Comment. In untreated rabbits, hemorrhage does not occur in skin sites prepared with meningococcus toxin until after an intravenous injection of toxin or other suitable "provocative" material is given, 18 or more hours later. A variety of apparently unrelated substances are capable of producing the phenomenon by intravenous injection, including bacterial toxins, whole bacteria, starch, agar, glycogen, kaolin, and antigen-antibody mixtures(2,3). The possibility cannot be excluded that ACTH and Cortisone produced the observed reactions by virtue of a comparable "non-specific" property which is not related to the known functions of these materials. It is unlikely that bacterial contaminants in the substances were responsible for the lesions, since cultures of the ACTH and Cortisone preparations yielded no growth.

It is possible that the skin reactions in ACTH- or Cortisone-treated rabbits may be based on a mechanism different from the Shwartzman phenomenon itself. In the absence of edema and erythema, and in the gradual progressive manner of their development, the lesions differed sharply from the typical Shwartzman reaction. Conceivably, the abatement of the usual inflammatory reaction to locally injected toxin may have increased the vulnerability of skin tissue to a primary damaging property of the toxin. There is evidence which suggests that the vessels of skin become less permeable following an injection of meningococcus toxin(4), and the possibility that further interference with permeability may occur following the administration of ACTH and Cortisone is under investigation.

Summary. The observations indicate that under certain circumstances the treatment of rabbits with ACTH or Cortisone results in a type of local skin damage by meningococcus toxin which is not seen in untreated animals. The application of these findings to other varieties of tissue damage by bacteria and their products is a field which merits further study.

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A Method for Visualization of Kidney Blood Vessels Applied to Studies of the Crush Syndrome.* (18061)

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A variety of conditions such as crush syndrome, severe trauma to muscles, non-traumatic muscular ischemia, transfusions with incompatible blood, heat stroke, toxemias of pregnancy, sulfonamide intoxications, poisoning of different types, etc., have one clinical manifestation in common, oliguria or anuria. Furthermore, the pathological finding of lower nephron nephrosis seems to be typical for all the above mentioned conditions. A drop in blood pressure below the necessary filtration pressure would itself be expected to cause anuria as filtration in the glomeruli stops. Arterial blood pressure determinations in the conditions mentioned will usually, in the very early stages, show a lowered blood pressure, but not always a pressure below that believed adequate for ultrafiltration. Often an increase in blood pressure is seen as the condition develops; despite this the patient may be completely anuric.

Three main theories are generally accepted as being possible explanations of oliguria and

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anuria: (a) renal ischemia, (b) tubular obstruction, and (c) unselective reabsorption of glomerular filtrates.

The first hypothesis (a), which claims that renal ischemia is the main factor leading to lower nephron nephrosis, has recently gained strength by the important investigations of Trueta and co-workers(1), at least as a complication of the crush syndrome in rabbits. The technic used in those experiments has been further perfected by Barclay(2).

Baker and Dodds(3) have supported the theory (b) that the mechanism of oliguria is caused by an obstruction of the tubules. Others have the same point of view(4,5). Most investigators, however, oppose this hypothesis(6-14).

The theory (c) of unselective reabsorption of glomerular filtrates was first put forward by Dunn and his co-workers(15,16), and later confirmed by Richards and his associates(17).

Inasmuch as the hypothesis (a) of renal ischemia has gained a growing acceptance as the cause of these conditions, it seemed important to study the kidney circulation in normal rabbits and rabbits which had been exposed to crush injury, using Trueta's method of producing crush. These studies appeared to be especially indicated inasmuch as there was available a method for intra vitam visualization of the walls of the blood vessels.

Method. The method used in these experiments has been described in a previous publication by Schlegel(18). The principle of the method is to inject intravenously a fluorescent dye, Thioflavine-S (methyldehydrothio-p-toluidine-sulfonate.† This dye has the property of being taken up by the walls of the blood vessels immediately after the injection, thus making these visible when observed in ultraviolet light. Initially the tissues were examined in ultraviolet light after freezing and dehydration, but recently we have introduced a modification which seems to be of value. Immediately after removal of the kidney, it is sliced with a sharp knife, and the slices are placed in 100% glycerin. This will clear the tissue so that the vascular details are clearly visible with the microscope when observed in reflected ultraviolet light. The diffusion of the fluorescent dye out into the glycerin is slow enough to allow observations for hours, and photographic recordings have routinely been carried out.

