PROTECTION AGAINST FATAL RENAL INJURY DUE TO CHOLINE DEFICIENCY BY RENAL DECAPSULATION

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Plates 16 and 17

(Received for publication, June 26, 1952)

Very impressive renal lesions develop regularly in young rats which have been maintained for about a week on diets deficient in choline (1). The kidneys rather suddenly become greatly enlarged, dark, and tense, and microscopic examination reveals necrosis and congestion in the cortex, and casts of homogeneous material in the distal tubules. The lesions resemble to some extent those which occur in the clinical syndrome of acute bilateral cortical necrosis (2). Many animals die in the acute stages of injury. Those which survive for 10 to 14 days on the diet usually recover, and the renal congestion, edema, and tenseness disappear rapidly. Many of the necrotic tubules are repaired. However, a variable portion of the glomeruli in the outer cortex have been damaged or destroyed, and certain of the tubules have been irreparably plugged and converted to cysts. The acute injury is sometimes followed by hypertension in the later stages (3).

In the course of studies on the pathogenesis of this interesting renal syndrome, the effects of renal decapsulation were investigated. This investigation was prompted by the tenseness of the kidneys in advanced stages of the acute lesions, and the apparent decrease in tenseness after removal of the thickened, edematous capsule. The observations presented here indicate that removal of the capsule from one kidney just prior to starting the deficient diet resulted in a significant decrease in damage to the decapsulated kidney compared with that occurring in the contralateral kidney of the same animal, and in a decrease in mortality of the animals compared with that in a control group. Some evidence was obtained concerning the nature of the protective effect.

Methods

The rats were 20 to 22 day old males of a Sherman strain which weighed 30 to 40 gm. at the beginning of the experiments. The choline-deficient diet was the same 20 per cent casein, sucrose-containing diet described previously (4), with Wesson's modification of the Osborne-Mendel salt mixture (5). The animals were maintained in individual cages with large mesh screen bottoms, in a room with controlled temperature.

Decapsulation Techniques.—In the majority of the experiments upon which the principal conclusions are based, the left kidney only was decapsulated, under ether anesthesia, through a

lumbar incision in the costovertebral angle immediately over the dorsal aspect of the kidney. The kidney was delivered through the incision and the capsule together with the superficial portions of the cortex was incised from pole to pole along the lateral curvature of the kidney. The cut edges of the capsule were then peeled toward the hilum anteriorly and posteriorly, and the portions of the capsule thus freed were trimmed away so that they could not slip back into place and cover the denuded surfaces of the kidney. The portion of the capsule near the renal pelvis was not disturbed and the hilar structures were avoided completely. It was not necessary to detach the kidney from the adrenal. The kidney was then returned to the abdominal cavity and muscle and skin were closed with interrupted cotton sutures. In each experiment, control animals were subjected to the same operation without actual decapsulation. The animals were started on the choline-deficient diet on the same day or the day following the surgical procedure.

In a smaller number of experiments, the left kidney was decapsulated through a midline abdominal incision. Adequate exposure of the kidneys was difficult with this operation. However, since the incision was located at a distance from the kidney, it provided a possible means of evaluating the effect of the adhesions which developed between the decapsulated kidney and the area of the incision following the lumbar operation.

In other experiments, decapsulation through the lumbar incision was delayed until the 3rd to 6th day on the deficient diet in order to determine the effects of late decapsulation.

Artificial Encapsulation.—After it became clear that decapsulation afforded protection to the decapsulated kidney, a number of attempts were made to determine whether or not adhesions between the decapsulated kidney and surrounding structures played a part in the protective effect. This was done in part by enclosing the freshly decapsulated left kidneys in loosely fitting artificial capsules made of plastic or rubber. The most satisfactory artificial capsules were made by cutting five-sixteenths of an inch surgical drainage tubing into three-fourths inch lengths, compressing the ends in planes at right angles to one another, and closing the ends with closely placed sutures. A slit one-eighth to one-fourth of an inch in length was then cut lengthwise in the side of each such preparation, and after stretching the slit over a wire frame, the tubing was slipped over the kidney and the wire frame was slipped out. The relationship of the slit, through which the kidney was introduced, to the plane in which the upper end of the capsule was flattened, was such that the flattened surface lay smoothly against the stomach.

