Muscle Extract Infusion in Rabbits

A New Experimental Model of the Crush Syndrome

YORAM BLACHAR, JACK S.C. FONG, JEANE-PIERRE DE CHADAREVIAN, AND KEITH N. DRUMMOND

SUMMARY Previous studies provide inconclusive data concerning the nephrotoxicity of myoglobin following muscle injury. We investigated the possibility that released muscle constituents other than myoglobin may be associated with renal damage, and studied accompanying hematological and coagulation changes. An extract of homologous or autologous muscle was infused intravenously in rabbits in a dose of 100 mg of muscle extract protein/kg; equine myoglobin was given to control animals. Experimental animals developed proteinuria, cylindruria, and a 50% reduction in glomerular filtration rate. Leukopenia, thrombocytopenia and evidence of intravascular coagulation also were seen. The muscle extract was shown to have thromboplastic activity; however, inhibition of this by phospholipase C did not prevent the changes induced by muscle extract infusion possibly because the intrinsic coagulation pathway still was activated. Although moderate hypotension and ECG changes developed in some rabbits, these were not consistent and the renal functional changes appeared to be independent of these factors. Pulmonary and glomerular microthrombi were seen in experimental animals and there was vacuolation of the renal proximal tubular cells. These studies indicate that a number of biological systems are activated following muscle extract infusion and that there may be more important than the nephrotoxicity of myoglobin in the pathogenesis of the renal injury.

The association of muscle injury and myoglobinuria with renal failure was first recognized by Bywaters in 1941 (Bywaters and Beall, 1941). This and subsequent observations relating the association of either hemolysis or rhabdomyolysis with acute renal failure has led to the widely held view that heme pigments may be nephrotoxic (Knochel, 1976).

Nevertheless, it has been shown both experimentally and clinically that renal failure may complicate rhabdomyolysis in the absence of myoglobinuria, and that myoglobinuria may develop following muscle injury without evidence of renal dysfunction (Jaenike, 1966; Knochel, 1976). Furthermore, attempts to cause renal failure in experimental animals by infusion of either myoglobin or hemoglobin have been inconclusive; supplementary manipulations, such as the induction of hypotension and metabolic acidosis, have been necessary for renal injury to result (Mason et al., 1963; Braun et al., 1970; Oken, 1972; Knochel, 1976).

The confusion concerning the role of heme pigments in the pathogenesis of renal failure associated with muscle injury led us to investigate the possibility that constituents of muscle cells other than myoglobin could play a pathogenetic role in the renal damage, and to examine the mechanisms by which muscle injury causes renal dysfunction (O’Regan et al., 1979).

The studies described in this paper were carried out in rabbits and consisted of (1) infusion of pooled homologous rabbit muscle extract, (2) infusion of autologous muscle extract, and (3) infusion of pooled homologous muscle extract with phospholipase C, a specific inhibitor of thromboplastic activity.

Our results indicate that myoglobin is not nephrotoxic in the normal rabbit, that muscle extract is nephrotoxic, and that the coagulation system is involved in the pathogenesis of the renal injury.

