commodate the moving plate (D). A 2-mm length is slotted to take a small screw driver. It is then screwed into the casing. The movable compressing plate is drilled and the hole countersunk. It is then cut and shaped to size. All edges are rounded. The plate is then peened to the screw. A facing plate (E) of 0.12 mm silver is formed and bent to fit the moving plate. Finally, the removable bottom plate is cut, shaped, and finished.

The smaller clamps have been applied bilaterally at one operation with aseptic technic to the renal arteries of 11 rats. The screw of the clamp was turned to give complete occlusion of the artery and then turned back 1 to 1½ turns. This setting of the clamp was not subsequently altered.

Blood pressure, before and after operation, was determined in the hind leg of the unanesthetized rat by a modification(2) of the method of Kersten *et al.*(3). Control blood pressures, before operation, varied from 100 to 125 mm Hg. Eight of the rats attained a blood pressure level of at least 160 mm Hg within a time period varying from 8 to 21 days postoperative. In 2 of the rats, such a pressure level developed somewhat later. One rat died postoperatively with massive infarcts of both kidneys. The pressures have remained elevated (160 up to a maximum of 220 mm Hg) for months in 9 of 11 rats.

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Occurrence of Renal Lesions in Guinea Pigs Given Parenteral PGA.* (17774)

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Human infants and guinea pigs on diets deficient in ascorbic acid and high in aromatic amino acids excrete p-hydroxyphenylpyruvic and p-hydroxyphenyllactic acids and small quantities of tyrosine in the urine(1,3). This abnormal excretion of metabolites is abolished by the administration of ascorbic acid(1,2,4). Since pteroylglutamic acid (PGA) increases the oxidation of tyrosine by suspensions of liver from sulfa-treated rats(5) and because the excretion of phenolic compounds by patients with pernicious anemia is reduced by liver therapy(6) the effect of PGA on the urinary excretion of hydroxyphenyl compounds by scorbutic guinea pigs was studied(7). Daily doses of 5 mg of PGA were found to abolish the hydroxyphenyluria in these scorbutic pigs(7,8). An anatomical study was made of the animals used in these latter investigations and in other experiments devised to elucidate the problem of hydroxy-

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phenyluria in scorbutic pigs and its reversal by therapy with ascorbic acid, pteroylglutamic acid and other compounds. It is the purpose of this paper to describe renal lesions which were observed in some of these experimental animals.

Materials and methods. Albino guinea pigs weighing approximately 300 g were used. The basal diet consisted of heated (100° for 2 hours) skim milk powder, 30; fortified rolled oats, 39; wheat bran, 20; cottonseed oil, 8; sodium chloride, 1; and cod liver oil, 2. This diet contains approximately 0.8 μg of PGA per gram. In the initial experiments tyrosine was added to the diets to a proportion of 5% and supplements of ascorbic acid, PGA, etc.. were added in the quantities and for the time intervals indicated in individual experiments. The animals were maintained in individual metabolism cages and the urines collected for determination of total hydroxyphenyl compounds(8).

Results. The first animals studied consisted of groups treated as follows: basal diet plus 5% tyrosine; basal diet and tyrosine plus 25 mg of ascorbic acid daily; and basal diet plus tyrosine plus 5 mg of PGA daily. The ascorbic acid was given orally, the PGA parenterally. There was abundant hydroxyphenyluria in the animals on the unsupplemented basal diet with tyrosine but no appreciable hydroxyphenyluria in the animals which received the diet with tyrosine supplemented with either ascorbic acid or PGA. These animals were killed and studied without reference to their treatment. Although changes were observed in other organs, the chief findings were in the kidneys. On gross examination the kidneys were slightly enlarged and their surfaces were smooth, mottled and pale. Microscopic sections revealed that clusters of nephrons showed dilation of their tubules. These groups of nephrons were related anatomically to collecting tubules which were also dilated. Casts were seen in all varieties of tubules but they were most numerous in the larger collecting ducts and in the ducts of Bellini which were greatly dilated. Most of the casts were yellow and granular but some were homogeneous and hyaline. No necrosis of tubular epithelium was seen but the lining cells of all the dilated tubules were flattened and basophilic. There were no glomerular changes.

Since the histologic studies were made without reference to the treatment that the animals had received, it was initially surmised that those kidneys showing the above-described lesion were from animals which had exhibited hydroxyphenyluria. On the contrary, however, the injured kidneys were from pigs which had received PGA and which had not shown hydroxyphenyluria. It was also of interest to note that no alterations were observed in the group treated with ascorbic acid. Because these lesions occurred only in the animals receiving PGA, it seemed advisable to determine the role played by PGA, tyrosine, and by the ascorbic acid deficiency. Consequently, groups of animals were placed on the basal diet plus 25 mg of ascorbic acid with and without added tyrosine. Half the animals in each group were given 5 mg PGA daily. It was impossible to determine presence of the biochemical lesion by observing hydroxyphenyluria which does not occur under these conditions. Autopsy studies showed that the renal lesion was present only in animals which had received PGA and that the presence or absence of tyrosine in the diet had no demonstrable effect on its development. Furthermore, the lesion in this series was identical with that observed in the earlier investigation in which all of the animals were scorbutic. The renal damage therefore, was associated with PGA administration and not due to either added tyrosine or to ascorbic acid deficiency.