In order to determine how much renal injury was caused by the artificial capsules themselves, rather than by choline deficiency, half of the animals subjected to the covering procedure for the left kidney in each experiment were placed on the diet with choline added, and the right kidney was removed in a second operation 4 or 5 days later, at the time that renal lesions were developing in the deficient animals. In the case of animals which died after having survived the immediate postoperative period (18 hours), it was assumed that the left kidney had been severely damaged by the artificial cover. This was borne out by autopsy studies.

Renal Denervation.—Attempts were made in several experiments to study the effects of renal denervation without removal of the renal capsule. Employing a long transverse incision across the left side of the abdomen, the artery and vein of the left kidney were isolated and carefully stripped as completely as possible. Some of the operations were done with the aid of a dissecting microscope. The kidney was then pulled away from the dorsal abdominal wall and separated from the adrenal and other surrounding structures. The kidney was left supported only by the stripped renal vessels and ureter, but the capsule was intact.

RESULTS

Effects of Renal Decapsulation.—The usual unilateral decapsulation on the left side through a lumbar incision caused a significant increase in rate of

survival of the experimental animals compared with a group subjected to the control incision without decapsulation. A majority of the animals with the left kidney decapsulated, like the control animals, became ill and began to lose weight after 4 to 7 days on the deficient diet. However, in about half of the decapsulated group, the illness was relatively short lived and the animals began to gain weight again in 2 or 3 days. About 68 per cent of the group subjected to decapsulation survived, compared with 17 per cent of the control group. Food consumption and growth rate of the controls and decapsulated animals were almost identical, up to the time of the illness. These results, as observed after 12 days on the deficient diet, are summarized in Table I (groups 1 and 2).

TABLE I

Effects of Various Decapsulation Procedures and of Artificial Encapsulation of the Kidney

Group No.	Operative procedure	No. of animals	Animals surviving
			per cent
1	Control	186	17
2	Lumbar decapsulation	189	68
3	Abdominal decapsulation	31	61
4	Lumbar decapsulation (3rd day)	13	46
5	Lumbar decapsulation (4th or 5th day)	39	21
6	Lumbar decapsulation with artificial encapsulation	53	64*
7	Lumbar decapsulation with artificial encapsulation (diet with choline, right kidney removed 5th day)	38	45*
8	Denervation	45	20

All procedures were done as described in text, prior to placing animals on the deficient diet unless otherwise specified. All groups were on choline-free diet except group 7.

* See text for interpretation.

Unilateral renal decapsulation not only increased the rate of survival, but the damage which occurred in the decapsulated kidney was consistently less than that which occurred in the contralateral, undecapsulated kidney of the same animal (Fig. 1). In a few cases, the decapsulated kidney of the autopsied animals appeared normal. Much more frequently, injury of some degree was present, but less than that which occurred in the contralateral kidney. In the control animals, the renal lesions invariably were of approximately equal severity on the two sides, and any considerable difference in the two kidneys following unilateral decapsulation was a result of the operative procedure. The use of one kidney as a control for the other within the same animal, eliminated any uncontrolled effects of the surgical procedure or other procedures on the animal as a whole; this was difficult to accomplish with certainty when results in different groups were compared.

The most normal areas of the decapsulated kidneys appeared on inspection in the gross to be located about areas of adhesions between the kidney and surrounding structures. This observation was made so consistently as to suggest that the protection afforded by decapsulation was in some way due to the adhesions, perhaps a result of collateral circulation through the adhesions. However, protection was not invariably accompanied by adhesions, and the relationship between adhesions and protected areas was not clear from examination of sections.

In cases in which there was a considerable degree of protection as a result of decapsulation, the decapsulated kidneys probably did not become as tense in the stage of the acute lesions as did the contralateral kidneys with capsules intact. Also, there appeared to be less edema fluid in adjacent tissues, particularly along the course of the renal vessels, in the case of the decapsulated kidneys.