The dye does not cause any change in blood pressure and the investigations of Algire and Schlegel(19) have shown that the dye causes no change in the state of blood vessels when observed by means of transparent windows in mice. This dye when injected intravenously into the living animal will give a picture of all vessels through which blood was flowing at the time of injection. Whatever happens during the removal of the organ to be studied

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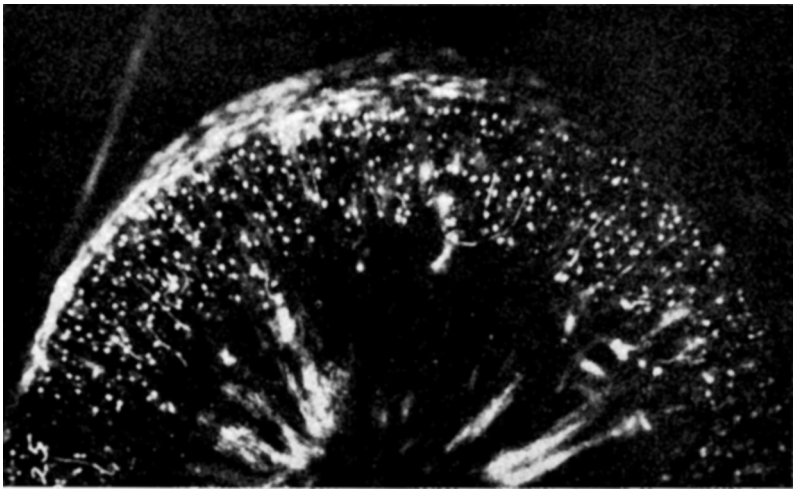

FIG. 1.
Photomicrograph taken in incident ultraviolet light of a kidney from a rabbit with a blood pressure of around 40 mm mercury. Note brilliant fluorescence of all vessels and practically no diffusion of dye (8×).

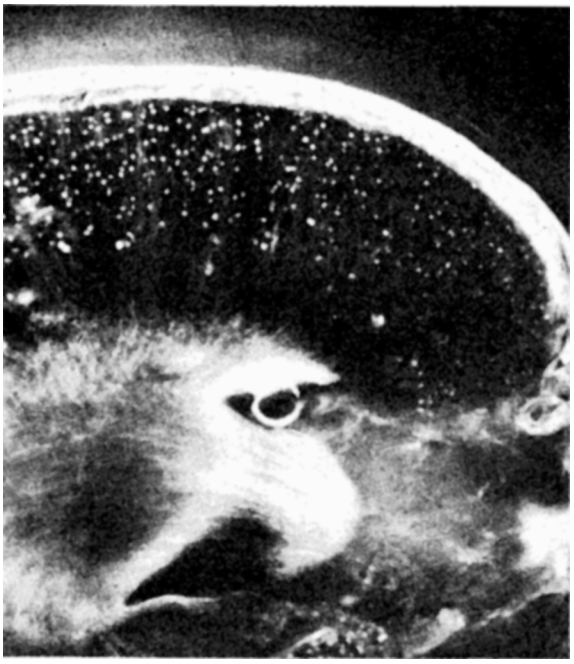

FIG. 2.
Photomicrograph taken in incident ultraviolet light from a normal rabbit kidney. Kidney was removed 15 sec. after injection of Thioflavine-S. Medulla shows a brilliant fluorescence (8×).

does not interfere with the interpretation of the results, since the dye is already in the walls of the vessels and will remain there for some time before it diffuses out (Fig. 1). No other fluorescent dyes we have tried have the property of making the blood vessels

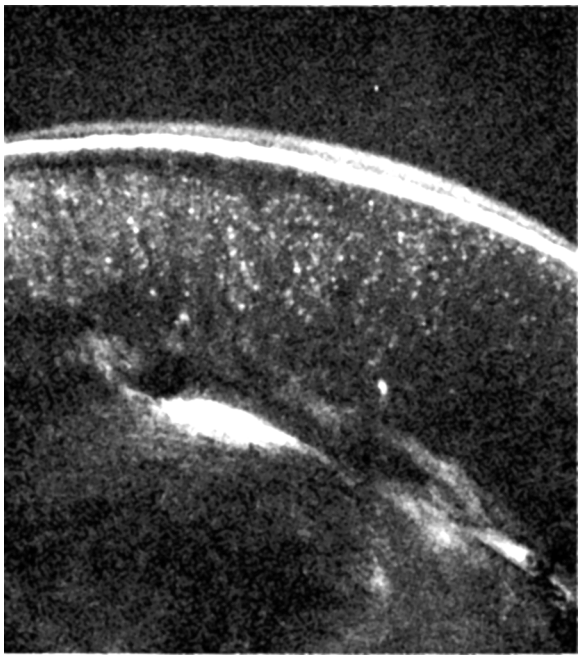

FIG. 3.
Photomicrograph taken in incident ultraviolet light of a kidney from a crush rabbit. Kidney was removed 15 sec. after injection of Thioflavine-S. Note lack of fluorescence of medulla as compared to Fig. 2 (8×).

