tion, which blocks the effect of endotoxin on the renal cortex in the Shwartzman reaction(11), does not prevent the renal lesion caused by exotoxin. Thus it appears that the kidneys remove a considerable amount of staphylococcal toxin from the circulation, for bilateral nephrectomy performed 7 to 10 hours after the toxin is given prevents death. The same conclusion follows from the observation that death from toxin is quicker if the kidneys are removed before the toxin is given. Hence it appears that in the intact animal, extraction of toxin by the kidneys delays but does not prevent death. One may conclude further that while the toxin is held by the kidneys, it is not acting elsewhere. But sometime afterward the toxin or a product of the toxin interacting with the kidney is released and that this released material kills. Since ischemic necrosis of both kidneys does not kill earlier than bilateral nephrectomy, the necrosis produced by toxin is not the cause of death. Presumably, then, the toxin or a modification of it acts on an unidentified site elsewhere to cause death. Vasospasm and other effects of the catechol amines appear not to be involved. The mechanism of death following administration of staphylococcal exotoxin remains unknown.

Summary. The R.E.S., which is the main defense against endotoxin, plays no part in the defense against staphylococcal toxin. The kidneys remove staphylococcal toxin from the circulating blood. The toxin or a modification of it is subsequently released, then exerts a lethal action elsewhere. For some hours after it is taken up by the kidney the toxin cannot act. Thus, the kidney is protective for some hours. Indeed nephrectomy prevents death from exotoxin. The renal necrosis produced by the toxin in itself is not the lethal lesion, because renal necrosis produced by arterial occlusion does not cause such rapid death as occurs in renal necrosis produced by the toxin.

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Renin and Angiotensinase Content of the Kidney of Normal and Renal Hypertensive Rats.*‡ (27044)

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The possible role of renin as a causative agent in experimental renal hypertension has been extensively investigated. Direct determination of the concentration of renin in the blood has been indecisive. It is to be expected that if renin, through its pressor effect, is the direct cause of experimental renal hypertension, it would be present in the blood of hyper-

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tensives in abnormally high concentrations. Actually its concentration in both normal and hypertensive conditions is so low that no agreement has been reached by these studies(1).

Assay of renin in the kidney, where it is present in higher concentrations than in the blood, has been extensive but the results of these studies have not shown sufficient uniformity to permit a clear picture of what happens to the renin content of this organ as hypertension develops(2).

The current study has 2 objectives: (1) to make further observations bearing on renin content of the kidneys in experimental hypertension, and (2) to make simultaneous determinations of renal angiotensinase activity. Since the pressor response to renin is mediated through production of angiotensin, and since this polypeptide is rapidly inactivated by angiotensinase in blood and tissues, both the physiologic effect and the assayed activity of renin are dependent on the rate of this enzymatic inactivation of angiotensin. Variations in angiotensinase activity in hypertension conceivably could alter the pressor activity of renin and could interfere with the biological assay of its content in the kidney.

Methods. Albino rats of the Sprague-Dawley strain, weighing between 190 and 340 g, were used in all experiments. Hypertension was produced by partial occlusion of the left renal artery by means of a silver clip, as described by Schaffenburg *et al*(3). Blood pressures were measured by the Friedman and Freed tail microphone technique(4).

Rats were kept under observation for about 6 weeks (range 1-2 months) following operation. During this time blood pressure measurements were made at weekly intervals. When an animal was ready for testing it was killed by a blow on the head, the kidneys were removed and homogenized, individually, in 10 ml of saline per gram of tissue. The homogenate was centrifuged and the supernatant used for determination of renin and angiotensinase.