Not only was protection as a result of decapsulation apparent in animals examined during the acute stage of the renal lesions, but also in the surviving animals examined weeks or months later. In the subacute stage of injury after congestion and edema had disappeared, the decapsulated kidney was larger and firmer than the contralateral kidney with the more severe injury. In many cases, the kidney with intact capsule appeared after several weeks as a small, scarred, atrophic organ with calcium-studded surfaces and a thin, irregular cortex, while the decapsulated kidney was large, hypertrophied, and fairly normal in appearance (Fig. 2). The relatively small size of the severely injured kidneys at this stage was in striking contrast to their great enlargement in the earlier acute stage of injury. In Fig. 3 a section from a damaged right kidney is shown for comparison with a section from the decapsulated left kidney of the same animal shown in Fig. 4.

3 to 6 months after development of the acute renal lesions, the undecapsulated kidney in most cases still was smaller and showed evidence of more severe damage than did the decapsulated kidney (Figs. 5–8). Particularly in animals with severe damage, growth rate through the late periods was less, and the weight of the two kidneys together was disproportionately greater, than in animals receiving choline from the start.

Effects of Varying the Decapsulation Procedure.—As shown in Table I (group 3), decapsulation of the left kidney through a midline abdominal incision afforded protection of a degree approaching that produced by the lumbar procedure. In earlier experiments before the decapsulation technique was well established, it had appeared that the abdominal operation did not afford very substantial protection. The abdominal operation was followed by less extensive adhesions between the decapsulated kidney and surrounding tissues than was the lumbar operation because of the distance between the kidney and the incised tissues in the case of the abdominal procedure. It should be stated in

this connection that adhesions usually occurred between decapsulated kidneys and surrounding tissues, while undecapsulated kidneys almost never developed adhesions in the course of development of the lesions.

Influence of Time of Operation.—In the experiments which have been described, the surgical procedures were done just prior to starting the deficient diets. Decapsulation probably was effective up to the 3rd day on the diet (Table I, group 4). However, when the procedure was delayed to the end of the 4th or 5th day (Table I, group 5), little protection resulted. Most of the animals operated on after 4 or 5 days on the diet already had developed renal lesions, though the lesions were quite early in about half of the cases. These results suggested that the damage actually occurred considerably ahead of the morphological evidence of damage, or else that in order for protection to result a period of time must elapse between the decapsulation and the development of the damage. The results may mean simply that closely superimposing the injury of the operation and the damage resulting from the renal lesions was more than the animals could tolerate.

Effect of Prevention of Adhesions.—Because of the observations suggesting that the protective effect of decapsulation might have occurred as a result of the development of adhesions, many attempts were made to prevent the adhesions without injuring the kidney and without otherwise influencing the results. This was never entirely successful in groups as a whole. The results of the most satisfactory experiments are included in Table I (groups 6 and 7). When the kidneys were completely covered, with only a small hole for the vessels and ureter, more than 50 per cent of the kidneys were severely damaged as judged by the results in animals on a normal diet in which the left kidney was covered and the right kidney was subsequently removed (Table I, group 7), and also as judged by autopsy examinations on the deficient animals. On the other hand, in experiments (not included in Table I) in which the kidneys were less completely covered, the kidneys often slipped partially out of the capsule or developed adhesions through the opening in the capsule. In spite of mechanical injury to many of the adequately covered kidneys, the mortality in deficient animals with the left kidney decapsulated and covered was not greatly increased as a result of the covering procedure (Table I, group 6). This result might appear to indicate the absence of any causal relationship between adhesions and protection due to decapsulation, but actually it cannot be interpreted at all. It was evident from examination of the animals that the artificial covers on the left kidneys influenced not only the covered kidneys but also the animals as a whole, with a reduction in the incidence and severity of the renal lesions in the contralateral kidneys which were not decapsulated or covered. This effect on the undisturbed kidney perhaps was a result of an "alarm reaction" which has been observed to afford some protection (6), and of an irregular decrease in rate of growth. In spite of these complications, it did