In another study, 8 animals on the basal diet were given orally a suspension of 350 mg of tyrosine twice daily. Two of these animals were supplemented with 25 mg of ascorbic acid daily; 3 with 5 mg of PGA daily; and 3 were given no vitamin supplements. Tyrosine so administered was poorly tolerated. The food intake decreased to about one-third of usual except for the animals receiving ascorbic acid. All of these pigs lost weight rapidly and became moribund at the end of 2 weeks. When tyrosine was mixed in the diet in comparable amounts the majority of the pigs survived at least 3 weeks. No renal lesions were observed in the ascorbic

TABLE I.

Group No.	Daily supplement: PGA (mg)	Daily supplement: Ascorbic acid (mg)	Diet	Duration of exp. (days)	Renal lesions	Wt. changes (avg, g)
I	0	0	Stock*	21	0	
	0	0	and	21	0	+112
	0	0	Cabbage	21	0	
II	5	0	Stock	21	0	
	5	0	and	21	0	+ 86
	5	0	Cabbage	21	0	
III	0	25	Basal	21	0	
	0	25	"	21	0	+ 29
	0	25	"	21	0	
IV	1	25	"	21	+	
	1	25	"	21	0	+ 42
	1	25	"	21	0	
V	3	25	"	21	+	
	3	25	"	9	0	± 9
	3	25	"	21	0	
VI	5	25	"	21	+	
	5	25	"	21	+	— 11
	5	25	"	21	+	
VII	1	0	"	21	+	
	1	0	"	16	0	— 57
	1	0	"	21	0	
VIII	3	0	"	21	+	
	3	0	"	17	+	— 82
	3	0	"	21	+	
IX	5	0	"	21	+	
	5	0	"	21	+	— 54
	5	0	"	3	+	
	5	0	"	11	+	

* Purina rabbit chow.

acid supplemented pigs and renal lesions were seen in all of the PGA treated animals. In the 3 animals receiving the tyrosine without supplementation one showed definite renal lesions while another showed questionable lesions. These animals are the only ones in which renal lesions have been observed in the absence of PGA administration. Inanition of this degree was not associated with PGA administration in the previous experiments. It seems improbable that inanition alone accounts for the appearance of the kidneys described above.

In all of the previous experiments, PGA had been administered parenterally in doses of 5 mg daily. The next series of experiments was designed to determine the amounts of PGA necessary for the production of the lesion and the effects of varying dietary conditions. While the available evidence indicated that neither excessive tyrosine intake nor the scorbutic state was essential for the development of renal lesions, a further study of the role of the scorbutic state was made. To study the effect of ascorbic acid on the development of the lesion, the animals were run in 2 major series, one without ascorbic acid (Groups VII-IX) one with daily supplements of 25 mg of ascorbic acid (Groups III-VI). In each of these series were groups of 3 animals receiving by injection 1, 3, and 5 mg of PGA daily. The animals were maintained on this regime for 3 weeks. The results are indicated in Table I. Since other dietary factors might be involved in the phenomenon, studies were made simultaneously of 2 groups of 3 animals each (Groups I and II) which were fed a stock diet consisting of Purina rabbit chow