visible because they diffuse out of the blood vessels almost simultaneously with the intravenous injection, whereas diffusion of Thioflavine-S takes several minutes, thus, allowing adequate time for removal of the organ to be studied. The blood, in itself, shows no fluorescence, so that squeezing of blood from the vessels will not interfere with the results. The rate of diffusion of the dye out of the blood vessels into the tissue is probably determined by the blood pressure, the rate of blood flow, and the permeability of the vessels. These conditions are to be investigated further. Blood pressure determinations have been carried out on most of the rabbits by inserting a needle in one of the carotid arteries, and recordings have been made on a kymograph by means of a mercury manometer. To determine the urinary excretion of the rabbit, collection of urine by catheterization has also been carried out in most of the experiments, usually in a half-hour period immediately prior to the injection of Thioflavine-S and removal of the kidneys.

Material. For these preliminary experiments, 46 rabbits have been used. Dial‡ in the amount of 0.6 cc per kg, given intravenously has been used for anesthesia.

Thioflavine-S in a 4% aqueous solution has been injected in an ear vein in the amount of 1 cc per kg of body weight. At different time intervals after the injection, the kidneys have been removed, sliced, placed in glycerin, and examined with the microscope in reflected ultraviolet light.

A tourniquet was applied to the left thighs of 16 rabbits according to the method described by Trueta, and left for 4½ hours. These rabbits will hereafter be referred to as "crush rabbits." In these animals the dye has been injected 1 to 2 hours after removal of the tourniquet.

Results. In all the kidneys examined, there has not been a single case in which the inter-

‡ Dial (each cc contains Diallylbarbituric acid 0.1 g, urethane 0.4 g, monoethylurea 0.4 g, distilled water *q.s.*) Furnished through courtesy of Ciba Pharmaceutical Products, Inc., Summit, N.J.

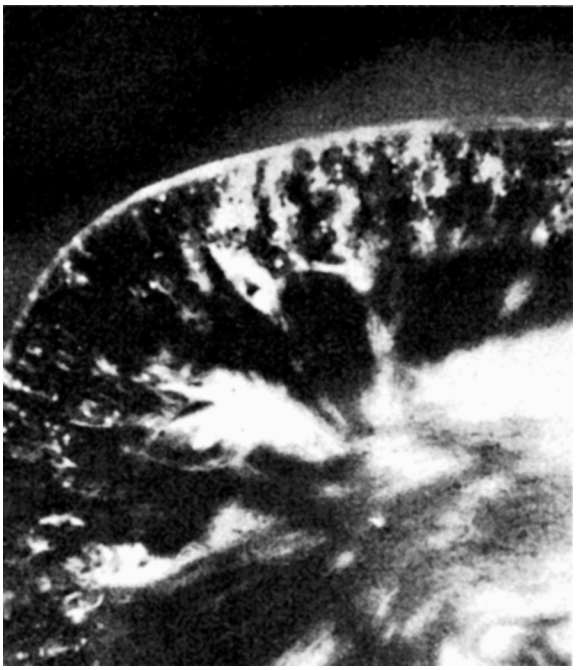

FIG. 4.
Photomicrograph taken in incident ultraviolet light of normal rabbit kidney after intravenous adrenalin injection (8×). Note that fluorescence is not limited to any particular area.

lobular arterioles supplying the glomeruli did not show definite fluorescence. The interlobular arterioles and all the glomeruli, the peripheral as well as the juxta-medullary, showed a high degree of fluorescence in the kidneys of normal as well as of crush rabbits. Fig. 2 and 3 are photographs of sections from kidneys removed 15 seconds after the injection of Thioflavine-S. Fig. 3 is the kidney of a rabbit which had been exposed to a crush injury according to Trueta's method, and Fig. 2 is from a kidney of a normal rabbit. From the photographs it can be seen that the glomeruli, in both instances, show a bright fluorescence, indicating that blood had been flowing through the glomeruli in the normal rabbit kidney and in the kidney from the crush rabbit, causing the same degree of fluorescence of the glomeruli. A difference exists in the degree of fluorescence of the medulla. There is a bright fluorescence of the medulla in the kidneys from the normal rabbits contrasting with only very slight fluorescence of the medulla in the kidneys from the crush rabbits. The urine output in the rabbits exposed to the crush injury within 1-2 hours after removal of the tourniquet was on the average much higher than that of the normal rabbits. Phenolsulfonphthalein kidney function tests showed a delayed and decreased excretion of the dye as compared to the excretion in normal rabbits.