seem possible to arrive at certain valid conclusions from these experiments with artificial encapsulation. While comparison of the results in groups as a whole meant little, the results in certain individual animals seemed conclusive. In a few animals in which the left kidney was decapsulated and covered satisfactorily without injury to the kidney, severe lesions developed in the non-decapsulated right kidney but not in the decapsulated and covered left kidney, which remained normal. This observation appeared to indicate that decapsulation was capable of affording protection in the absence of adhesions, though it did not eliminate the possibility that part of the protective effect of decapsulation under other circumstances may have resulted from adhesions. It did not seem likely that protection in the covered kidneys was a result of the artificial encapsulation rather than of the decapsulation.

Effect of Renal Denervation on Development of Lesions.—The possibility of nervous influences was considered in looking for the explanation of the protective effect of renal decapsulation. In performing all the renal decapsulation operations, care was exercised in avoiding the area about the pelvis, vessels, and ureter, and even the connections with the adrenal. While we never could be certain that the surgical procedure designed to sever the autonomic nerves to the kidney without removing the capsule actually accomplished complete renal denervation, it seemed certain that the denervation operation was at least as effective in this respect as was the decapsulation operation. Not only did denervation fail to increase significantly the rate of survival (Table I, group 8), but also it appeared from a comparison of the denervated kidneys with the undisturbed contralateral kidneys of the same animals that the damage was of equal severity on the two sides in nearly all cases.

DISCUSSION

The experiments described here demonstrated conclusively that renal decapsulation done prior to placing young rats on a diet deficient in choline afforded protection against renal injury and death. A similar observation was described previously in a brief report by Dessau and Oleson (7), but this observation was not confirmed by Hartroft (8). In support of the hypothesis that the renal injury of choline deficiency is on the basis of a vascular syndrome mediated through the autonomic nervous system, Dessau and Oleson observed that the renal lesions also were prevented by atropine—an observation which we have been unable to confirm in experiments in which food intake of control and treated animals was maintained at the same level (6).

The mechanism of the protective effect of renal decapsulation in the present experiments was not determined conclusively. Since it was demonstrated, by comparing decapsulated kidneys with the contralateral undecapsulated kidneys of the same animals, that the protective effect of decapsulation was due to some effect on the decapsulated kidney rather than to some general effect on the animal as a whole, the likely mechanisms of the effect seemed to be limited.

It appeared improbable that decapsulation influenced directly the processes going on within the renal cells and its effects more likely were due to influences on pressure or circulation within the kidney, though other mechanisms are conceivable.

From the experiments on renal denervation, it appeared that the protective effect of decapsulation was not a result of disturbing the renal nerves.

As already noted, certain observations suggested that the protective effect of decapsulation might have been due to the development of adhesions, functioning perhaps by conducting collateral circulation to the cortex or by delaying the regeneration of a rigid capsule. Suggestive of this mechanism were the appearance at times of some correlation between the extent of adhesions and the degree of protection, the frequent appearance in the gross of relatively normal tissue about areas of adhesions, and perhaps the failure of late decapsulation to afford a significant degree of protection. None of this evidence was conclusive and possibly the apparent relationship between adhesions and protection indicated only that both phenomena were related to the effectiveness of the decapsulation. The experiments with artificial capsules, and occasional other experiments, demonstrated that protection due to decapsulation could occur without adhesions.

Perhaps the most probable explanation of the protective effect of decapsulation, on the basis of *a priori* considerations, is that decapsulation protected by removing the restraining effect of the rigid capsule, thus reducing the increase in intrarenal pressure which occurred as a result of edema and swelling. This explanation probably implies that renal decapsulation protected against secondary disturbances rather than against the primary disturbances which resulted from choline deficiency. In favor of this explanation were the obvious enlargement and tenseness of the kidneys with well developed lesions. Perhaps against it was the failure of decapsulation performed at the stage of early renal injury to afford significant protection. We are inclined at present by way of elimination to favor this latter explanation, based on the removal of a limiting capsule, perhaps together with some participation of the adhesions which developed about the decapsulated kidneys.