Methods

Preparation of Muscle Extract

Muscle extract was prepared from female New Zealand white rabbits weighing 2–3 kg. After the rabbits had been anesthetized with pentobarbital sodium, 30–50 mg/kg, iv, the abdominal aorta was catheterized above the bifurcation and the catheter secured. The inferior vena cava was severed and the lower limbs were flushed via the catheter with...
cold normal saline until the perfusate from the inferior vena cava was clear; this required a volume of 200–300 ml of saline. The animal was killed with an intravenous bolus injection of pentobarbital sodium and the muscles of the lower limbs were dissected and immersed in ice-cold saline. The dissection was meticulous to remove as much as possible of the non-muscle tissue such as fat, tendons, and nerves. The muscle was ground with an ordinary meat grinder and then homogenized in a beaker immersed in an ice bath using 1- to 2-minute bursts with a SDT-Tissumizer (Tekmar Co.). The total time for homogenization was 10–12 minutes, and care was taken to avoid warming of the homogenate during the homogenization procedure. The resultant homogenate had a paste-like consistency; it was centrifuged at 48,000 g for 60 minutes at 4°C using a J-21B Beckman centrifuge (Beckman Instruments, Inc.). The supernate was clear and had a light pink color; it was designated as stock muscle extract for the purposes of these studies and was stored in 10-ml aliquots at −70°C until used. Microscopic examination of the stock muscle extract at 400× showed it to be free of visible particles. Each batch was tested for osmolality, bacterial growth, endotoxin content by the Limulus amebocyte-lysate assay (Yin et al., 1972) with an E-Toxate kit (Sigma Chemical Co.), and thromboplastin activity using the muscle extract instead of commercial tissue thromboplastin in the two-stage prothrombin time test. Standard biochemical techniques were used to measure the concentrations of the following substances: sodium, potassium, calcium, phosphorus, protein, creatinine, glutamic oxalacetic transaminase (GOT), and creatine phosphokinase (CPK). Fibrinogen concentration was measured by an established method (Ratnoff and Menzie, 1951) on each batch. Pooled stock muscle extract was tested for possible immunological cross-reaction with serum from eight different rabbits by a standard immunodiffusion technique (Ouchterlony, 1958).

Preparation of Autologous Muscle Extract

Rabbits were anesthetized as before; a tight pressure bandage was applied to the right leg to reduce the blood content, and the femoral artery and vein were clamped. The muscles of the right leg then were dissected out and the stock muscle extract prepared as before. During this procedure, blood loss was minimal and there was no change in blood pressure; the rabbits were maintained on a normal saline infusion at a rate of 0.3 ml/kg per min.

Histopathology, Immunopathology, and Electron Microscopy

Histopathological studies were done on kidney, lung, liver, heart, and spleen tissue using standard techniques; immunopathological studies were done on kidney tissue using unfixed tissue that was snap frozen to −70°C and was then sectioned at −20°C and directly stained with fluorescein isothiocyanate-labeled goat antiserum to rabbit fibrin, IgG, and C3 (Cappel Laboratories, Inc.) using standard immunopathological techniques (Michael et al. 1966). Kidney tissue was also processed for electron microscopic examination by the method of Barré et al. (1977).

Miscellaneous

Pure equine myoglobin, type I, (Sigma Chemical Co.) from equine skeletal muscle was used as a control infusate in one of the experiments. The myoglobin, initially salt-free, crystallized, and lyophilized (Sigma Chemical Co.), was diluted with saline to the same concentration of myoglobin as was present in the stock muscle extract—3.5 mg/ml. The concentration of the myoglobin was determined by its absorption at 435 nm (SP 30 UV spectrophotometer, PYE Unicam Ltd.).

Phospholipase C (EC 3.1.4.3) was obtained from Boehringer Mannheim Canada Ltd. It is a specific inhibitor of thromboplastin activity (Otnaess et al., 1972). It also causes hemolysis in vivo, and hemolyzes heparinized blood in vitro (Colley et al., 1973). The hemolytic activity is inhibited by thromboplastin (Bjørklid et al., 1973). These biological effects were made use of in determining the amount of phospholipase C necessary to inhibit the activity of any thromboplastin present in the stock muscle extract, as indicated by a two-stage prothrombin time exceeding 2 minutes. In a dose-response experiment in which stock muscle extract was added to serial dilutions of phospholipase C, the amount of the enzyme that would inactivate the thromboplastin activity of the muscle extract was determined. Heparinized rabbit whole blood then was added to detect the presence of any hemolytic activity; this was used as an indicator of an excessive amount of phospholipase C. The optimum amount of phospholipase C was considered to be that which would inactive the thromboplastin activity of the stock muscle extract without causing any hemolysis.

Fibrin split products were determined on serum specimens using the staphylococcal clumping test (Erickson et al., 1972). Platelet and total white blood cell counts were done on heparinized whole blood using standard techniques. For statistical analysis of the data obtained, Student's t-test was performed.

