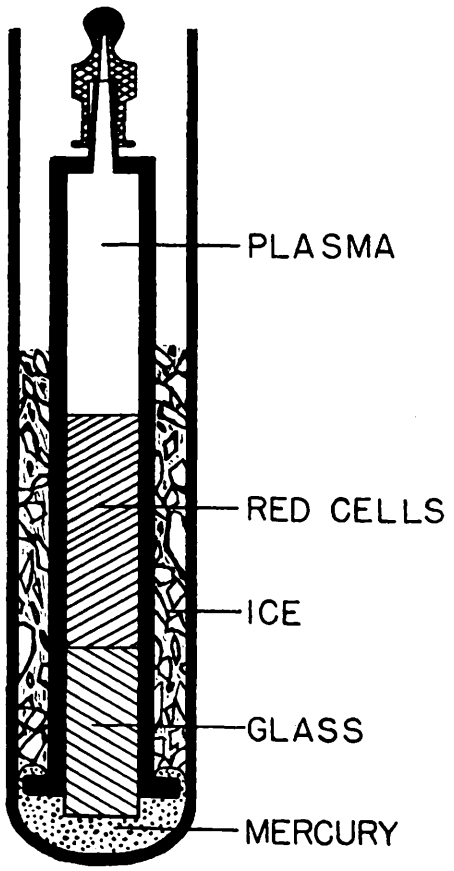

FIG. 1. Apparatus as it appears immediately following centrifugation.

lower portion of the glass unit before centrifuging, or the volume of mercury in the centrifuge tube should be decreased.

Following centrifugation it will be found (Fig. 1) that the red cells have been centrifuged into a compact layer just above the plunger of the syringe. If only minimal amounts of mineral oil have been used, the mineral oil will be found in the small tip of the syringe where it can easily be ejected. The remainder of the plunger is returned to its normal position and the samples are delivered into pipets.

With Krogh-Keys pipets(1) and the usual volumetric pipets this is easily accomplished by connecting the pipet to the syringe tip with a 1 cm length of small latex tubing, and filling the pipet with positive pressure from the syringe. With Scholander-Roughton blood pipets(2) it has been found convenient to grind the syringe tip so that the pipet tip fits into it. Between aliquots the cap was returned to the syringe tip.

This work was carried out under a contract between the University of Minnesota and the Atomic Energy Commission.

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Glomerular Intermittence in Rats Demonstrated by Use of a New Direct Visual Technic. (19947)

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Incidental to a broader study of the renal vascular bed in various physiological and pathological states of function, we have gathered material which seems clearly to demonstrate glomerular intermittence in the rat kidney.

Glomerular intermittence was first observed in the frog by Richards *et al.*(1) and its occurrence in amphibia is widely accepted. However, the belief that "all glomeruli function all the time" in mammals has been widely held. Smith(2) and others believe their work utilizing clearance technics constitutes firm proof of this statement if not in all mammals at least in the dog and humans. The work of Kaplan and Smith(3) using clearance technics has demonstrated glomerular intermittence in rabbits. This work has been both confirmed and disputed by others.

Methods. Our technic consisted in the following: 1) At a preliminary operation (under ether anesthesia) rats were opened, the abdom-

inal aorta visualized, and a clamp placed on it a few mm above its bifurcation. Using a straight cutting edged needle, a small opening was made in the aorta just above the bifurcation and a No. 10 polyethylene tube (previously filled with normal saline) was inserted into the opening. The clamp was then removed from the aorta and the tubing was pushed craniad so it would terminate a few (5 to 10) mm above the renal arteries. The tubing was secured by ligatures to the muscles of the posterior belly wall. The other end of the tube was brought out of the lower end of the abdominal incision and by means of blunt dissection was carried beneath the skin to a point on the back behind the head where a small incision was made and the free end of the tube exteriorized. Before closing the tube, we would demonstrate that we were in the aorta by observing a back flow of blood under pressure in the tube. The tube was then cleared with normal saline and sealed. The abdomen was then closed and the animals were allowed to recover in individual cages for not less than 2 and usually 3 to 5 days. (Animals which did not appear fully recovered after 2 or 3 days were discarded). 2) After the 2 to 5 day recovery period, various agents (2% saline, 5% urea, distilled water, pitressin, and epinephrine) intended to modify the function of the kidneys were introduced through the tubing. Dehydration for 48 hours was also used as an experimental agent. At appropriate time intervals, 1 ml India ink was rapidly (5 to 10 sec.) injected through the tubing and while the injection was proceeding the animal was guillotined at a point between the kidneys and the heart. In this manner we believe we introduced ink into the kidneys at normal pressure over the pathways which were functioning during the 5- to 10-second injection period and that by guillotining the animal, as we did. we stopped, at that moment, all movement of blood in the kidneys and therefore had an India ink outline of the vascular bed which was operative during the brief 5- to 10-second interval. The kidneys were immediately removed, fixed in 10% formalin and sections prepared for histologic study.