Two bioassay procedures were used for determination of renal renin concentration: (1) Direct Method. Six-tenths ml of kidney homogenate were administered intravenously to an assay rat. This animal was anesthetized with urethane (1-1.5 g/kg intraperitoneally) and blocked with pentolinium (3 mg/kg subcutaneously). Its arterial pressure was recorded continuously from a float mounted on a mercury manometer which was connected to the rat's femoral artery by means of a polyethylene cannula. The renin content of the homogenate was determined by referring the pressor response it produced to a dose-response curve constructed from the pressor responses recorded when a standard renin preparation was studied in a series of 38 rats. Each assay rat was used for only one renin determination, thus avoiding any effects of tachyphylaxis. Renin content was expressed in Goldblatt Units.

(2) Indirect Method. This method is based on production of angiotensin on incubation of renin with substrate *in vitro*. The angiotensin formed in presence of sufficient substrate and in absence of angiotensinase, is proportional to the amount of renin in the original sample. Angiotensinase was inactivated, with minimum destruction of renin, by acidifying with phosphoric acid to pH 2.0 for 60 min at 24°, then returning the mixture to pH 6.5 with sodium hydroxide. The angiotensinase-free mixture was then incubated for 2 hours with excess angiotensinogen prepared from normal dog plasma, according to the methods of Fasciolo and Taquini(5). This reaction was stopped by placing the sample in boiling water for 3 min. Three-tenths ml of the supernatant solution were injected into an assay rat prepared as described previously. A standard hog renin preparation** was treated in a similar way to produce angiotensin *in vitro*. From a comparison of the pressor response of the standard with those of the test preparations the concentration of renin in the kidney extract was calculated, in terms of Goldblatt Units.

For determination of angiotensinase the kidney homogenate was incubated with synthetic valine-5 angiotensin II amide*** (specific activity: 3 Goldblatt Units/μg) for 2 hours at 37°, then injected into an assay animal.

Individual kidneys from 3 groups of rats were studied: Group 1, normal, control rats; (average arterial pressure 111 mm Hg), 7

** We are indebted to Dr. Oscar Helmer, Eli Lilly and Co. for hog renin used.

*** Obtained from CIBA Laboratories, Basel, Switz.

animals; Group 2, rats which developed hypertension following unilateral partial renal ischemia, (blood pressure 150 mm Hg or above), 12 animals; Group 3, rats which failed to develop hypertension after the procedure, (blood pressure less than 150 mm Hg), 11 animals. Rats of Group 3 will be referred to as "normotensives".

Results. The renin concentration as determined by the 2 methods (direct and indirect) was in satisfactory agreement (Fig. 1). This agreement was used as justification for averaging the values obtained by the 2 methods; these averages are used in the graph of Fig. 2. It is apparent that: (1) There is no difference between right and left kidneys of normal animals with respect to renin concentration. (2) Concentration of renin in the kidneys of animals subjected to partial occlusion of the renal artery was lower than that in the kidneys of control animals. This was independent of blood pressure elevation. The difference between kidneys of normal animals and kidneys of hypertensive animals with (P value <.025) or without (P value <.005) partial occlusion of the renal artery was statistically significant as also was the difference between kidneys of normal animals and kidneys of normotensive animals with (P value <.0005) or without (P value <.0005) partial occlusion of the renal artery. (3) Mean renin concentration was higher in the ischemic than in the non-ischemic kidneys. This was not dependent on blood pressure elevation. The difference was not statistically significant (P value >.1), possibly due to the small number of observations.

The data concerning concentration of angiotensinase in the kidneys of the several groups of rats (Fig. 3) show that: (1) There is no difference between right and left kidneys of normal animals in this respect. (2) Concentration of angiotensinase in kidneys of experimental animals which became hypertensive was equal to that in control animals. (3) Concentration of angiotensinase in kidneys of experimental animals which did not become hypertensive was less than in hypertensive or control groups. The difference was not statistically significant (P value >.1). Normotensive rats with contralateral nephrectomy (not represented in Figure) did not show this decreased angiotensinase concentration (this angiotensinase activity was 1153 μg of synthetic angiotensin inactivated/g, SE ± 164). (4) There was no difference in angiotensinase concentration between kidneys with arterial supplies partially occluded and those non-