Experimental Design

General

Blood pressure was recorded continuously by means of an indwelling femoral artery catheter which was connected to a blood pressure monitor (A-V pressure monitor; Med-Science). Electrocardiographic studies were done using lead II on a standard ECG machine with electrodes placed in the left and right axillae and on the right leg. Both
ureteral orifices were exposed and no. 5 French umbilical artery catheters were introduced for collection of urine. All urine was collected in sequential 15-minute periods and the volume recorded. Urine specimens were tested for protein and glucose by dipstick test, and a microscopic examination was done on the centrifuged sediment. Creatinine was determined for measurement of creatinine clearance. Blood specimens were obtained from an ear artery and all infusates were given via a peripheral ear vein; the total volume of blood removed from each animal was less than 5 ml/kg over the entire duration of the experiment.

Infusion of Homologous Muscle Extract (Experiment 1)

The experimental group of 11 rabbits was injected with homologous muscle extract at a dose of 100 mg protein/5 ml infusate per kg body weight. The protein content of the stock muscle extract was diluted to the appropriate concentration of 100 mg/5 ml with physiological saline immediately before use. The five rabbits that comprised the control group received pure equine myoglobin diluted to 3.5 mg of myoglobin/ml in saline, given in the same volume (5 ml/kg) as the experimental animals received.

The animals were anesthetized with pentobarbital sodium, 30-50 mg/kg, iv, and the femoral arterial and ureteral catheters were inserted. During recovery from anesthesia (1-2 hours) and the equilibration period, defined below, normal saline was infused at a rate of 0.3 ml/kg per min. The equilibration period was that period during which two sequential 30-minute urine collections of similar volume were obtained and the blood pressure was stable. Following the equilibration period, three 15-minute control urine collections and a blood specimen were obtained. Rabbits in the experimental group then were given an infusion of muscle extract and those in the control group received myoglobin at a rate of 0.3 ml/kg per min. After the muscle extract or myoglobin infusion, normal saline was infused at 0.3 ml/kg per min until the termination of the experiment.

Continuous ECG and blood pressure recordings were obtained. After initiation of the muscle extract or myoglobin infusion, urine was collected in consecutive 15-minute aliquots and blood specimens were obtained at 5, 15, 30, 45, and 60 minutes for platelet and WBC count; blood specimens also were taken before muscle extract infusion and at the end of the experiment for determination of urea nitrogen, creatinine, sodium, potassium, calcium, and phosphorus. A microscopic examination was done on the centrifuged sediment of each urine specimen; each was tested for proteinuria and glycosuria, and the creatinine was measured for calculation of the creatinine clearance. At the end of each experiment, 180 minutes after initiation of the infusion, each animal was killed by an infusion of pentobarbital sodium, and tissue was taken for pathological study.

Infusion of Autologous Muscle Extract (Experiment 2)

The same protocol as described for the infusion of homologous muscle extract was followed. Six rabbits comprised the experimental group; each received all of the muscle extract prepared from its own right leg given at the same infusion rate as in experiment 1. Six control rabbits underwent the same surgical procedure but the same volume of normal saline, instead of muscle extract, was infused.

In addition to the blood studies done in experiment 1, the following measurements were carried out: plasma fibrinogen, prothrombin time, partial thromboplastin time, and fibrin split products.

Infusion of Muscle Extract and Phospholipase C (Experiment 3)

The protocol was the same as that used in experiment 1. Five rabbits received homologous muscle extract that had been mixed with phospholipase C 30 minutes prior to infusion and kept at room temperature. Five control animals received only phospholipase C. These same studies as in experiment 2 were done.

