SOME ASPECTS OF EXPERIMENTAL AND HUMAN PHENACETIN NEPHRITIS

by

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TO MY WIFE

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CHAPTER I.

INTRODUCTION.

Modern pathology is forced to deal more and more with changes brought about by modern therapy on the one hand and by the abuse of drugs by the laity on the other.[1] New diseases have been added, others altered. Skin allergies, collagen diseases, frightening foetal abnormalities due to thalidomide, peptic ulceration with cortison therapy are but a few of the end results of the abuse of the numerous pharmaceutical substances available with or without prescription to the public. To this list may be added the nephrotoxic effects of phenacetin.

Phenacetin was first used in 1887 for its antipyretic properties[2]. Later it was found to have an analgesic effect. In a few cases, mainly when large doses had been taken, toxic effects on the red cells were observed, but these were rarely serious and readily reversible when the drug was withdrawn.

In 1950 Zollinger and Spuhler[3] noted an increase in chronic interstitial nephritis in their autopsy material. This was based upon the finding of a diffuse lympho-plasmo-cellular infiltration at the site of the transition between cortex and medulla as originally described by Zollinger in 1945[4]. Being aware of an increased consumption of

phenacetin in Switzerland, Zollinger and Spuhler found that a history of phenacetin containing analgesic tablets could be obtained from relatives of these patients [5,6]. Thus after phenacetin had been widely used for over sixty years, there came the first allusion to a nephrotoxi effect. Since that time many reports dealing with an association between excessive phenacetin consumption and chronic interstitial nephritis have appeared in the Swiss and Scandinavian literature[7,8,9,

Not only has phenacetin been incriminated in causing an intei stitial nephritis, but it is also felt to be responsible for an increase in renal papillary necrosis [11,12,13] in the absence of diabetes mellitus and obstructive nephropathy. Renal papillary necrosis was first described by von Friedreich eighty-six years ago [14]. Gunther (1937) [1] and Froboese (1937) [16] pointed out that renal papillary necrosis was often associated with diabetes mellitus or obstruction of the urinary tract. Mandel [17] upheld this view and in a review of 160 collected cases found 90 of the patients to be diabetic and 48 of the remaining 64 to be suffering from some form of obstructive uropathy. However, recent observations have not borne out this close relationship between diabetes mellitus and obstructive uropathy as being causes of renal papillary necrosis. In the more recent literature most of the patients

had neither diabetes nor obstructive uropathy [18]. Simon [19] in 1957 collected 42 autopsy cases of renal papillary necrosis from the files of the Mayo Clinic and found that only 19% had diabetes mellitus. In a series of 155 cases of renal papillary necrosis, Lindvall [20] reports the incidence of associated conditions as: diabetes 19, urinary tract obstruction 3, abuse of phenacetin 86, absence of these 12 and incomplete data as regards addiction to phenacetin as 35. So that attention has now been drawn to a relationship between excessive phenacetin consumption and the development of renal papillary necrosis.

The original reports of a specific phenacetin induced nephropathy emanated from Europe. More recently workers in Australia [13,21], South Africa [22,23], Canada [24], New Zealand [25] and America [26,27,28] have observed a similar association.

Although there has been great interest in the problem of an association between "chronic interstitial nephritis" and prolonged ingestion of phenacetin containing compounds, there are many workers who do not believe that phenacetin causes a specific renal change. Most of these authors feel that the condition is a form of chronic pyelonephritis [29,30,31,32].

Conclusive proof that phenacetin causes renal lesions is lacking and most of the evidence incriminating it is circumstantial. For this reason many attempts have been made to induce interstitial nephritis and papillary necrosis by feeding large quantities of phenacetin and its breakdown products to animals. In these early experiments it became obvious that excessive phenacetin intake alone was not sufficient to produce renal lesions [1,33,34], however, if phenacetin and its breakdown products were fed first and this followed by intravenous inoculation of organisms, interstitial nephritis was produced [35,36,37]. Renal papillary necrosis did not occur when phenacetin was fed alone.

The drug assumed to be the specific cause of renal disease is phenacetin [38] and its breakdown products. Schreiner in an editorial entitled "The Nephrotoxicity of Analgesic Abuse" [39] noted that despite interest in phenacetin, renal consequences occurred in combination with other drugs such as aspirin and caffeine. As yet there is no solution to the problem of the specificity of phenacetin nephritis. There has also been no clarification of the role played in the development of renal disease by the individual substances with which phenacetin is combined.

The aim of this thesis was an attempt to elucidate some of these problems. Experiments were planned in which rats were fed phenacetin and tablets containing aspirin, phenacetin and caffeine (A.P.C.) for long periods in order to produce renal lesions and in particular papillary necrosis.

In the first experiment (Experiment I) the effects of feeding phenacetin alone and tablets containing aspirin, phenacetin and caffeine (A.P.C.) were compared. In view of renal lesions being produced in Experiment I, and in an attempt to clarify the role of the individual components of the A.P.C. tablet, a second experiment was undertaken (Experiment II). Rats were fed powders consisting of combinations of aspirin, phenacetin and caffeine. The results obtained will be discussed and the experiments compared with others reported in the literature. In addition, a comparison will be made between these lesions and those of human phenacetin nephritis. Finally, the possible pathogenetic mechanisms underlying the cortical and papillary lesions and their relationship to the drugs will be discussed.

CHAPTER II

MATERIAL AND METHODS OF EXPERIMENTS

I. EXPERIMENT I

Male Albino rats one year old and of a Wistar strain were divided into 3 groups. Each group received a standard laboratory diet of mouse biscuits and rolled oats.

Group I

Thirty two rats were placed into two communal cages and were fed pure phenacetin powder. Four grams of the powder were mixed in a dish of rolled oats and were replaced when the dish containing the oats was empty. The average daily intake of phenacetin per rat was 30 mgms.

Group 2

Twenty three rats were placed into a communal cage and fed tablets of aspirin, phenacetin and caffeine (A.P.C.). Each tablet contained 160 mgms. phenacetin, 225 mgm. aspirin and 30 mgms. caffeine.

Ten tablets were crushed in a mortar and mixed with fine rolled oats. When the feeding dish was empty a further ten tablets were added. The average intake of phenacetin per rat per day was 17 mgms.

Group 3

This consisted of a control group of twenty two rats which

were fed the standard laboratory diet of mouse biscuits only. These animals received in addition a dish of fine rolled oats once a week.

2. EXPERIMENT II

Group 1

This group consisted of 12 rats which were fed aspirin and phenacetin in the same amounts as they occur in an A.P.C. tablet, i.e 160 mgms phenacetin and 225 mgms aspirin. Four grams of a powder consisting of phenacetin and aspirin in the above ratio was mixed with the oats and replaced when the dish containing the oats was empty. The total daily intake of phenacetin was 17 mgms per rat.

Group 2

This group consisted of 11 rats which were fed phenacetin and caffeine in the same amounts as they occur in an A.P.C. tablet i.e. 160 mgms phenacetin and 30 mgms caffeine. Four grams of a powder consisting of phenacetin and caffeine in the above ratio were fed as outlined in Group 1. The total daily intake of phenacetin was 17 mgms per rat.

Group 3

This group consisted of 9 rats fed aspirin and caffeine also

in the same amounts as they appear in an A.P.C. tablet, i.e. 225 mgms. of aspirin and 30 mgms. of caffeine. Four grams of a powder consisting of aspirin and caffeine in the above ratio were fed as outlined in Group 1.

3. METHOD OF HISTOLOGICAL EXAMINATION

In an attempt to assess the natural development of lesions, rats were sacrificed at random intervals throughout the duration of the experiment and autopsies were performed. In addition animals dying spontaneously during the course of the experiment were autopsied. The kidneys were sectioned coronally from upper to lower poles and through the renal papilla. The two halves of each kidney were fixed in 10% formol saline, sections were cut and stained with haematoxylin and eosin.

A golden brown granular pigment was present in tubular epithelial cells and was investigated using Perl's stain, Pickworth's stain [40], Stein's technique for bile [41], reduced ferricyanide method of Chevremont and Frederic [42], and Sudan Black B stain [43]. In addition the pigment was examined for autofluorescence.

4. ELECTRONMICROSCOPY

The kidneys of occasional experimental and control animals were examined electronmicroscopically. The animals were sacrificed with ether, the abdomen rapidly opened and the kidney removed. The kidney was cut coronally with a sharp blade and blocks of tissue 1 mm in size were removed from cortex and papilla. The tissues were prepared by fixation in 1 per cent buffered osmium tetroxide for one hour in an ice bath, dehydrated in serially increasing concentrations of alcohol, infiltrated in a mixture of uncatalysed methacrylate (five parts butyl methacrylate to one part methyl methacrylate) followed by infiltration in the same mixture catalysed with luperco. Embedding was carried out in a partially polymerised methacrylate mixture in gelatin capsules. Polymerisation was completed at 60^{o} C overnight. Sections were cut with a Porter-Blum microtome and examined in a Siemens electron microscope

CHAPTER III

RESULTS OF EXPERIMENTS

1. EXPERIMENT I

The whole of the cut section of each kidney was examined and the cortical and papillary lesions present noted. In addition the cortical lesions were graded.

i. Cortical Lesions

a. Types of Cortical Lesions. The following were the types of cortical lesions:

1. Thickening of the tubular basement membrane with atrophy of epithelial cells.

This lesion occurred scattered throughout the cortex and subcortical zones of the kidney (fig. 1). The distal convoluted tubules were predominately effected. At times the thickening of the basement membrane was so severe it could be referred to as a "tubulosclerosis" (fig. 2). A golden brown pigment was observed in occasional tubular epithelial cells. This pigment stained positively for iron. However, on occasions the Perl's stain was negative. The pigment was then investigated and showed negative results for haemoglobin and bile, it stained blue-green with the reduced ferricyanide method, was negative to Sudan Black B and feebly P.A.S. - positive. These findings indicated a highly oxidised lipofuscin.