The technic used in these experiments is, we believe, superior to most previous experimental work of a similar nature because: (a) It requires no anesthetic agent which suppresses urine formation. (b) It requires no surgery which might disturb the nerve supply to the kidney. (c) The India ink which at the termination of the experiments

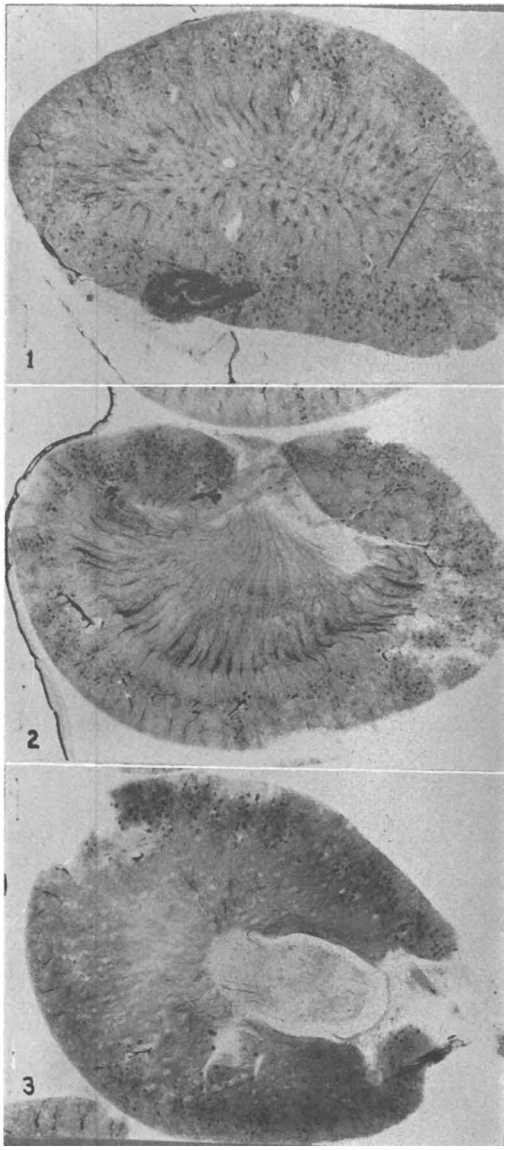

FIG. 1. Rat received standard chow and tap water until 2 hr before sacrifice when water was removed.

FIG. 2. Rat received 2% saline (1 cc/100 g) 30 min. before being sacrificed.

FIG. 3. Rat received .2 U pitressin in ½ cc saline followed immediately (in about 5 sec.) by sacrifice procedure.

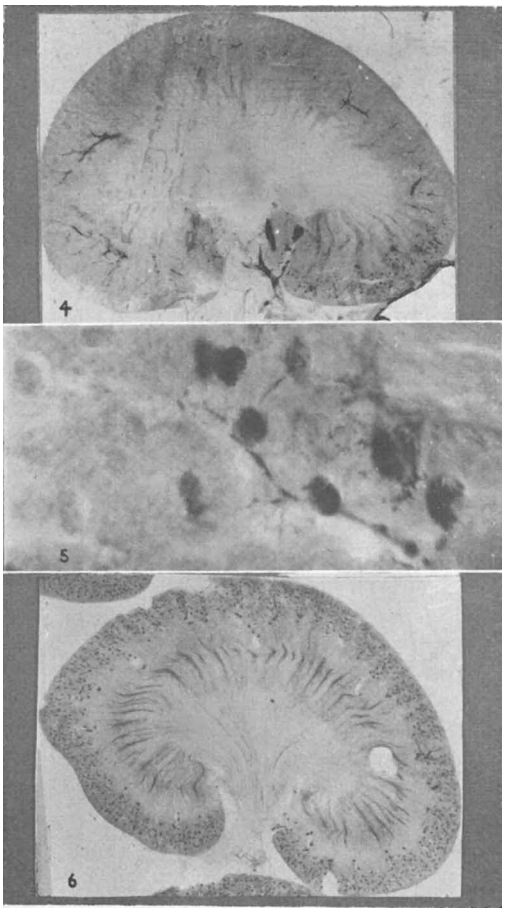

FIG. 4. Rat received ½ cc of 1/250000 epinephrine followed immediately (in about 5 sec.) by sacrifice procedure.

FIG. 5. Enlarged microphotograph of a portion of section shown in Fig. 3.

