CHAPTER III

MATERIALS AND METHODS

Study Design

Animal experimental study

Materials and Methods

Lyophylized Russell's viper venom obtained from the Thai Red cross Society, Bangkok was used. The dried crystal was dissolved in sterile physiologic saline (0.9 % NaCl) just prior to injection at a concentration of 1 mg. per ml.

Highly inbred male wistar rats weighing 200 - 300 gm., obtained from the National Laboratory Animal Center, Saraya, Nakornprathom, were used. These rats were devided into 3 groups as follow :

Group A : Russell's viper venom 1 mg/kg.

Group B : Russell's viper venom 2 mg/kg.

Group C : 0.9 % NaCl solution 1 ml./kg.

All were injected intramuscularly at the site of quadriceps muscle.

Nephrectomies were done at intervals of 2 hour, 6 hour, 1 day, 3 day, 10 day and 30 day after injection under ether anesthesia. Each interval consisted of 4 animals in group A and B and 2 animals in group C.

Preparation the Tissues for Examination

Each of renal tissues was processed for both light and electron microscopy.

For light microscopy, the tissues were fixed in 10 % buffered formalin solution, embedded in paraffin, and stained with hematoxylin-eosin (H&E), periodic acid-schiff (PAS) and silver methenamine methods. The phosphotungstic acid hematoxylin (PTAH) technique was used for special fibrin stains.

Tissues for electron microscopy were diced into 1 cumm, fixed in freshly prepared cold 2.5 % glutaraldehyde in phosphate buffer, postfixed in 1 % osmium tetroxide in 0.1 M phosphate buffer. Dehydration was carried out in a graded series of ethanol. Then the tissues were embedded in Epon. The thick sections of 1 micron, stained with toluidine blue, were examined under the light microscope. The ultrathin sections were stained with uranyl acetate and lead citrate and then examined by Hitachi HU-12A electron microscope.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

For light microscopy, the tissues were fixed in 10 % buffered formalin solution, embedded in paraffin, and stained with hematoxylin-eosin (H&E), periodic acid-schiff (PAS) and silver methenamine methods. The phosphotungstic acid hematoxylin (PTAH) technique was used for special fibrin stains.

The tissues for electron microscopy were diced into 1 cumm, fixed in freshly prepared cold 2.5 % glutaraldehyde in phosphate buffer, postfixed in 1 % osmium tetroxide in 0.1 M phosphate buffer. Dehydration was carried out in a graded series of ethanol. Then the tissues were embedded in Epon. The thick sections of 1 micron, stained with toluidine blue, were examined under the light microscope. The ultrathin sections were stained with uranyl acetate and lead citrate and then examined by Hitachi electron microscope.