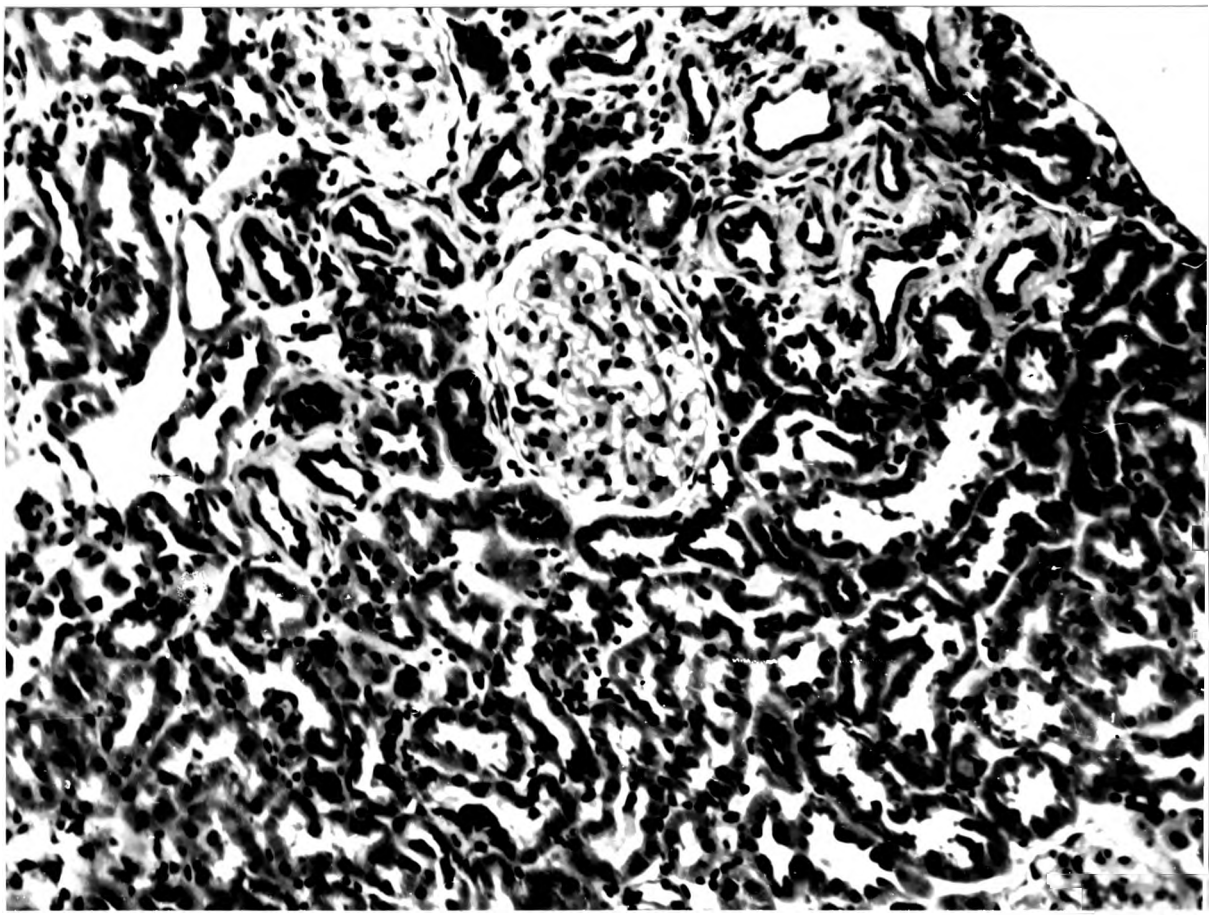

Fig. 1 Low power view of focal areas in the renal cortex of tubular basement membrane thickening with atrophy of tubular epithelial cells in rat fed phenacetin for $6\frac{1}{2}$ months. H. and E. X 600.

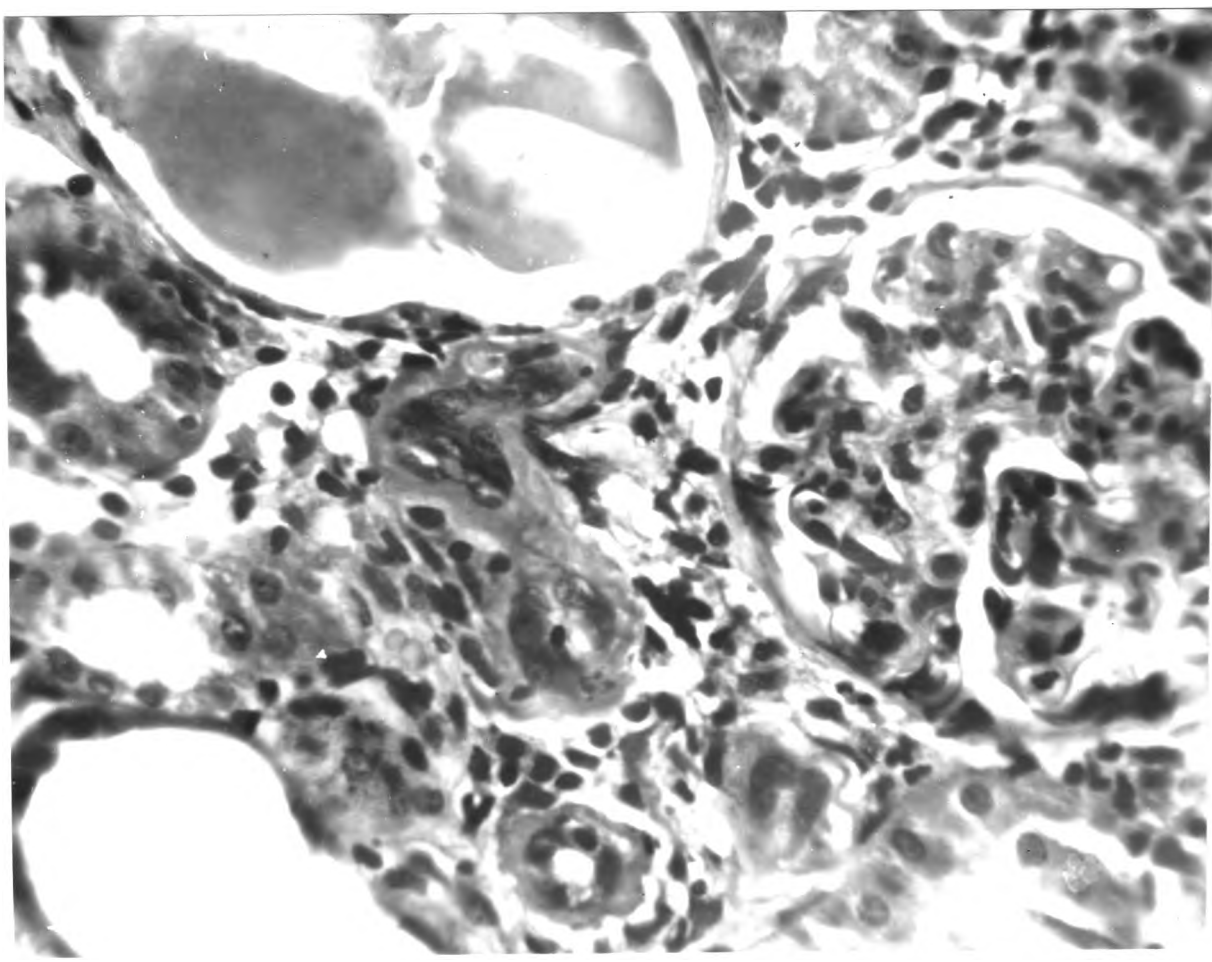

Fig. 2 Marked thickening of tubular basement membrane with atrophy of tubular epithelial cells ("tubulosclerosis"). There is also interstitial fibrosis with nongranular cell infiltration. Note the large dilated tubule with colloid cast in the left uppermost corner. Rat fed A.P.C. tablets for 7 months.

H. and E. X 2,1000

2. Interstitial Fibrosis

The interstitial fibrosis was always rather loose, consisting of a few reticulin fibres with an occasional fibroblast nucleus (fig. 3). Never did it adopt the characteristic dense nature of the interstitial fibrosis seen in human cases of phenacetin induced interstitial nephritis. At times, however, the fibrosis did become more dense (fig. 4). This could be seen in the zone of demarcation, at the edge of an area of papillary necrosis and was probably part of the reaction to the surrounding necrosis.

3. Dilated tubules with colloid casts

These often occurred alone and were then found most frequently at the cortico-medullary junction or in the cortex (fig. 5). At times they were grossly distended, giving rise to the "thyroid-kidney" (fig. 6). When this occurred the glomeruli often underwent glomerular tamponade with total atrophy and disappearance of the glomerular tuft (fig. 7). Although glomeruli were sometimes hyalinised, periglomerular fibrosis was not a feature.