FIG. 6. Rat received no water for 48 hr prior to sacrifice.

is introduced into the kidneys, reaches the kidneys under a pressure which must be approximately the normal for the rat being sacrificed. (Most injection studies have been done on post-mortem material often at very high pressures.) Many of those in which the ink was introduced by the animal's own heart activity involved some very unphysiologic procedure such as opening the chest in order to introduce a needle into the aorta(4), etc. Most of the previous studies of a similar nature have been open to criticism on one or more of the above grounds, and we believe the technic described above successfully avoids these objections.

Results. As previously stated, this is a report of an incidental finding made during the course of a larger study not yet fully completed. Of the 20 kidneys thus far studied, 9 show what we interpret as clear cut proof of glomerular intermittence.

These results were obtained under 4 different conditions: (a) One of our normals which had had the water removed from the cage 2 hours before sacrifice (Fig. 1). (b) Three animals receiving 2% saline (1 cc/100 g) Fig. 2 is typical of these. (c) Two animals receiving Pitressin (one received 0.2 U and one received 1.0 U) Fig. 3 is typical of these. (d)Three animals receiving epinephrine in 3 different dosages as follows: 1 cc—1/250000, ½ cc—1/250000, ½ cc—1/25000. Fig. 4 is typical of these.

With greater magnification (about 20×), each of these kidneys shows areas such as are seen in Fig. 5 in which some glomeruli filled with ink and other "empty" glomeruli are clearly visible. The kidneys seen in Fig. 1 to 5, which we believe constitute a demonstration of intermittence, may be contrasted with Fig. 6 which is typical of the 11 kidneys in which all glomeruli were functioning at the time ink was injected.

Comments and conclusion. In analyzing our results two principal questions arose: 1) Was the ink being thoroughly mixed with blood? We believe this technic, in which the ink is injected into the aorta in a direction counter to the blood flow, would set up such an eddy as to insure very adequate mixing. (We believe the ratio of ink to blood at the point of injection is in the range of 1 to 5). The fact that all glomeruli of 11 kidneys contain ink supports this belief. Furthermore, in the 9 kidneys which show intermittence, most of the large arteries (arcuate and interlobular) contain ink. 2) Could "the empty" glomeruli have contained ink which had flowed on to the venous side? Except in a few instances we succeeded in guillotining our animals *while* the ink was being injected and we believe this stopped all pressure behind the ink column. Furthermore, India ink contains a high percentage of particles too large to pass through capillaries. In addition, the glomeruli which contain ink in those kidneys

showing intermittence are well filled.

For these reasons, we *conclude* that no blood (and ink) was flowing through the "empty" glomeruli during the time (5 to 10 sec.) while the injection of ink was being made. In other words, the "empty" glomeruli were not functioning or more precisely blood was not flowing through them as it was in those glomeruli which contain the ink.

Attention is drawn to the fact that the functioning glomeruli in the 9 kidneys showing intermittence seem to be predominantly though not exclusively of the juxta-medullary type.

Summary. A new direct visual technic for studying the vascular supply of the rat kidney is described. Photographs of kidneys which demonstrate glomerular intermittence are presented.

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Effect of Estrogens on Serum Precipitable Iodine.* (19948)

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It is known that the serum precipitable iodine (SPI), a reliable index of the level of the circulating thyroid hormone, increases during normal pregnancy(1). The rise occurs early in pregnancy, is maintained until after delivery, and is not associated with clinical hyperthyroidism(2). The cause of the increase in the level of the circulating thyroid hormone has not been explained.

Some instances of abortion or threatened abortion in early pregnancy have been reported to be associated with levels of the SPI below that found in normal pregnancy and the administration of exogenous thyroid has been advocated in these cases(1,2). On the other hand the Smiths(3) and others have suggested the use of estrogens in cases of habitual abortion. In other studies the pregnant state appeared partially to ameliorate clinical hyperthyroidism(4). Because of these observations and because estrogens appear to depress the respiration of tissue homogenates(5) it seemed pertinent to investigate the influence of estrogens on the SPI.

Materials and methods. Sixteen patients (5 women and 11 men) whose ages ranged from 18 to 74 years were given either diethylstilbestrol† (14 patients) or Premarin† (2 patients). None was acutely ill although the majority were chronically ill with carcinoma of the breast or prostate. From 20 to 100 mg of diethylstilbestrol per day and 5 mg of Premarin per day were administered in divided doses. The SPI was determined by the method of Barker(6) as modified by Danowski and associates(7). Our normal range, obtained on 63 individuals, varies from 3.5 to 7.3 μg per 100 ml of blood (mean 5.2 μg) and is

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† Stilbestrol was kindly supplied by Mr. Robert Thompson and Mr. J. W. Schma of the Upjohn Co. The Premarin (conjugated estrogens-equine) was supplied by Dr. J. S. Scanlon of Ayerst, McKenna and Harrison.