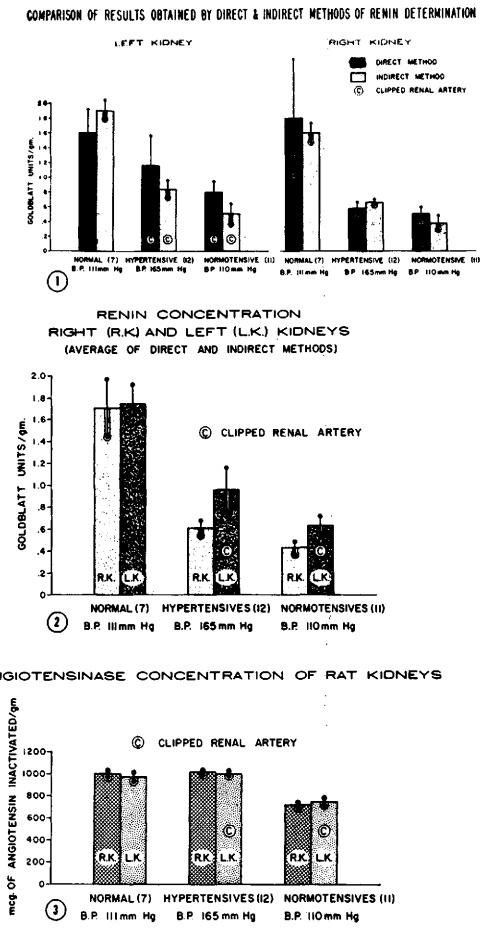

FIG. 1. Mean values (vertical bars) for renin concentrations for left and right kidneys of normal, hypertensive and "normotensive" rats. Vertical lines at top of bars represent standard error of mean. B.P. in each case is average mean blood pressure of the group. Numbers in parentheses are numbers of kidneys studied.

FIG. 2. Average mean values for renin concentrations in left and right kidneys of normal, hypertensive and "normotensive" animals. Vertical line at top of each bar represents standard error of mean. B.P. in each case is average mean blood pressure of group; in parentheses, number of animals in group.

FIG. 3. Mean values for angiotensinase concentrations for right (RK) and left (LK) kidneys of normal, hypertensive and "normotensive" rats. Standard error of mean is indicated as in previous figures. B.P. in each case is average mean blood pressure of each group; in parentheses, number of animals in group.

occluded. This was true whether or not the blood pressure became elevated.

Discussion. If the assumption that renin secretion parallels renin content in the kidney is correct, one is forced to conclude from our data that experimental renal hypertension in the rat is not caused by increased renin secretion; kidneys of renal hypertensive rats were consistently lower in renin concentration than kidneys of control rats.

This is in agreement with Beckwith and Chanutin(6) and Williams *et al*(7), who, working in the same species, found decreased pressor properties of kidney extracts in renal hypertension. Gross *et al*(8) and Tobian *et al*(9) on the other hand, found that the decrease in renin concentration was present only in the "untouched" kidney, while the ischemic or "clipped" kidney had a normal renin concentration.

Gross *et al*(8) and Tobian *et al*(9) found a higher concentration of renin in the ischemic kidney than in the contralateral. This is in accord with our results although we did not find the difference to have statistical significance, possibly due to the number of observations. The latter author has suggested that the decreased concentration is associated with increased stretch of the juxtaglomerular cells (the proposed source of renin) caused by increased arterial pressure(10). This interpretation is not in accord with the present data where the "untouched" kidneys of the experimental groups of rats had a similar renin concentration whether the pressure was elevated or not. The stimulus for regulating renin secretion remains undiscovered; it is unlikely that it is a circulating substance since after unilateral "clipping" there are differences in concentration between the 2 kidneys though they are both circulated by the same blood. Increasing amounts of evidence suggest that Na metabolism is closely related to renin and we are inclined to believe that this line of research is likely to disclose the stimulus for regulating the secretion of this enzyme.