4. Nongranular cell infiltration

The infiltration of nongranular cells was found scattered within the tissue throughout the cortex (fig. 8). It usually consisted

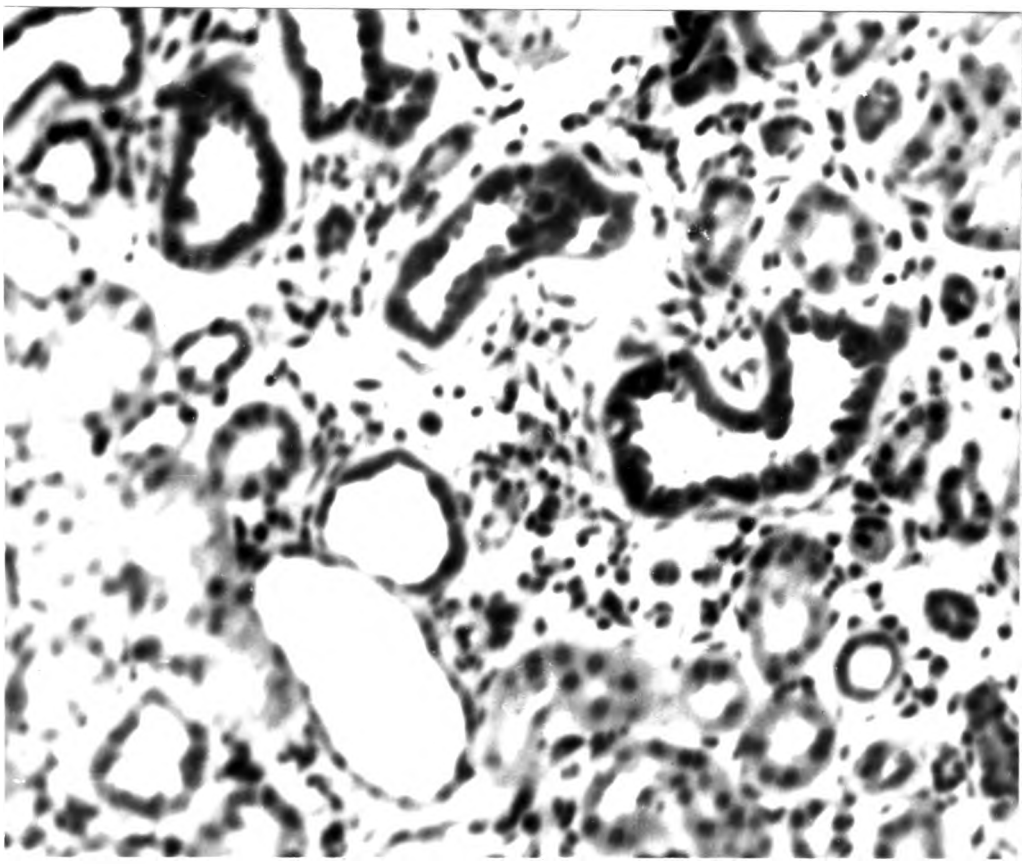

Fig. 3 Note the loose interstitial fibrosis between the tubules in a rat fed phenacetin for 12 months.

H. and E. X 1,000

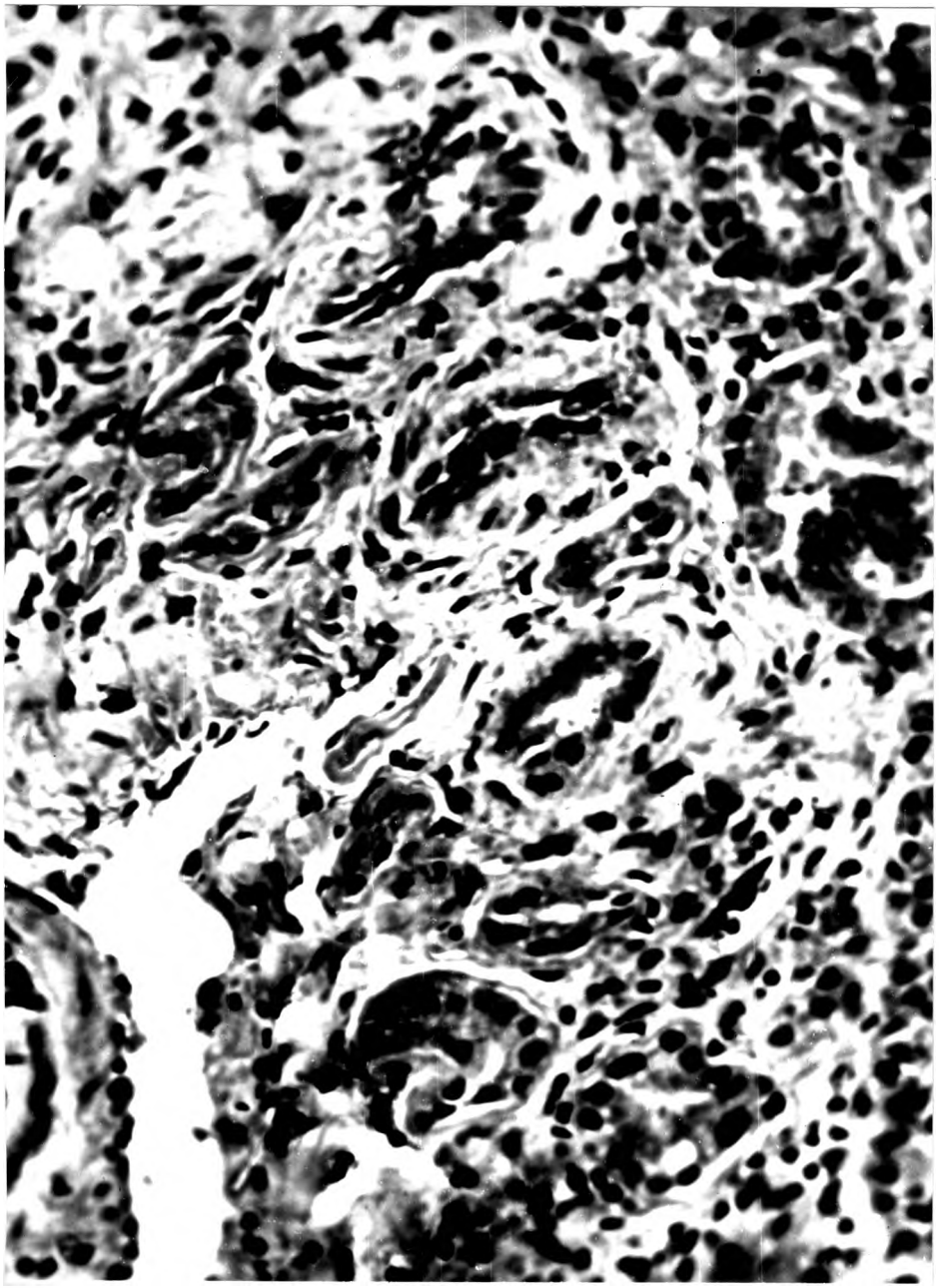

Fig. 4 Marked interstitial fibrosis between tubules at the edge of an area of papillary necrosis in a rat fed A.P.C. tablets for 13 months.

H. and E. X 960

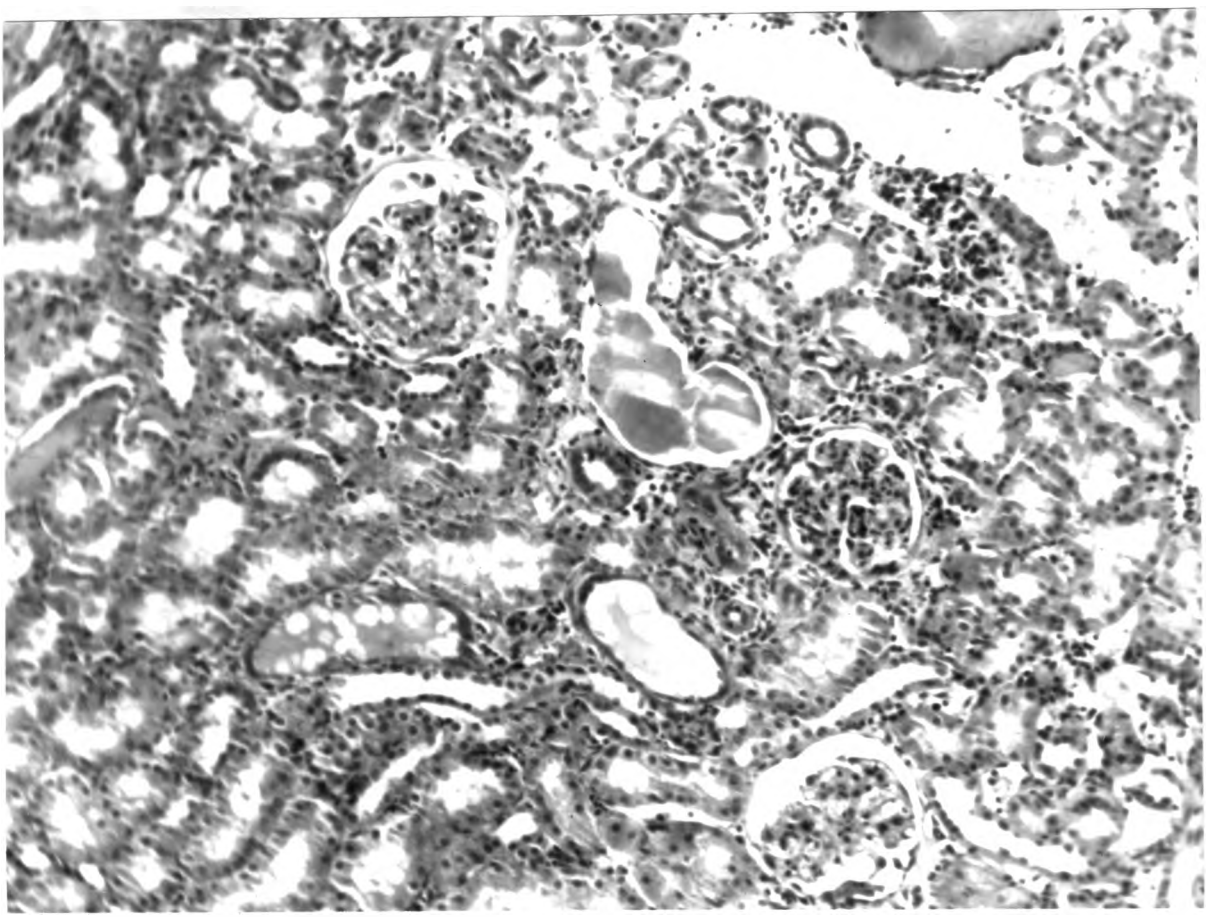

Fig. 5 Occasional diluted tubules with colloid casts are present in the cortex of this rat fed phenacetin for 7 months.

