glycerol,” thus showing that treatment by captopril modifies the effect of glycerol on PGE₂ synthesis.

PGE₂ Synthesis by Papillary Homogenates from Control and Glycerol-Treated Rats at Different Times after Administration of the Drug

In order to appreciate whether the modifications in PGE₂ synthesis induced by glycerol administration were specific to glomeruli or more general in the kidney, we also studied PGE₂ synthesis by papillary homogenates, since papilla is the main renal site for PGE₂ synthesis. A limited number of time intervals were studied (0.5 and 48 hours were omitted). There was a late increase (72 hours) in PGE₂ production both under basal conditions and in the presence of arachidonic acid (P < 0.05) by the papilla prepared from glycerol-treated rats (Fig. 5). No difference was observed when the stimulatory effect of arachidonic acid was tested.

Discussion

The decrease in renal blood flow and glomerular filtration rate reported in glycerol-induced ARF prompted investigation of the possible role played by the prostaglandins. These products have been implicated since PGE₂ protected (Papanicolaou et al., 1975; Werb et al., 1978) and indomethacin worsened (Torres et al., 1975; Papanicolaou et al.,...
1975) glycerol-induced acute renal failure. The effect of indomethacin was observed in the rabbit but not in the rat (Torres et al., 1974b) and was interpreted as consistent with the opposite properties of PGE2 in both species, renal vasodilation in the rabbit and vasoconstriction in the rat (Malik and MacGiff, 1974). However, measurements of renal PGE in rabbits treated by glycerol demonstrated an increased production (Torres et al., 1974a). Since PGs are multiple and act differently on the blood vessels, any new approach to the problem must include assays of the various PGs synthesized by the kidney. Furthermore, since PGs act locally on targets close to their site of synthesis, we felt that PG production by the glomeruli themselves must be evaluated. Finally, since a possible reason for the discrepancies in literature could have been the different times of study after glycerol administration, we investigated PG production by the glomeruli at eight different times after glycerol injection.

The present results show that PG production in this experimental model of ARF depends mainly on two factors: the time elapsed from glycerol administration and the administration itself of either glycerol or its solvent alone. Our aim was to study the latter, but we cannot neglect the fact that PGE2 production by glomeruli from the two groups of rats clearly decreased at 2 hours, both under control conditions and in the presence of arachidonic acid. This fall must be interpreted as the consequence of factors which were common to the two groups of rats. Two factors can be put forward: anesthesia with ether and, more probably, the change in water supply. Indeed, the rats were kept without water for 24 hours before glycerol administration to enhance the effects of this drug, but they were allowed free access to tap water after the injection. It cannot be excluded that passage from a dehydrated to a hydrated state may transiently modify PGE2 production by the glomeruli. This decrease in synthesis was clear only for PGE2, not for the other PGs measured, PGF2α and TXB2.