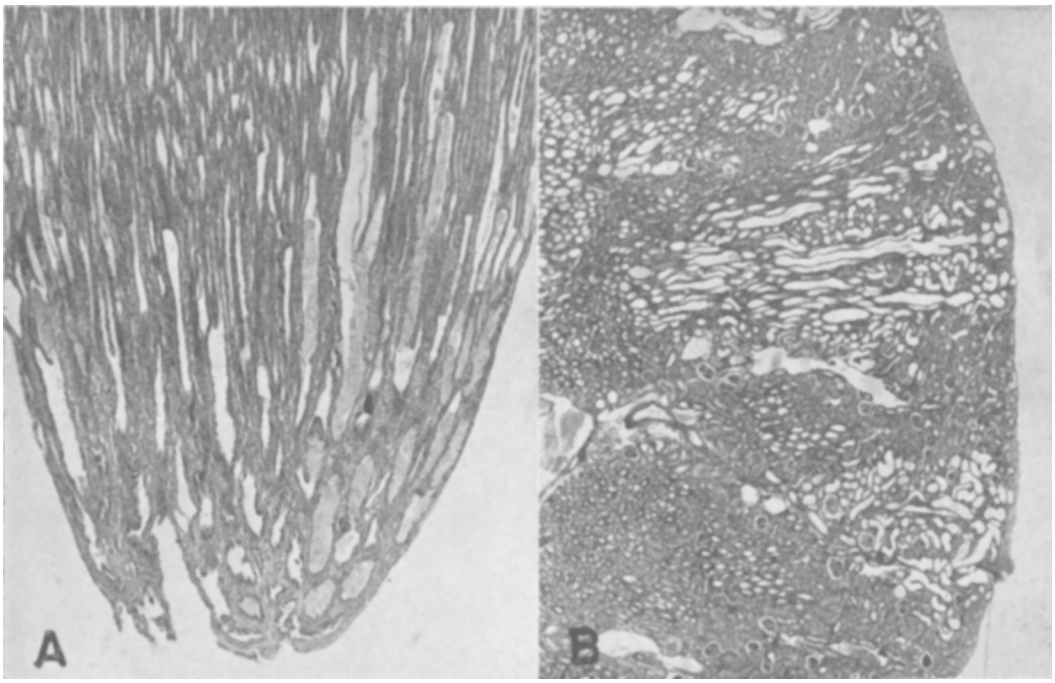

FIG. 1.
(a) Pyramid showing dilated, cast-filled ducts of Bellini. Hematoxylin and eosin × 30.
(b) Cortex showing dilatation of tubules of nephron and collecting tubules. Hematoxylin and eosin × 30.

plus cabbage *ad lib*. Three of these animals were given daily injections of PGA in amounts of 5 mg. In none of these 6 pigs did renal lesions develop in a 3 week observation period (Table I).

Three features of Table I deserve comment. First, both scorbutic and non-scorbutic guinea pigs developed kidney injury when injected with PGA. If any difference exists, the data indicate that the scorbutic animals are slightly more susceptible to the renal injury. The second point is that no lesions developed in the PGA-treated animals which were fed the stock diet and cabbage. The third point is the relationship between weight gain and renal injury. It will be noted that the lesions are least severe in animals which gained the most weight (those on the stock diet) and most severe in animals which lost the most weight (those on the experimental diet without ascorbic acid). It is also evident that administration of these quantities of PGA is associated with loss of weight or a reduction in the amount of weight gained.

In order to study the time required for the development of the renal lesions and to determine whether or not the lesions were reversible, another series of animals was given the basal diet plus tyrosine and 5 mg of PGA but without ascorbic acid or other supplements. Eighteen animals were used. Two animals were killed on the second, 4th, 6th, 8th, and 10th day after the start of the experiment. On the 12th day, the PGA was discontinued and animals were killed after 2, 4, 7, and 10 additional days. None of these animals exhibited renal lesions. Previous experience had shown that these lesions appeared after as little as 5 days treatment and that by two weeks all animals showed severe changes. In examining the details of this experiment in order to see where it might have differed from previous ones, it was determined that these animals were conditioned in the laboratory for several weeks prior to the start of the experiment whereas previous studies were made on pigs recently purchased from dealers. While they were held in our laboratory, they had

received a diet which was supplemented with unlimited amounts of cabbage. It will be recalled that in a previous experiment cabbage was used as a source of vitamin C in the diet which prevented the development of the renal lesions.

Discussion. The lesions described in this paper are similar to those seen by Harned, Cunningham, Smith, and Clark(9) in their experiments to determine the toxicity of PGA. These workers assumed that the casts are composed of PGA. The same assumption has been made by others(10,11). It is not unlikely that this is true in so far as the yellow casts are concerned, but the hyaline, eosinophilic casts are probably of different composition. We have no information on this point. The development of dilated atrophic tubules is almost certainly due to obstruction by these casts.

The factors which govern the formation of these casts are not known; the influence of fluid intake on their formation and the manner in which a stock diet and cabbage prevent them remain to be studied.

Summary. 1. The administration of PGA to the guinea pig may lead to the development of casts in the renal tubules and to dilation of the obstructed nephrons.

2. The lesions may develop with parenteral doses of 1 mg of PGA daily for 21 days.

3. With daily parenteral doses of 5 mg of PGA the lesions may develop in 5 days.

4. The formation of these casts is not related to the feeding of tyrosine.

5. Scurvy is not a prerequisite for the appearance of the injury.

6. The lesions were not observed to occur on a stock diet containing cabbage. It appears that such a diet offers some protection against the development of the injury.

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Further Studies on Mechanism of Auricular Fibrillation.* (17775)

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The topical application of aconitine on the dog's auricle induces auricular flutter or auricular fibrillation. These arrhythmias are immediately stopped when the area of application is isolated from the rest of the auricles by a clamp, or cooled with the aid of a thermode. Removal of the clamp or cessation of the cooling leads to reappearance of the arrhythmias. The analysis of these tracings does not support a circus movement as the responsible mechanism(1-4). The presence of a focus of stimulus formation must be assumed. In ventricular flutter precipitated by the topical application of aconitine, cooling also stopped the flutter and made sinus rhythm reappear for the duration of the cooling. Ventricular fibrillation, however, never responded to cooling. Evidence was advanced to show that with higher rates of ventricular flutter many centers became active in the ventricles. Therefore, cooling of the original center on which aconitine had been applied did not change the form of the arrhythmia(5).

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