H. and E. X 480

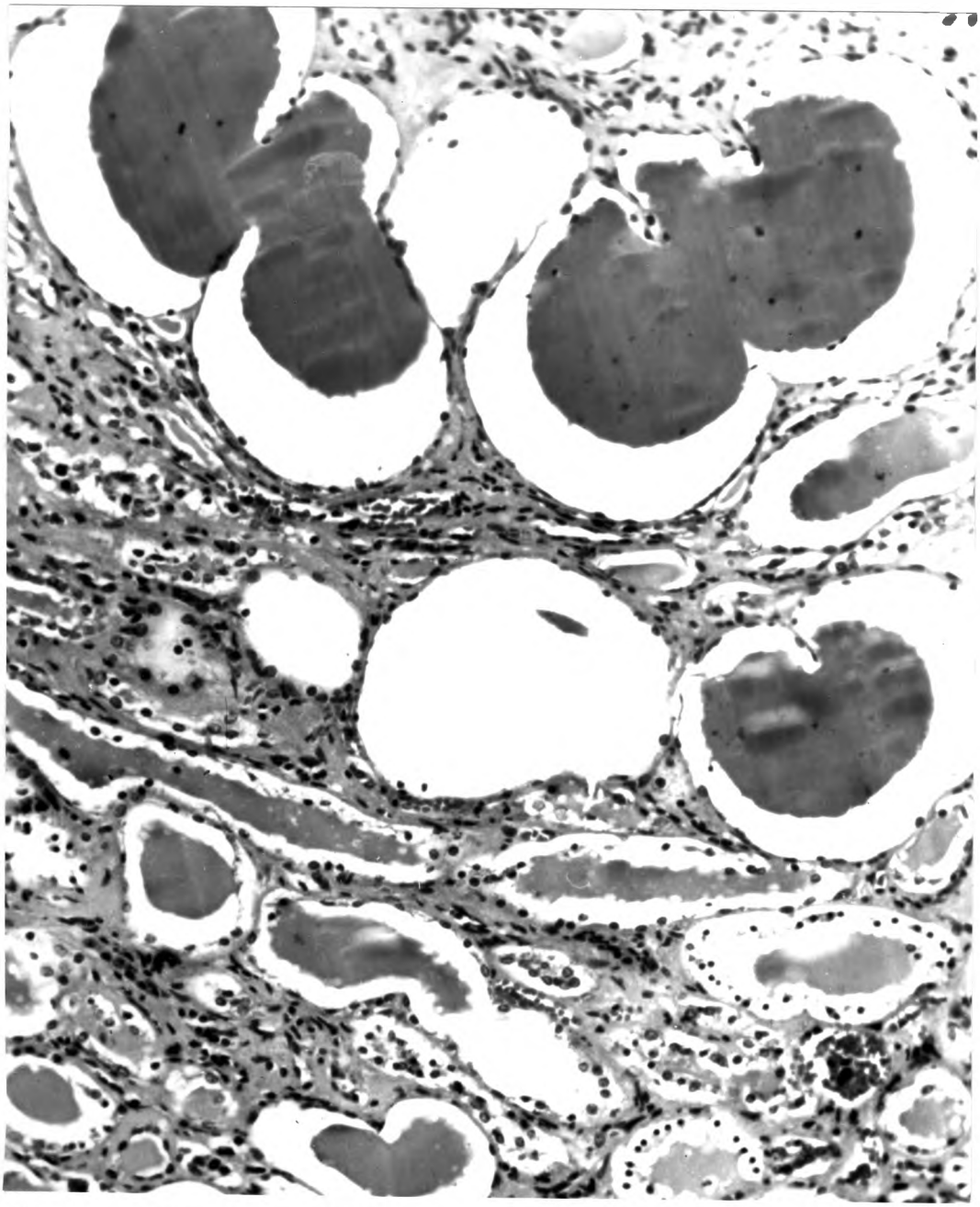

Fig. 6 "Thyroid-like" appearance due to dilated tubules with colloid casts in rat fed phenacetin for 10 months.

H. and E. X 700

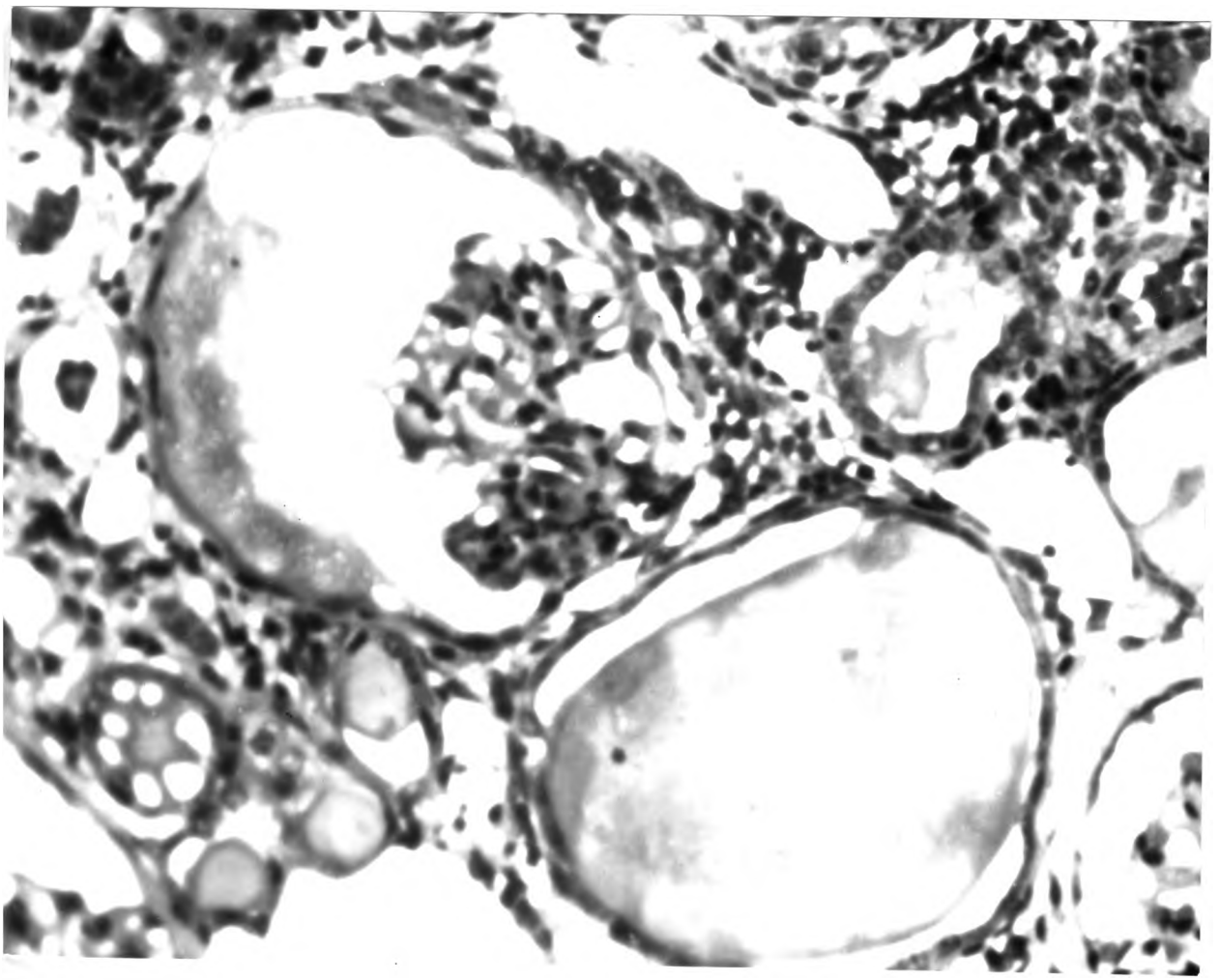

Fig. 7 Glomerular "tamponade" leading to atrophy of glomerular tuft. Note also the dilated tubules.

H. and E. X 1,250

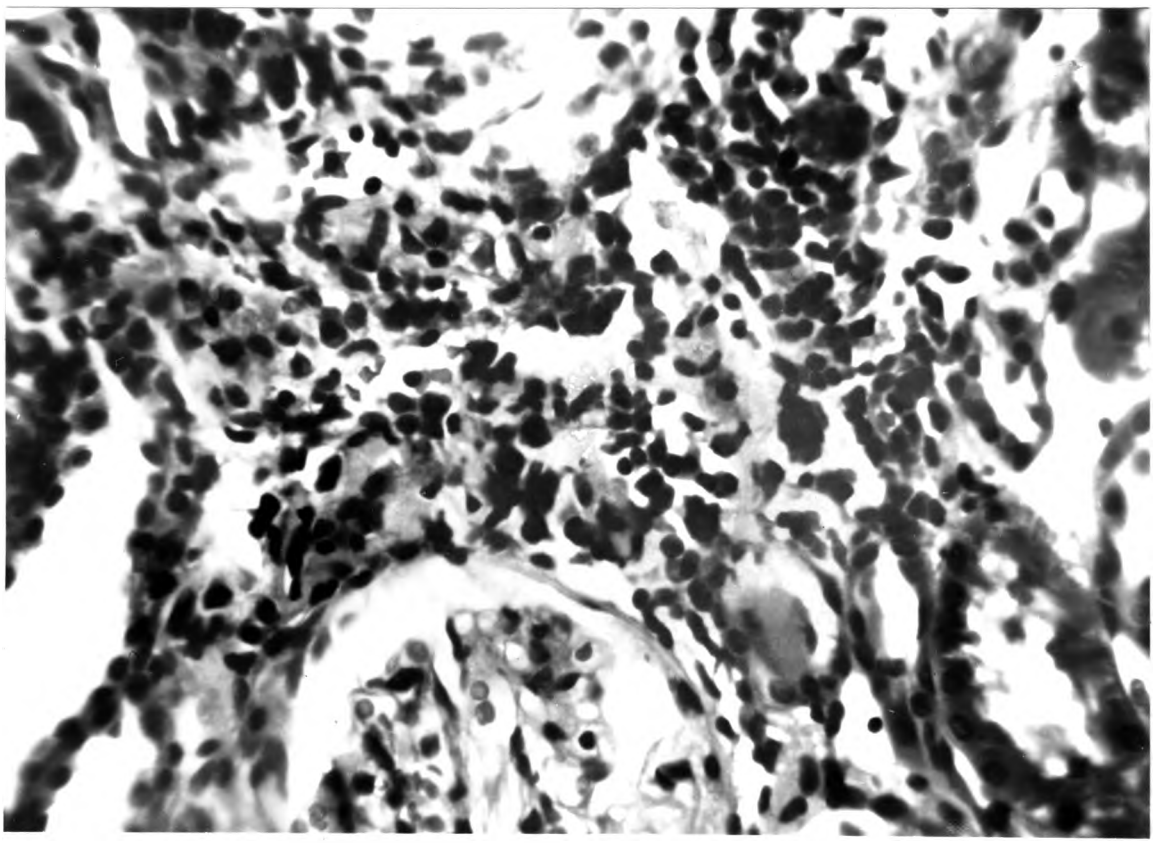

Fig. 8 Small focus of nongranular cell infiltration into the interstitial tissue of rat fed A.P.C. tablets for 11 months.