The increase in the synthesis of all PGs by glomeruli isolated from glycerol-treated rats killed 24 hours after administration of the drug is clear in the present study. HPLC associated to specific RIA showed a marked difference between the glomeruli prepared from the two groups of rats. This was confirmed by further assays of three PGs, PGE2, PGF2α, and TXB2, in a great number of individual experiments. The experiments also provided supplementary results. They showed that the increase in PG production was clearer in the presence of arachidonic acid than under basal conditions. For that reason, we tested the stimulatory effect of arachidonic acid estimated as the difference between PG productions observed with and without arachidonic acid. This stimulatory effect was much more marked in glycerol-treated rats for the three PGs studied. This was clear at 24, 48, and 72 hours for PGE2 and at 24 hours for PGF2α and TXB2. It is noteworthy that, in control rats, arachidonic acid markedly stimulated PGE2 and PGF2α production, but was inactive on TXB2 production. A stimulatory effect of arachidonic acid for this latter PG could be observed only using glomeruli from glycerol-treated rats. An increase in TXB2 production by the isolated perfused kidney has also been observed at 24 hours by Benabe et al. (1980). These data and ours may be compared to those reported by Morrison et al. (1978), who observed an increase in renal TXB2 production after ureteral ligation, which is another experimental model involving vasoconstriction of the glomerular capillaries. Precursor availability is not the factor modifying PG synthesis in glycerol-treated rats, since the difference between both groups of rats was more marked in the presence of an excess of arachidonic acid. The factors involved must act at a stage beyond the deacylation of phospholipids, probably on cyclooxygenase activity. We tested the role of angiotensin II by studying glomeruli isolated from rats pretreated with captopril, an inhibitor of angiotensin I-converting enzyme. The stimulatory effect of glycerol on PG synthesis at 24 hours was no longer observed when glycerol-injected rats were pretreated with captopril. This result favors a role for angiotensin II in the stimulation of PG synthesis, but does not exclude an opposite role for bradykinin, since the converting enzyme both decreases the synthesis of angiotensin II and increases that of bradykinin. The role of these two peptides would be different from their direct mode of action, which is the stimulation of PG production through an increase in the activity of phospholipase (Limas et al., 1979). Thus, preparation of the tissues included several washings with large volumes of buffer and, subsequently, all samples were incubated in the same buffer. The observed difference between glycerol-treated and control rats at 24 hours could then not be attributed to a direct and immediate effect of angiotensin II or bradykinin in glycerol-treated rats. It can be attributed only to an in vivo modification in cell function persisting in vitro at least during 1 hour. A similar late increase in PG production was observed for PGE2 synthesis by the papilla. Only PGE2 was measured. As for the glomeruli, this increase was observed both under basal conditions and with arachidonic acid. The fact that two different regions of the kidney, the glomeruli and the papilla, behave similarly is also in favor of the role of a change in the composition of plasma.

The consequences of the late increase of PG production by the glomeruli on the glomerular function remain unclear. Acute renal failure is associated at 24 hours with a marked decrease in renal blood flow and glomerular filtration rate (Oken et al., 1966; Ayer et al., 1971; Reineck et al., 1980). At this time, the rats have not yet begun to recover. Since the synthesis of all PGs was increased, irre-
spective of their effect on vasoactivity, it is difficult to predict the consequences. Indeed TXA₂, precursor of TXB₂, and PGF₂α, are considered as producing vasoconstriction, whereas the effect of PGE₂ in the rat is still open to discussion. In some studies, PGE₂ has been reported to have a vasoconstrictor effect in the rat (Malik and MacGiff, 1974), whereas it is considered elsewhere as a vasodilatory agent (Dunham, 1976; Rosenthal and Pace-Asciak, 1980). Furthermore, Baylis et al. (1976) have shown that PGs of the E series could produce a decrease in the glomerular filtration coefficient. It is possible that the simultaneous activation, at this time of the disease, of the renal renin-angiotensin system (Baranowski et al., 1978) and of vasoconstricting PGs participates in the maintenance phase of acute renal failure. Alternatively, the effects of the vasodilatory PGs could be predominant. This would be a response to compensate for a vasoconstrictor effect by keeping the glomerular capillaries patent. This latter hypothesis is supported by the observation of Papanicolaou et al. (1975), who reported that indomethacin pretreatment enhanced the glycerol nephrotoxicity.

A decrease in the stimulatory effect of arachidonic acid on PGE₂ production was observed at 1 hour in glycerol-treated rats and raises the question as to whether this plays a role in the mechanism of this model of ARF. The decrease of synthesis was observed only for PGE₂. This PG represents the main PG produced by rat isolated glomeruli (Sraer et al., 1979b) and also rat epithelial (Sraer et al., 1979a; Pettrulis et al., 1980) and mesangial (Sraer et al., 1979a) cultured cells. If, as proposed in several studies (Dunham, 1976; Rosenthal and Pace-Asciak, 1980), PGE₂ is a vasodilatory agent in the rat, it would be possible that this early decrease in PGE₂ production at a time when the renin-angiotensin system is not yet activated, should produce vasoconstriction of the glomerular capillaries and a fall in GFR. This early modification is not a direct toxic effect of glycerol on glomeruli, since glycerol added in vitro did not modify PGE₂ synthesis (data not shown), but it could reflect the glomerular vasoconstrictor response to myohemoglobininemia and reduction in plasma volume which follow glycerol injection.

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