Results

Analysis of Stock Muscle Extract (Table 1)

The yield of muscle extract was approximately 365 ml/kg of muscle. As anticipated, the concentration of major intracellular elements such as potassium and phosphorus was high, as was the concentration of the muscle enzymes GOT and CPK.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Biochemical Analysis of Stock Muscle Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dl)</td>
<td>5.1 ± 1.0</td>
</tr>
<tr>
<td>Sodium (mEq/liter)</td>
<td>492 ± 1.2</td>
</tr>
<tr>
<td>Potassium (mEq/liter)</td>
<td>111.7 ± 5.7</td>
</tr>
<tr>
<td>Magnesium (mEq/liter)</td>
<td>181.1 ± 1.8</td>
</tr>
<tr>
<td>Calcium (mEq/liter)</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>158.6 ± 18.7</td>
</tr>
<tr>
<td>GOT (units/liter)</td>
<td>62200 ± 106</td>
</tr>
<tr>
<td>CPK (units/liter)</td>
<td>Greater than 1,000,000</td>
</tr>
<tr>
<td>Protein (mg/dl)</td>
<td>45.0 ± 4.5</td>
</tr>
<tr>
<td>Thromboplastin activity (sec)</td>
<td>26.8 ± 5.2</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>491 ± 20.4</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± 1 sd.
high osmolality of the preparations did not represent the endogenous intracellular condition of the muscle tissue; instead it served to define a physical characteristic of the extract preparation. The quantity of potassium and phosphorus infused into each animal was not great—less than 0.1 mEq/kg and 1.5 mg/kg, respectively. A trace of endotoxin was present in the stock muscle extract but the test was negative beyond a 1 in 2 dilution. Several bacterial cultures were sterile, whereas others showed minimal growth of different types of bacteria on subculture only. Immunodiffusion studies for circulating antibodies to muscle constituents were negative. Tissue thromboplastin activity in the muscle extract was equivalent to that of a 1:32 dilution of commercial thromboplastin (Fibroplastin, BBL) used for prothrombin time determination in routine coagulation studies.

Infusion of Homologous Muscle Extract (Experiment 1)

Effects on Renal Function (Table 2). Of the 11 experimental rabbits studied, one died 10 minutes after the infusion was started. The remaining 10 experimental animals developed abnormalities detected by urinalysis. These consisted of cylindruria, proteinuria (all exceeding 200 mg/dl), hemepigmenturia, and, in three animals, glycosuria. These abnormal findings appeared in the first urine specimen collected after beginning the muscle extract infusion and increased during the subsequent 60-90 minutes. The glomerular filtration rate, as determined by endogenous creatinine clearance, decreased immediately and reached a level of about 50% of the preinfusion value within 60 minutes; a continued fall was seen thereafter for the duration of the experiment.

By contrast, the control animals receiving myoglobin showed only transient hemepigmenturia and proteinuria (all less than 100 mg/dl); no change in glomerular filtration rate was observed.

In addition to changes in urinalysis and glomerular filtration rate, the experimental group showed the following changes: a decrease in serum calcium, a rise in serum phosphorus, and a rise in serum potassium. Each of these changes was statistically significant.

Hemodynamic Effects (Fig. 1). A precipitous drop in blood pressure occurred within 5 minutes of initiation of the muscle extract infusion. By this time, the systolic pressure had decreased from 116 ± 9 mm Hg in the control period to 87 ± 15 (P < 0.001) and the diastolic pressure from 83 ± 10 mm Hg before infusion to 58 ± 14 (P < 0.001). The blood pressure tended to rise after this initial rapid fall, but remained below the preinfusion values for the duration of the experiment. Blood pressure in the control group did not change. Concomitant with the time of maximal blood pressure fall there were marked electrocardiographic changes which consisted of alteration of the S-T segment, increased QRS voltage, and arrhythmias. In most instances, these changes were transient and disappeared within 15 to 30 minutes. No electrocardiographic changes were seen in the control group.

Hematological Changes (Fig. 2). Within 5 minutes of initiation of muscle extract infusion, the platelets fell from a preinfusion level of 481,000 ± 259,000/μl to 105,000 ± 65,000 (P < 0.001), whereas no change was noted in the control group. The total white cell count fell from 4380 ± 2800 to 1520 ± 910 (P < 0.05); the myoglobin-injected controls showed no change in white cell count.