H. and E. X 1,500

of lymphocytes and rarely of plasma cells. Polymorphonuclear leucocytes were only present in very occasional tubules.

These cortical lesions could be found alone or in combinations with one another (fig. 9).

b. Grading of cortical lesions

The cortical lesions were graded as follows:

O	:	no lesions
+	:	Small focal lesions not filling a low power microscopic field.
++	:	larger focal lesions almost filling a low power microscopic field.
+++	:	diffuse lesions merging with one another.

c. Analysis of results

An analysis of the cortical lesions occurring in the experimental and control animals appears in the histograms (figs. 10, 11. 12, 13). As can be seen all four cortical lesions are found in both the experimental and control groups of rats. However, there is a difference between these two groups in the grade of severity of the lesions. The histograms indicate that in the animals fed phenacetin

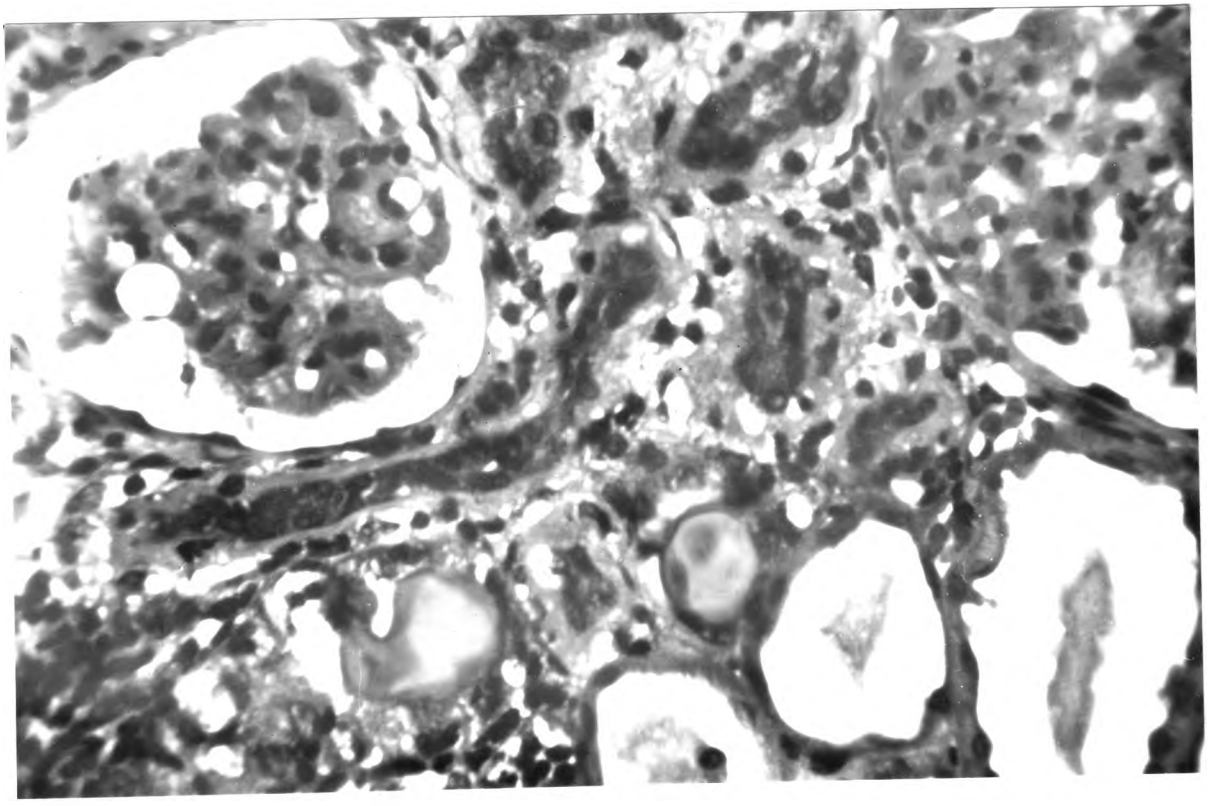

Fig. 9 All four cortical lesions are present in this section of kidney of a rat fed phenacetin for 12 months. Note the interstitial fibrosis, basement membrane thickening with tubular atrophy, dilated tubules with colloid casts and nongranular cell infiltration.

H. and E X 1,500

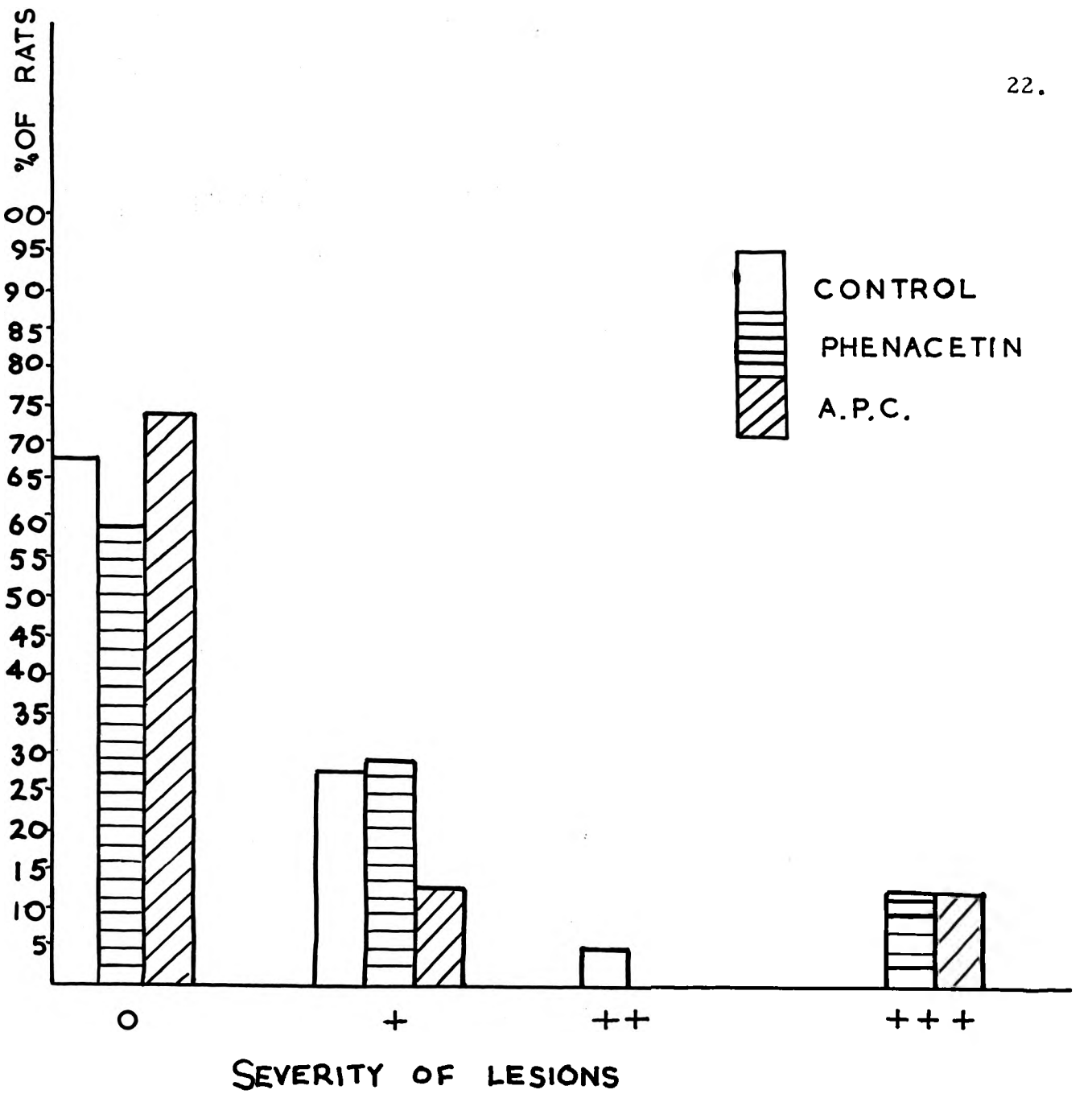

Fig. 10
Histogram of the comparison of the interstitial fibrosis occurring in Experiment I.