SUMMARY

Decapsulation of the left kidney through a lumbar incision immediately prior to placing young rats on a choline-deficient diet afforded a significant degree of protection against animal mortality, compared with that which occurred as a result of the deficiency in the control groups. Decapsulation also was effective in reducing greatly the renal injury which developed in the decapsulated kidneys, as judged by a comparison of the decapsulated kidneys with the contralateral undecapsulated kidneys of the same animals. Decapsulation through an abdominal incision also was efficacious.

Decapsulation after 4 or 5 days on the deficient diet, at which time relatively

early renal lesions were present in most of the animals, afforded little if any protection.

Experiments in which the usual adhesions between the decapsulated kidneys and surrounding tissues were prevented by covering the decapsulated kidneys with artificial capsules demonstrated that protection due to decapsulation could occur in the absence of adhesions.

Renal denervation without removal of the renal capsule was not associated with a decrease in renal damage or animal mortality.

Possible mechanisms of the protective effect of renal decapsulation in choline deficiency are discussed.

BIBLIOGRAPHY

1. Griffith, W. H., and Wade, N. J., *J. Biol. Chem.*, 1939, **131,** 567.
2. Christensen, K., *Arch. Path.*, 1942, **34,** 633.
3. Best, C. H., and Hartroft, W. S., *Fed. Proc.*, 1949, **8,** 610.
4. Baxter, J. H., *J. Nutrition*, 1947, **34,** 333.
5. Wesson, L. G., *Science*, 1932, **75,** 339.
6. Baxter, J. H., *Fed. Proc.*, 1951, **10,** 279.
7. Dessau, F. I., and Oleson, J. J., *Proc. Soc. Exp. Biol. and Med.*, 1947, **64,** 278.
8. Hartroft, W. S., *Brit. J. Exp. Path.*, 1948, **29,** 32.