### TABLE 2 Experiment 1: Changes in Glomerular Filtration Rate and Serum Biochemical Values after Infusion of Homologous Muscle Extract*

<table>
<thead>
<tr>
<th></th>
<th>GFR (% decrease)</th>
<th>Calcium (% decrease)</th>
<th>Phosphorus (% increase)</th>
<th>Potassium (% increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental (n=10)</td>
<td>53 ± 21</td>
<td>16 ± 6</td>
<td>37 ± 31</td>
<td>31 ± 16</td>
</tr>
<tr>
<td>Controls (n=5)</td>
<td>12 ± 26</td>
<td>8 ± 4</td>
<td>11 ± 19</td>
<td>5 ± 7</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.02</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Absolute values at time zero =</td>
<td>8.4 ± 2.6</td>
<td>12.0 ± 0.7</td>
<td>4.6 ± 0.7</td>
<td>2.7 ± 0.4</td>
</tr>
</tbody>
</table>

* Comparison of studies done before infusion and at the end of the experiment (180 min).
† Since urine was collected from each kidney separately, the number of collection periods used for this analysis was actually twice the value of n.
‡ Mean values were derived from both control and experimental groups before infusion. Respective values from these groups did not differ significantly.
Infusion of Autologous Muscle Extract
(Experiment 2)

Effects on Renal Function. All experimental animals developed abnormalities detected by urinalysis; these consisted of proteinuria, hemepigmenturia, and cylindruria. No urinary changes were seen in the control group except for transient microscopic hematuria resulting from the procedure of ureteral catheterization. The glomerular filtration rate fell to about 50% of the preinfusion values within 15 minutes of infusion of the muscle extract and remained at this level throughout the experiment; in the control group there was no change in glomerular filtration rate.

Hemodynamic Effects. Both systolic and diastolic blood pressure fell significantly within 5 minutes of initiating the muscle extract infusion; the preinfusion and 5-minute postinfusion systolic values were 120 ± 5.6 and 98 ± 8.5 mm Hg (P < 0.001), and the diastolic values were 87 ± 8.6 and 70 ± 12.2 (P < 0.01). In the control group, the preinfusion systolic and diastolic values were 121 ± 6.5 and 90 ± 6.1 mm Hg; there was no significant change throughout the experiment. With the exception of one experimental animal that died 42 minutes after muscle extract infusion was begun and whose electrocardiogram showed progressive bradycardia, neither the experimental nor control groups demonstrated any electrocardiographic changes.

Hematological and Coagulation Changes (Table 3; Fig. 3). In the experimental group, the following changes were observed: prolongation of the prothrombin time and partial thromboplastin time, fall in plasma fibrinogen level, rise in plasma fibrin split products, and fall in total white blood cell and platelet count. No changes were observed in the control animals.

Infusion of Muscle Extract and Phospholipase C (Experiment 3)

Effects on Renal Function. Within 15 minutes of initiating the infusion of muscle extract and phospholipase C, proteinuria and hemepigmenturia were noted; these findings were of the same severity as in the experimental animals in experiments 1 and 2. Cylindruria was not a prominent finding. The glomerular filtration rate fell from preinfusion values of 11.7 ± 3.9 to 4.8 ± 0.8 ml/min at the end of the experiment (P < 0.001). In the control animals given phospholipase C alone, there were no urinary changes nor did the glomerular filtration rate fall.

Hemodynamic Effects. No changes in blood

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**Table 3** Experiment 2: Changes in Hematological and Coagulation Parameters after Infusion of Autologous Muscle Extract

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% decrease in WBC*</th>
<th>% decrease in platelets†</th>
<th>PT (prothrombin time) (sec)</th>
<th>PTT (partial thromboplastin time) (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental</td>
<td>Control</td>
<td>Experimental</td>
<td>Control</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>64 ± 23</td>
<td>1 ± 9</td>
<td>77 ± 5†</td>
<td>1 ± 8</td>
</tr>
<tr>
<td>15</td>
<td>59 ± 16</td>
<td>5 ± 10</td>
<td>76 ± 12‡</td>
<td>11 ± 14</td>
</tr>
<tr>
<td>30</td>
<td>51 ± 28</td>
<td>3 ± 13</td>
<td>58 ± 28§</td>
<td>6 ± 8</td>
</tr>
<tr>
<td>60</td>
<td>47 ± 51</td>
<td>2 ± 13</td>
<td>11 ± 15</td>
<td>7 ± 10</td>
</tr>
<tr>
<td>90</td>
<td>43 ± 51</td>
<td>1 ± 12</td>
<td>23.5 ± 11.6§</td>
<td>7.2 ± 1.5</td>
</tr>
</tbody>
</table>