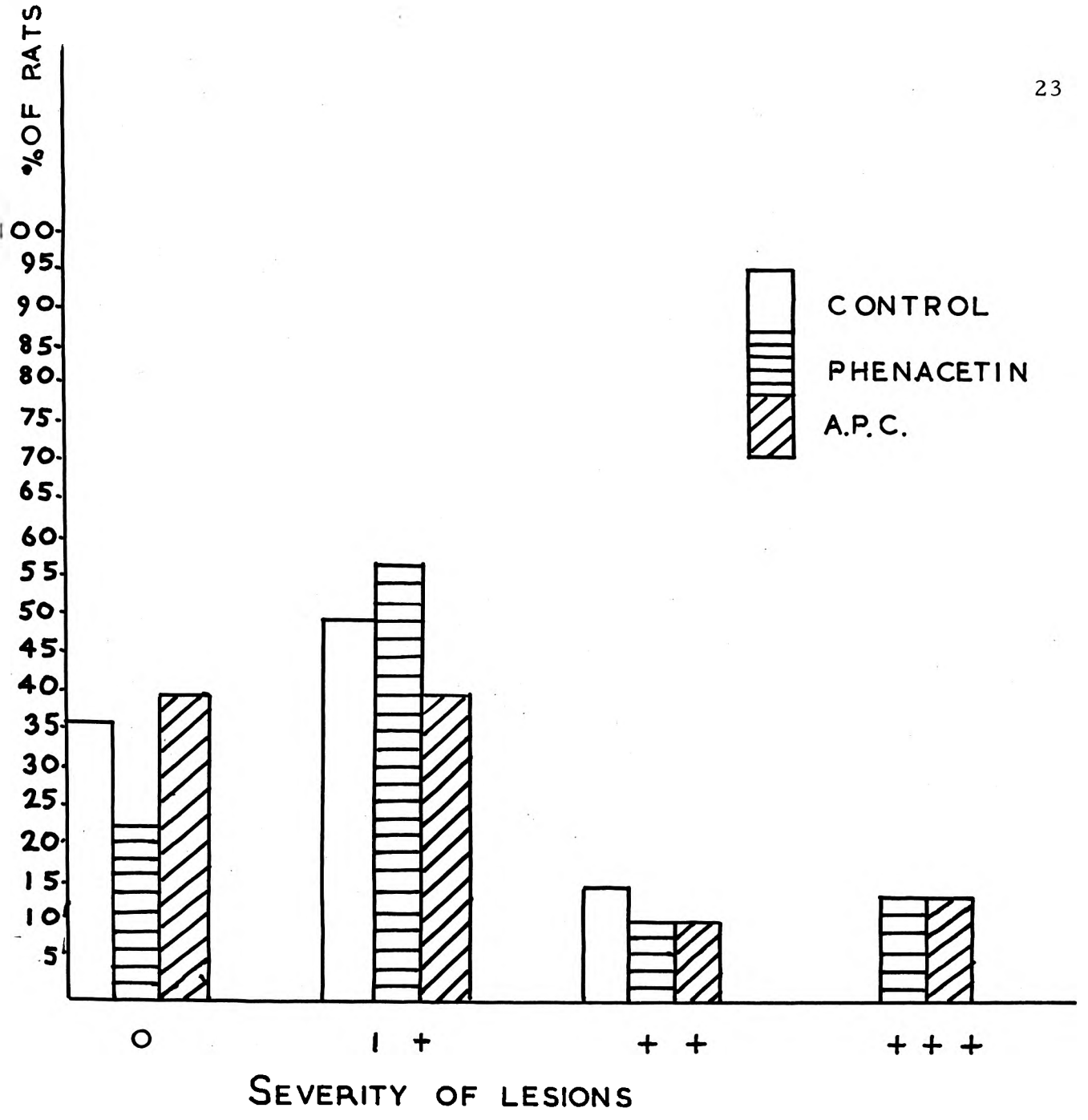

Fig. 11. Histogram of the comparison of the thickened tubular basement membrane and atrophy of tubular epithelial cells occurring in Experiment I.

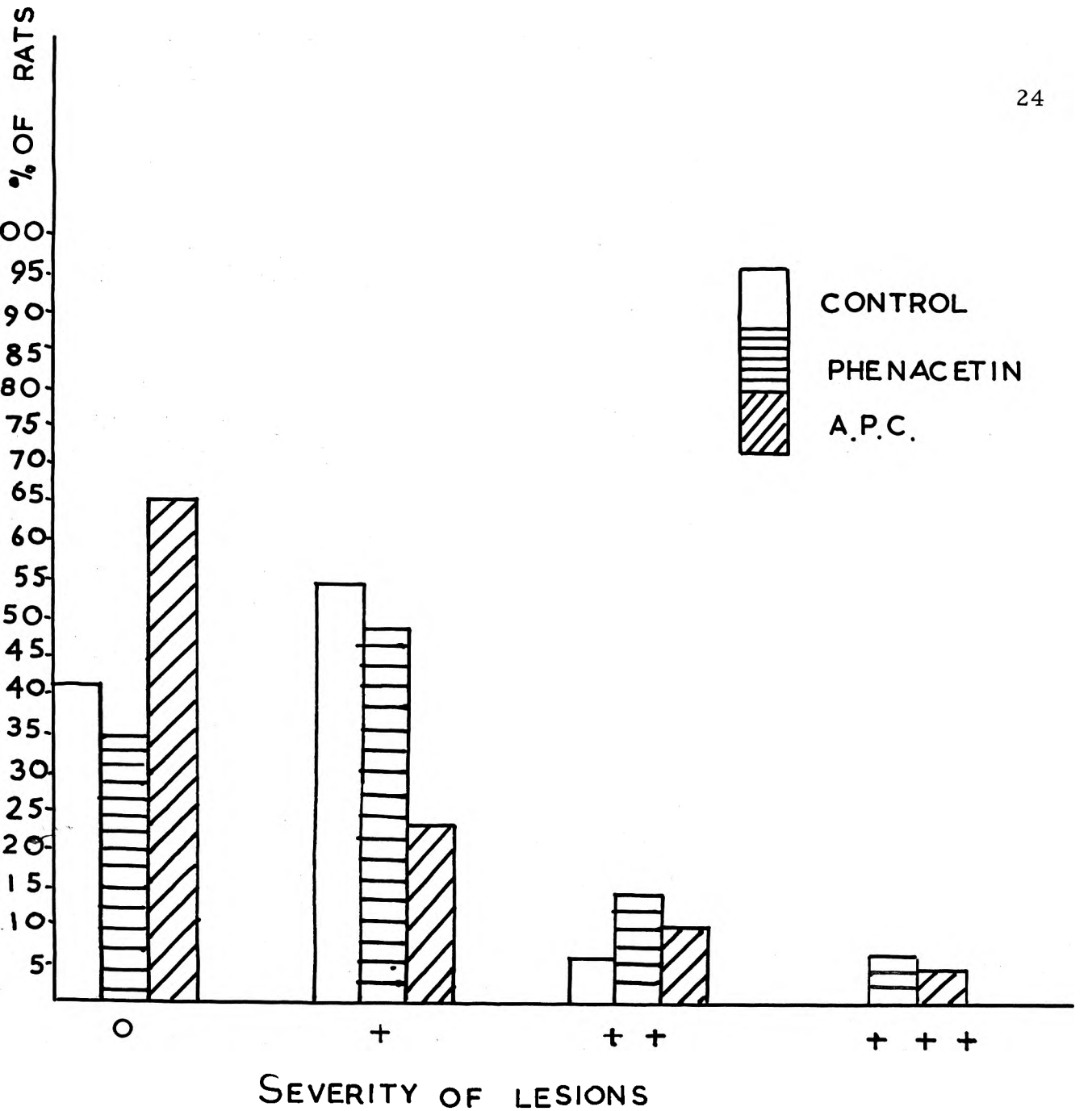

Fig. 12. Histogram of the comparison of dilated tubules with colloid casts occurring in Experiment I.

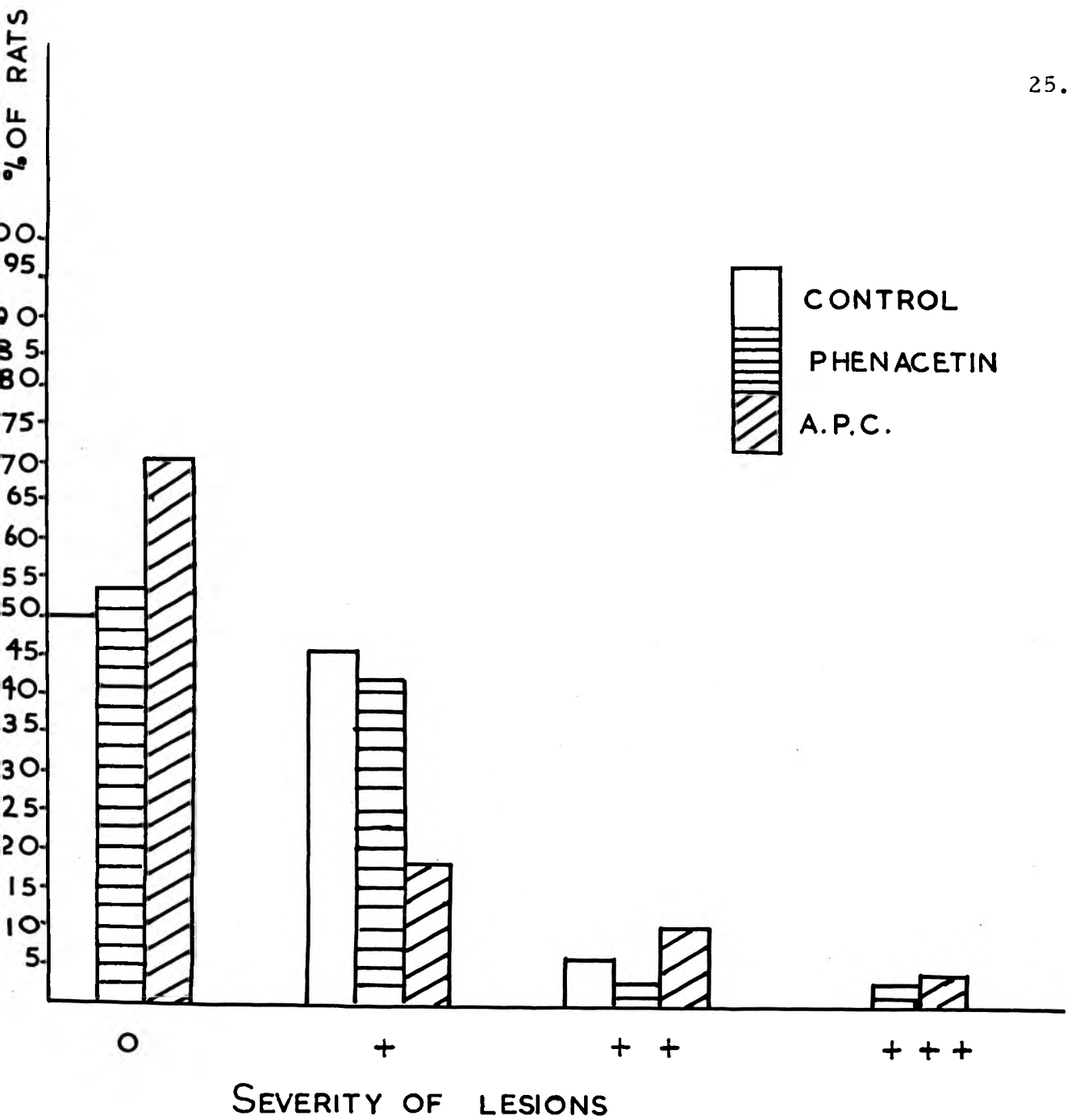

Fig. 13. Histogram of the comparison of the nongranular cell infiltration occurring in Experiment I.