EXPLANATION OF PLATES

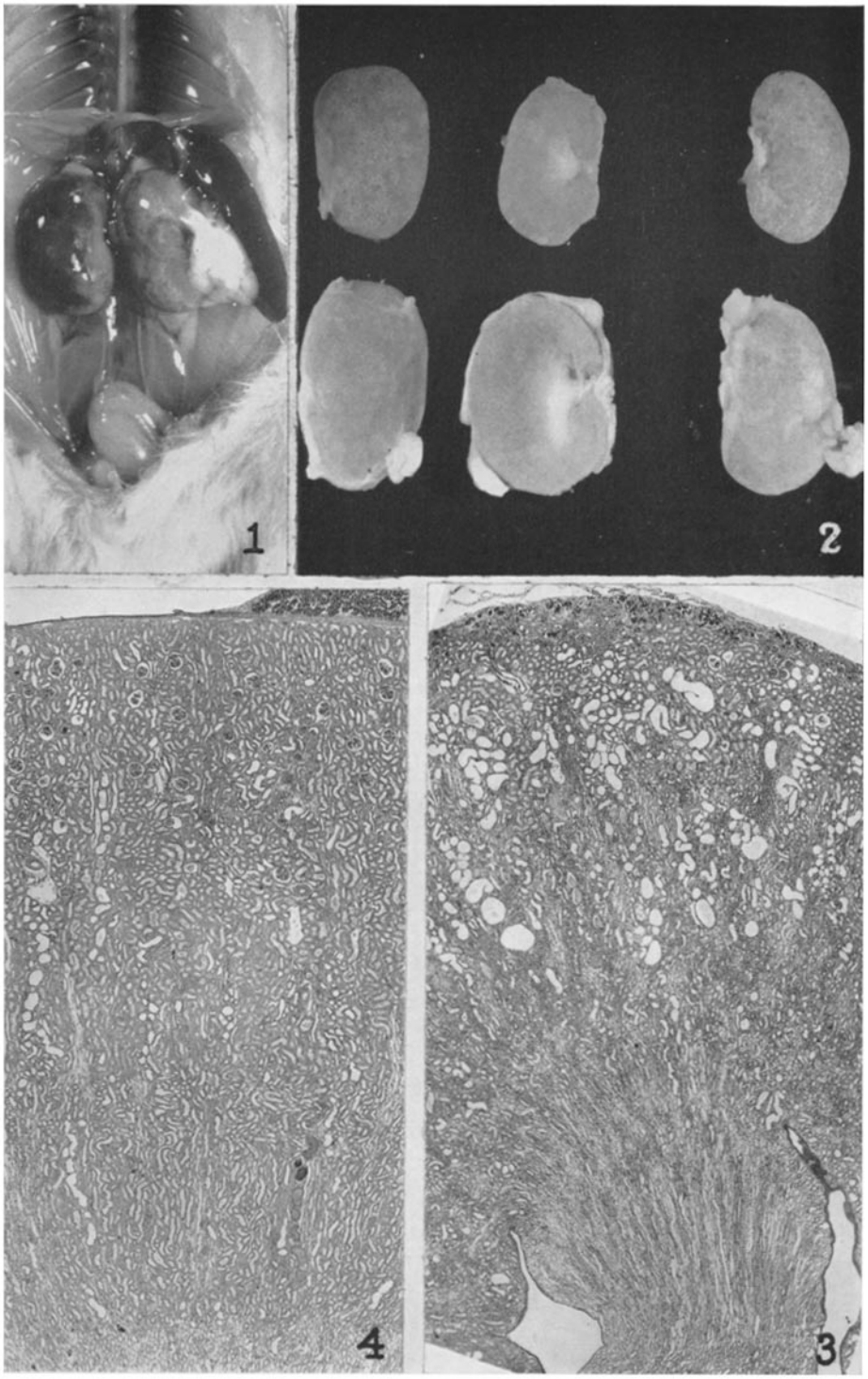
PLATE 16

FIG. 1. Photograph of the kidneys *in situ* of a rat autopsied after 5 days on the choline-deficient diet. The left kidney (at right in photograph), which had been decapsulated through a lumbar incision before starting the deficient diet, appeared more nearly normal than the right kidney. Firm adhesions bound the left kidney to the spleen and to the area of the incision. × 1.5.

FIG. 2. Photograph of kidneys from animals which survived the acute damage due to choline deficiency after previous decapsulation of the left kidneys. The animals were autopsied 3 weeks after starting the deficient diet. They were gaining weight and appeared healthy. The right kidney is shown above the left (decapsulated) kidney of the same animal in each case. The right kidneys were small and atrophic, with thin, calcified cortices. The left kidneys, on the other hand, were large and hypertrophied, with very thick cortices. × 1.5.

FIG. 3. Section from one of the right kidneys shown in Fig. 2. The outer two-thirds of the cortex had been destroyed; condensed, calcified debris remained. Only juxtamedullary glomeruli were intact. Compare the thickness of the cortex with that of the left kidney of the same animal shown in Fig. 4. Many tubules in the deeper portions of the cortex and in the medulla were dilated, thin walled, and filled with casts. Hematoxylin and eosin stain. × 19.

FIG. 4. Section of the left (decapsulated) kidney from the same animal as in Fig. 3. A few dilated, cystic tubules were present but the damage on the whole was slight. The cortical hypertrophy is apparent. Hematoxylin and eosin stain. × 19.