* Mean preinfusion value of both experimental and control groups: 4700 ± 2940/µl.
† Mean preinfusion value of both experimental and control groups: 515,000 ± 253,000/µl.
‡ Comparison of experimental vs. control group at each time interval, significant at P < 0.01.
§ Comparison of experimental vs. control group at each time interval, significant at P < 0.01.
sure or electrocardiographic findings were seen in either the experimental or control group.

Hematological and Coagulation Changes (Table 4; Fig. 3). There was a precipitous fall within 5 minutes in both total white blood cell and platelet count in the experimental group. These findings were not present in the control rabbits receiving phospholipase C alone. The experimental animals showed a significant rise in partial thromboplastin time and in fibrin split products, as well as a decrease in the level of plasma fibrinogen within 5-15 minutes of infusion of muscle extract and phospholipase C. These changes were not seen in the control animals.

However, in contrast to the findings in experiment 2 in which infusion of autologous muscle extract was associated with a marked rise in prothrombin time, only a minor prolongation was seen in the experimental group receiving muscle extract and phospholipase C; no change was seen in the control animals receiving phospholipase C alone.

Histopathology, Electron Microscopic and Immunopathological Findings

Light Microscopy (Figs. 4-6). Histopathological changes were not detected in the liver, spleen, or heart of experimental or control animals. Pathological findings were confined to the lungs and kidneys.

Multiple thrombi were seen in the lungs of all rabbits in each of the experimental groups, but in none of the controls. Thrombi occluded multiple capillaries and occasionally involved larger pulmonary vessels; a moderate to marked degree of edema fluid was present in the alveolar spaces.

Renal lesions affected glomeruli and tubules. The glomerular lesion was primarily thrombotic in nature. Fibrin-rich microthrombi occluded a variable number of glomerular capillary loops; some glomeruli were spared. This microangi thrombotic change was confined to the glomeruli and was of moderate to marked severity in most of the animals in experiments 1 and 2; none of the rabbits in experiment 3 and none of the control rabbits showed any renal thrombi.

The renal tubular changes affected primarily the proximal convoluted tubules. The cells were swollen, the cytoplasm was clear, and the intracytoplasmic organelles were prominent. In the more severely affected animals, the tubular changes were diffuse with intense "watery" vacuolation of the tubular cells without frank necrosis. The brush border was usually recognizable and the lumen was not distended, appearing empty or containing moderate amounts of finely granular proteinaceous material. A large number of homogeneous eosinophilic casts were present in many collecting ducts; the lumina of these ducts were often distended, but there was no significant alteration of the lining epithelium. Apart from moderate interstitial edema, there were no other changes. The tubular changes were variable in severity and were sometimes mild. In some animals, despite marked glomerular microangi thrombosis, there were no detectable tubular changes. Thus, there was a discrepancy between the severity of the tubular and glomerular lesions in any given animal. Tubular pathology was most marked in experiment 1, less severe in experiment 2, and barely recognizable or absent in experiment 3. No tubular abnormalities were detected in any of the control animals.