and A.P.C. tablets all four cortical changes occur in the +++ grade of severity, however, none of the control animals had such severe lesions. In both experimental and control animals there would appear to be a pattern of development of these cortical changes. The scattergrams (fig. 14 to 17) indicate the severity of the lesions as they occur in time. The pattern which emerges from these graphs shows a gradual increased severity of the lesions with time. Thus it is fair to expect that these cortical changes would occur with greater severity in the older rats, and on the other hand younger animals would normally have lesions of lesser severity. The increase in severity in the experimental group thus becomes more significant, because 84% of the control animals died or were killed in the period from 8 months to 1 year 4 months of the experiment, whereas only 68% of the experimental group were killed in the same time. Thus although more control rats were alive during the time in which the lesions of greater severity would be expected to occur, only lesions of lesser severity were observed. Also despite the fact that the lesions became more severe with time, a view of the scattergrams show that occasional lesions of +++ severity occur in the experimental groups at an early age.

Thus although these results must be treated with the greatest

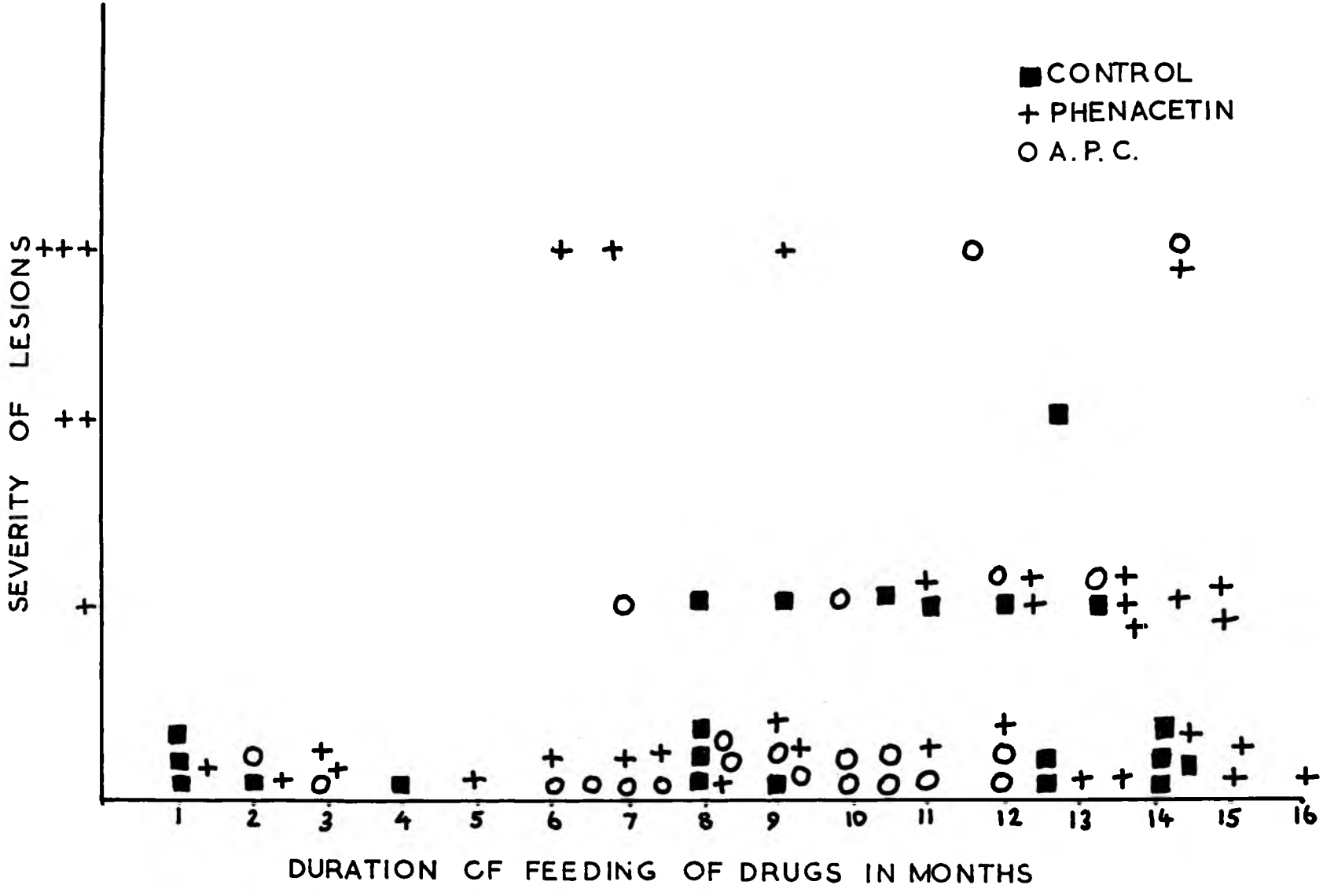

Fig. 14. Scattergram comparing the severity of interstitial fibrosis with the duration of feeding of the drugs in months.

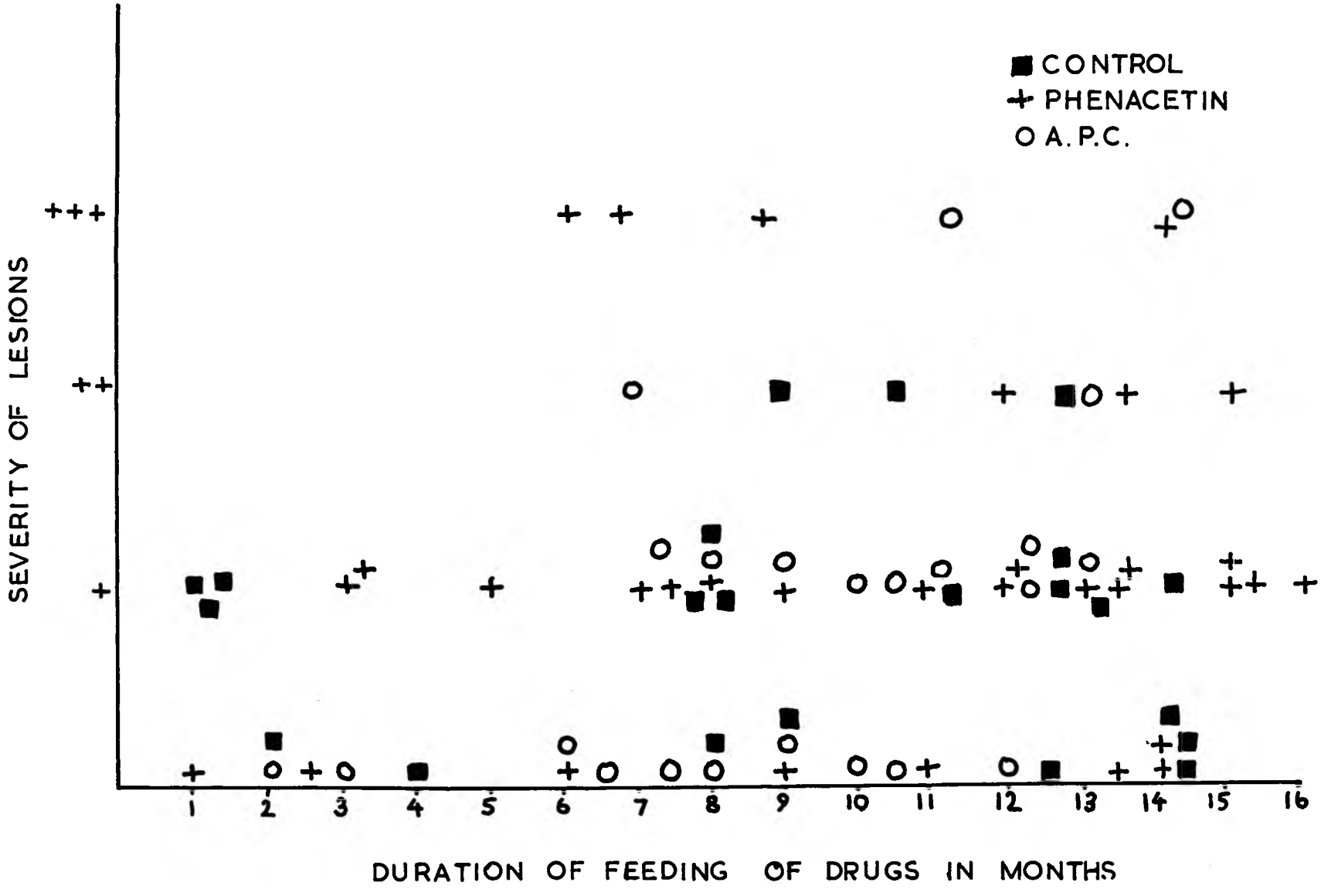

Fig. 15. Scattergram comparing the severity of the thickened tubular basement membrane and tubular atrophy with the duration of feeding of the drugs in months.