Blood pressure determinations were carried out in most of the experiments and showed on the average a pressure of around 100 mm mercury in the normal anesthetized rabbits and around 70 mm mercury in the crush rabbits.

Discussion and conclusion. We have not been able to confirm the results of Trueta's experiments in rabbits exposed to a crush injury. He interprets his results as indicating the exclusion of the renal cortex from the circulation and a maintenance of a blood flow through the medulla. The results we have obtained seemed rather to indicate a decrease in blood flow in the medulla of the crush rabbits; a decrease which is seen at a time

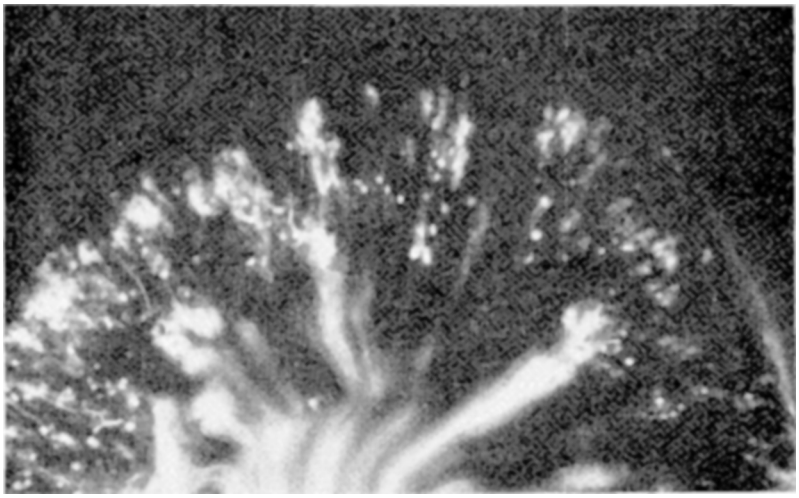

FIG. 5.
Photomicrograph taken in incident ultraviolet light of a crush rabbit after intravenous injection of adrenalin in a dosage equivalent to that given the rabbit whose kidney is seen in Fig. 4. Note that there is no significant difference from Fig. 4.

when the blood pressure is usually around 70 mm mercury. There does not seem to be any decrease in the blood flow through the glomeruli, as their fluorescence is practically equivalent to the fluorescence of the glomeruli of a normal rabbit kidney. The increased output of urine 1-2 hours after removal of the tourniquet indicates that the glomeruli are functioning. Whether the increase in urine production is caused by an increase in ultrafiltration or by a decrease in tubular reabsorption of water cannot be answered yet; the delayed and diminished output of phenolsulfonphthalein is most likely caused by a decreased tubular function.

In order to show whether adrenalin would cause any distribution of the blood flow as indicated by Trueta in the crush, adrenalin in the amount of 3.7 cc per kg (1:10,000) was given intravenously to a normal rabbit (Fig. 4) and to one which had been exposed to crush injury (Fig. 5). It will be seen from these pictures that there is an obvious vasoconstriction with a diminution of the blood flow; moreover the picture seen does not show any indication of a juxta-medullary by-pass. We must therefore conclude that in our experiments we have not confirmed Trueta's results indicating a by-pass in case of a crush injury. Our experiments seem to indicate that the changes in kidney function in rabbits seen 1-2 hours after a crush injury are a result of a decrease in blood flow, particularly in the medulla, caused by a drop in systemic blood pressure, most likely combined with a constriction of the efferent arterioles.

Summary. The technic for visualization of blood vessels by intravenous injection of the fluorescent dye, Thioflavine-S, has been used in a study of kidney circulation in normal rabbits and rabbits exposed to crush injury.

The results obtained show that the blood flow in the medulla is decreased. We have not been able to confirm Trueta's results indicating a by-pass through the juxtamedullary glomeruli.

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