Electron Microscopy (Fig. 7). Kidney tissue from experiments 2 and 3 was examined. The ultrastructural findings confirmed the changes seen by light microscopy. The glomeruli showed no significant alteration of the endothelium or capillary basement membranes, but there was a variable degree of swelling of the epithelial cells. The principal change consisted of focal and segmental capillary thrombosis. The affected capillary loops had narrowed or
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% decrease in WBC*</th>
<th>% decrease in platelets†</th>
<th>PT (prothrombin time) (sec)</th>
<th>PTT (partial thromboplastin time) (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6.8 ± 0.6</td>
<td>16.8 ± 2.5</td>
</tr>
<tr>
<td>5</td>
<td>61 ± 35</td>
<td>76 ± 13</td>
<td>6.6 ± 0.1</td>
<td>17.2 ± 2.5</td>
</tr>
<tr>
<td>15</td>
<td>68 ± 17</td>
<td>70 ± 17</td>
<td>7.3 ± 0.9</td>
<td>35.2 ± 10.7</td>
</tr>
<tr>
<td>30</td>
<td>59 ± 11</td>
<td>73 ± 23</td>
<td>7.9 ± 0.4</td>
<td>101.8 ± 34.9</td>
</tr>
<tr>
<td>60</td>
<td>50 ± 6</td>
<td>76 ± 22</td>
<td>8.2 ± 1.2</td>
<td>85.5 ± 34.9</td>
</tr>
<tr>
<td>90</td>
<td>50 ± 6</td>
<td>76 ± 22</td>
<td>8.8 ± 1.7</td>
<td>87.5 ± 62.5</td>
</tr>
<tr>
<td>120</td>
<td>50 ± 6</td>
<td>76 ± 22</td>
<td>8.8 ± 1.7</td>
<td>92.3 ± 84.1</td>
</tr>
</tbody>
</table>

* Mean preinfusion value of both experimental and control groups: 6880 ± 2408/jd.
† Mean preinfusion value of both experimental and control groups: 680,000 ± 165,000/jd.
‡ Comparison of experimental vs. control group at each time interval, significant at P < 0.001.
§ Comparison of experimental vs. control group at each time interval, significant at P < 0.05.
|| Comparison of experimental vs. control group at each time interval, significant at P < 0.01.

Totally occluded lumina containing fibrin aggregates; platelet clumps were often seen. There was no recognizable endothelial cell change separating the thrombus from the normal-appearing basement membrane. An unusual and unexpected finding was observed in three experimental rabbits (two from experiment 2 and one from experiment 3). This change consisted of the deposition of small, markedly dense electron aggregates of amorphous material predominantly in a subendothelial position; some of these deposits were seen on the epithelial side of the membrane. The size of the deposits approximates that of the pores of the membrana fenestra.

As was seen by light microscopy, the tubular changes were most pronounced in the epithelial...
cells of the proximal convoluted tubule. The changes were more often patchy than diffuse, and consisted primarily of swelling of the cytoplasm with rarefaction of cytoplasmic density. In many of the markedly swollen cells, mitochondria, lysosomes, and endoplasmic reticulum appear to have been lost or significantly displaced by the cytoplasmic swelling. In contrast to the cytoplasmic alteration, the brush border was generally well-preserved, although there were focal areas in which the brush border was denuded. In some tubules, detached or desquamated swollen cells containing almost no organelles were present in the lumen. A variable degree of interstitial edema also was present.

Immunopathology. Two of 11 and two of six experimental animals in experiments 1 and 2, respectively, showed marked glomerular fluorescence for fibrinogen affecting all glomeruli. One animal in experiment 1 showed positive glomerular fluorescence for IgG. No glomerular or tubular fluorescence was seen in the other experimental animals nor in any of the controls.

Discussion

Our study demonstrates the development of acute renal dysfunction with associated morphological change in well-hydrated rabbits following infusion of muscle extract. The data suggest that myoglobin is not responsible for the changes observed, and that there is perturbation in other biological systems, which include hematological and coagulation disturbances as well as hemodynamic changes. Immunological or histocompatibility factors do not appear to be operative.

Following muscle injury or rhabdomyolysis, other intracellular constituents in addition to myoglobin are released into the circulation; this is reflected in increased plasma levels of enzymes such as CPK and GOT (Wright et al., 1971). It may be anticipated, as well, that proteolytic lysosomal enzymes, collagen, and tissue thromboplastin also are released (Glenn and Lefer, 1971).