Agreement was found between the direct and indirect methods for renin determination, making it unlikely that differences in these assay techniques account for the conflicting results in the literature.

Our data show that the angiotensinase concentration of kidneys of animals with renal hypertension remains in the normal range. Haynes and Dexter(11) found normal values for angiotensinase in plasma of patients with hypertension. Quinby *et al*(12) observed no difference in concentration of angiotensinase in renal arterial and venous blood of humans before and after complete occlusion of the renal artery for periods of 10 to 12 min. Weinstein *et al*(13) observed a diminution of angiotensinase in the venous blood from acutely ischemic kidneys. This observation was not confirmed by Braun-Menendez *et al*(1).

Our findings indicate that the concentration of angiotensinase is independent of renin content of the kidney. This is not surprising since it has been shown by Helmer *et al*(14) that the so-called "angiotensinase" represents a group of different substances which have the common action of inactivating angiotensin. In any event the observed differences in renin content of the kidney cannot be attributed to variations in their angiotensinase concentration.

Summary. Kidney renin and angiotensinase concentrations were determined for the following groups of rats: normals(7); rats with renal hypertension produced by unilateral clipping of the renal artery(12); and rats unilaterally clipped which failed to develop hypertension(11). Kidney extracts were assayed for renin by 2 methods: 1) direct intravenous injection into a rat sensitized by ganglionic blockade, and 2) *in vitro* production of angiotensin which was subsequently assayed in a similar manner. Angiotensinase concentration was determined by incubation of the kidney extract for 2 hours at 37° with a known amount of synthetic angiotensin and subsequent assay of the residual angiotensin. Renin concentration was reduced in kidneys of both groups of rats with renal artery clips. The reduction in the untouched kidney was greater than that in the clipped kidney. Concentration of angiotensinase in the kidneys of experimental animals which became hypertensive was equal to that in control animals.

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Studies of Congenital Transmission of a Leukemia Virus in Mice.* (27045)

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Gross reported in 1951(1,2) and in more detail later(3-5) that some of the extracts and filtrates prepared from leukemic tissues of AK strain mice induced lymphocytic leukemia in certain recipient mice, particularly in the C3H/Bi and C3Hf/Bi strains. Experiments were described(2, 6, 7, 8) of an attempt to study the "natural" transmission of the Gross virus in C3H mice. The concept was developed that the virus isolated from AK leukemic mice was responsible for the high frequency of lymphocytic neoplasms within that strain, and that the virus was transmitted through the germ lines resulting in successive generations of leukemic AK mice with similar frequencies and latent periods (See 7). It had to be assumed also, but this was not discussed in considering the concept, that the paternal line was as effective a means of transmission of virus as the maternal line(9). The results of the early "natural" transmission studies were equivocal(2). The frequencies of leukemia among the uninoculated offspring from virus-inoculated parents were suggestive of transmission of virus in some matings(7) but not in others, the latent period was long and too few mice were observed beyond one generation.

Recently, Gross has reported on certain aspects of the so-called "vertical" transmission of the Gross virus using a more highly potent, serially passaged virus(10).

All C3Hf/Bi offspring born to virus-inoculated females developed leukemia and average age at leukemic death was similar to that of the inoculated parents. None of the offspring born to non-inoculated females mated with virus-inoculated males had developed leukemia up to 9 months of age. The experiments were not designed to determine the manner of transmission of virus through the maternal line.

The isolation and concentration of a leukemogenic virus from transplantable Sarcoma 37(11,12) presented the opportunity to study certain epidemiologic aspects of this neoplastic disease in mice, including that of natural transmission of the virus. This report presents the results, preliminary in some aspects, of the fate of offspring in successive generations, born to parenterally-infected parents.

Materials and methods. Virus. The preparation designated LT(V) 96 used throughout these studies was prepared as follows: A pool of leukemic lymph nodes and spleens from

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