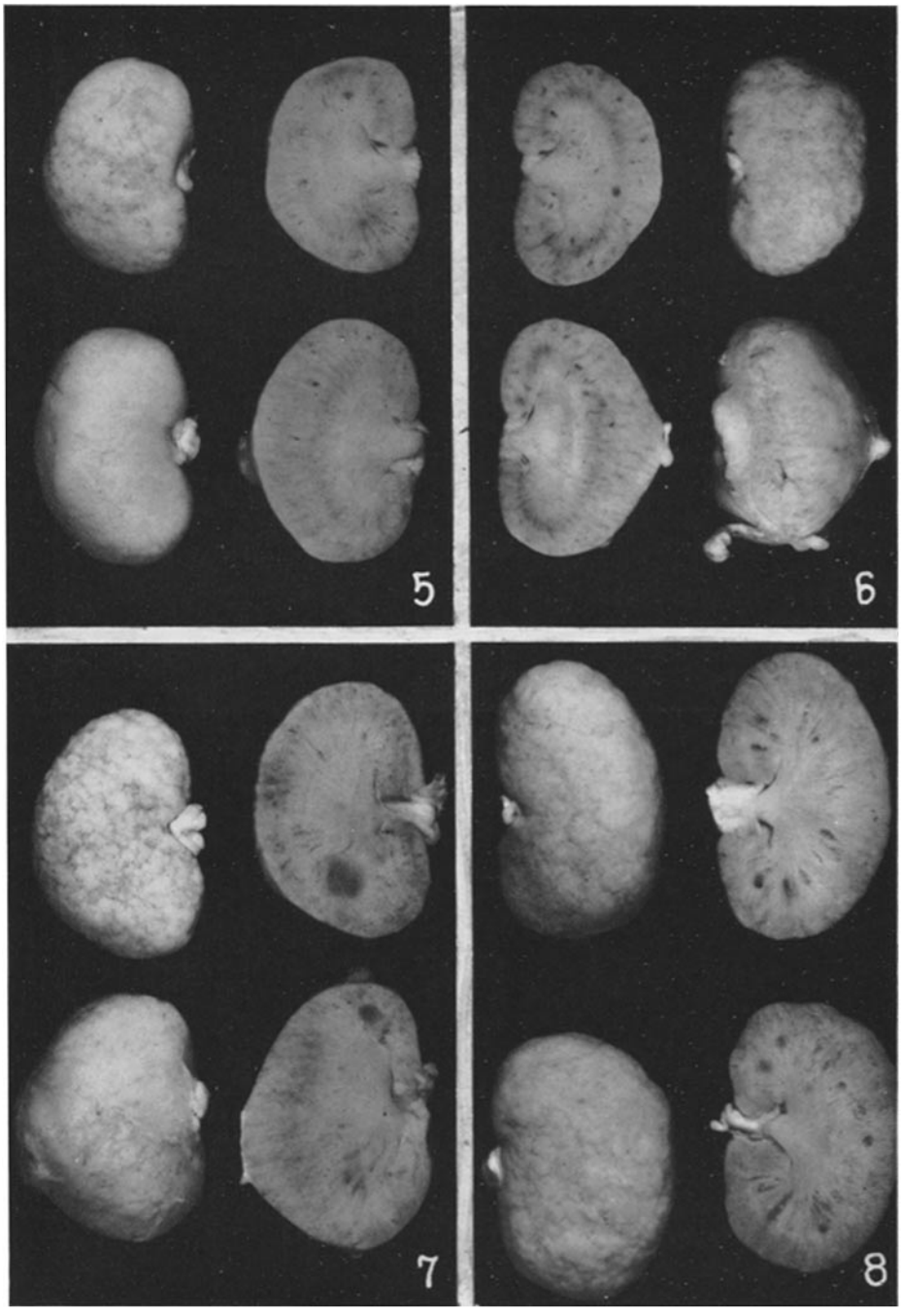


(Baxter: Renal decapsulation and choline deficiency)

PLATE 17

FIGS. 5 to 7. Kidneys from animals autopsied after an initial 2 weeks on the choline-free diet followed by 6 months on stock diet. In each figure, the two halves of the right, undecapsulated kidney are placed above, with the corresponding portions of the contralateral, decapsulated kidney from the same animal beneath. The successive figures are arranged to show undecapsulated kidneys with progressively greater damage. Note the pitted surfaces, relatively thin cortices, and dark areas which represent cystic areas filled with fluid. In each case, the degree of injury was considerably less in the decapsulated kidney; note the relatively smooth surfaces and the thick cortices. × 1.7.

FIG. 8. Right and left kidneys from a similarly treated control animal not subjected to renal decapsulation. Note that equally severe damage was present in both kidneys of this animal. × 1.7.



(Baxter: Renal decapsulation and choline deficiency)