The changes in platelet count and the coagulation disturbances seen after muscle extract infusion are compatible with intravascular coagulation initiated via both the intrinsic and extrinsic pathways by muscle constituents such as tissue thromboplastin, collagen, and enzymes either present within the extract itself or released from platelets. The mechanisms by which disturbances in these biological systems lead to renal dysfunction are not yet understood, but our data suggest that both tubular
and glomerular dysfunction occur within a very short period after intravenous infusion of muscle extract.

In clinical practice, extensive trauma or major surgical procedures may be complicated by what is referred to as post-traumatic lung insufficiency (Eeles and Sevitt, 1967); that intravascular coagulation may play a pathogenetic role is suggested by the finding of platelet aggregates and fibrin in the pulmonary microcirculation. In addition, intravascular coagulation may present as a complication of other forms of tissue injury, notably that seen in obstetrical problems such as abruptio placenta (Page et al., 1951), amniotic fluid embolism (Ratnoff and Vosburgh, 1952), and septic abortion (Crisnic et al., 1971). A fall in plasma fibrinogen and factor VIII within 4 hours after normal delivery suggests a minor degree of intravascular coagulation (Kleinert et al., 1970). Experimentally, a defibrination syndrome can be induced in dogs by infusion of an aqueous extract of human placenta (Page et al., 1951). Extracts of other body tissues vary in their ability to induce in vitro blood coagulation (Mills, 1921).

Release of tissue thromboplastin may be the principal common denominator in these coagulation changes, and thromboplastin activity was indeed demonstrated in the muscle extract used in our studies. Others have shown that intravenous infusion of purified tissue thromboplastin in a number of species may induce coagulation disturbances and pathological changes indicative of intravascular coagulation, presumably by activation of the extrinsic coagulation pathway (Hartmann et al., 1951; Giercksky et al., 1976a).

Tissue thromboplastin is a protein-phospholipid complex whose biological activity is effectively inactivated by the enzyme phospholipase C which causes hydrolysis of the phospholipid moiety (Otnaess et al., 1972). Recently it has been shown that rats that are pretreated with phospholipase C are protected from the lethal effect of intravenous infusion of tissue thromboplastin (Giercksky et al., 1976b). In experiment 3, in which muscle extract was preincubated with phospholipase C in a concentration sufficient to inactivate thromboplastin activity, there was only a minor difference between the prothrombin time in the experimental and control animals. However, the partial thromboplastin time was markedly prolonged in the experimental group, and renal dysfunction was observed. These findings are compatible with inactivation by phospholipase C of thromboplastin activity present in the muscle extract; activation of the intrinsic coag-
ulation pathway remained intact. The intrinsic coagulation cascade starts with activation of factor XII by surface contact (Macfarlane, 1972). Collagen and fatty acids can also activate factor XII in vitro and in vivo (Conner et al., 1963; Niewiarowski et al., 1966; Wilner et al., 1968), and aggregation of rabbit platelets is seen when they are exposed to a suspension of connective tissue such as collagen (Zucker and Borrelli, 1962) or a saline extract of tendons (Nachman and Ferris, 1969). Platelet aggregation leads to release of intraplatelet proteolytic enzymes (Zucker and Borrelli, 1962) which can initiate intravascular coagulation via the intrinsic system.

The pathological and immunopathological findings provide supportive evidence for the operation of intravascular coagulation in the pathogenesis of the changes seen after muscle extract infusion. What is not clear, however, is the mechanism by which the kidneys appear to be selected as the principal target organ. Furthermore, the striking tubular changes appear to be out of proportion to those which could be explained solely as a consequence of local ischemia induced by impaired circulation resulting from intravascular coagulation. The possibility remains that the muscle extract contains specific nephrotoxins which are themselves primarily responsible for the renal alterations we have observed.

A satisfactory explanation for the hemodynamic alterations is not at hand, nor is it clear what role the blood pressure drop seen in experiments 1 and 2, and the cardiac depolarization abnormalities seen in experiment 1, played in the pathogenesis of the renal dysfunction. The fact that renal disturbances such as a fall in glomerular filtration rate and abnormalities in urine sediment were seen in each of the experiments despite differences in the hemodynamic alterations between experiments suggests that these factors are not of paramount importance.

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