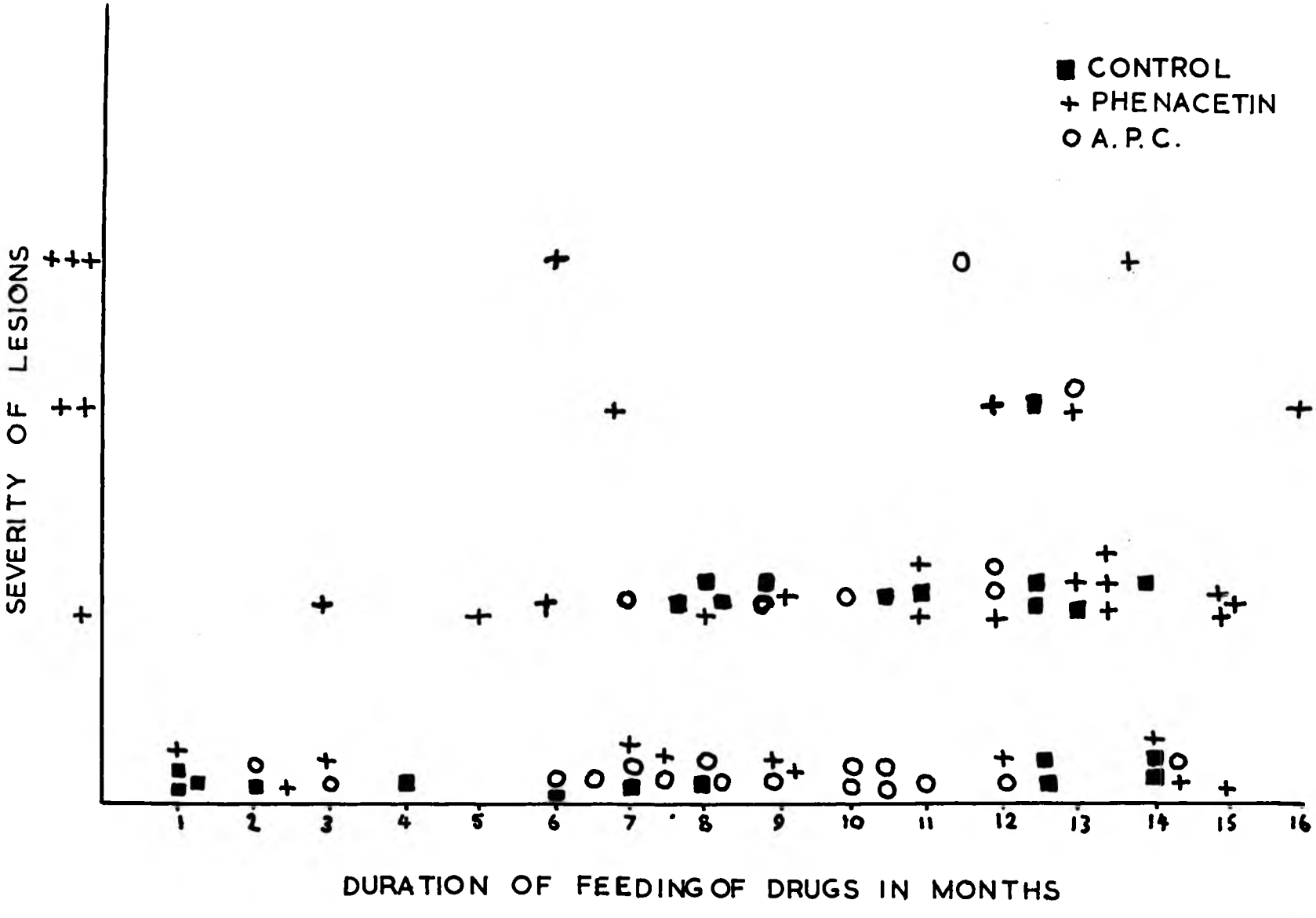

Fig. 16. Scattergram comparing the severity of dilated tubules with colloid casts with the duration of feeding of the drugs in months.

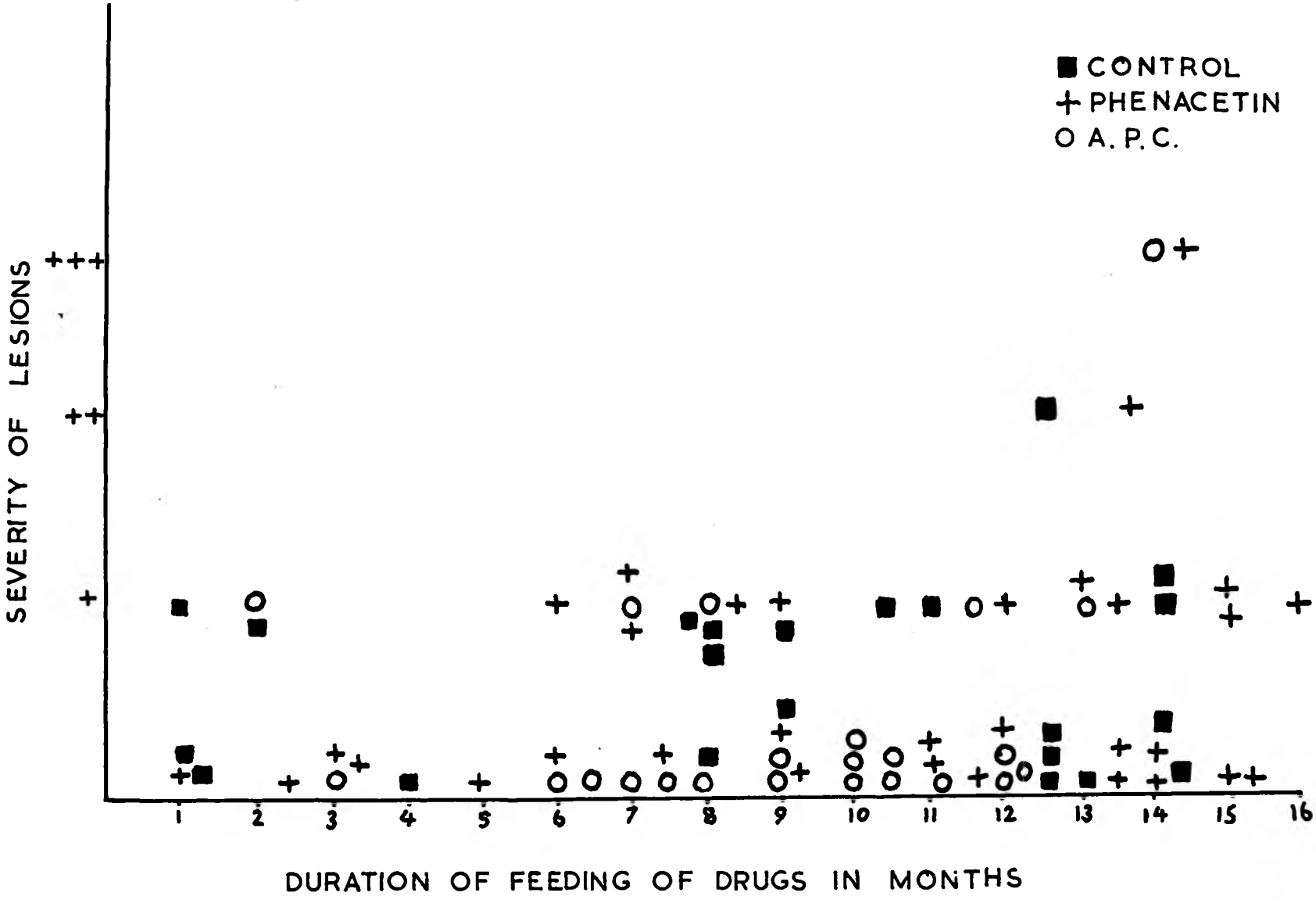

Fig. 17. Scattergram comparing the severity of nongranular cell infiltration with the duration of feeding of the drugs in months.

reserve, it would appear that phenacetin and A.P.C. tablets are responsible for causing a naturally occurring lesion to occur earlier and more severely than in the control animals.

The finding of naturally occurring renal lesions in these laboratory animals is not unique. Magee [44] in a study of the pathological changes occurring in rats at the age of approximately 2 years states that the commonest spontaneous disease in the ageing rat is probably the chronic inflammatory lung lesion, followed by a chronic kidney lesion, which in its fully developed form is quite characteristic. It has been described by Saxton and Kimball [45] as a nephrosis. It is found in 44% of rats dying naturally. These features very closely resembled those found in the animals in the present experiment both macroscopically and microscopically. Macroscopically the kidneys are often enlarged, yellowish brown in colour and have finely granular surfaces, a change seen in the present experiment. The condition is always bilateral. Microscopically there is gross dilatation of tubules, in cortex and medulla which contain large hyaline casts, varying degree of glomerular sclerosis and often interstitial infiltration of mononuclears. There is also an increase in peritubular interstitial fibrosis. The number of affected nephrons varies with the severity of the lesion and in badly

affected kidneys may represent a high proportion of the total.

From the histological features therefore a similar lesion is occurring in the experimental rats in this experiment. This lesion appears to be occurring earlier, more frequently and more severely in the drug fed animals.

ii. Papillary Lesions

a. Types of papillary lesions

Two types of papillary lesions were observed.

1. Haemorrhage and serous exudate into the renal papilla

The amount of blood in these haemorrhagic areas varied greatly, from small foci (fig. 18) to extensive areas occupying almost the whole of the renal papilla (fig. 19). In such severe haemorrhage the tubular epithelium could not be identified and was at times displaced, obscured or necrosed (fig. 19). The blood which was usually situated in the middle of the renal papilla sometimes extended toward the periphery.

On occasions it was difficult to separate true haemorrhage from marked congestion of the vessels of the renal papilla. Any

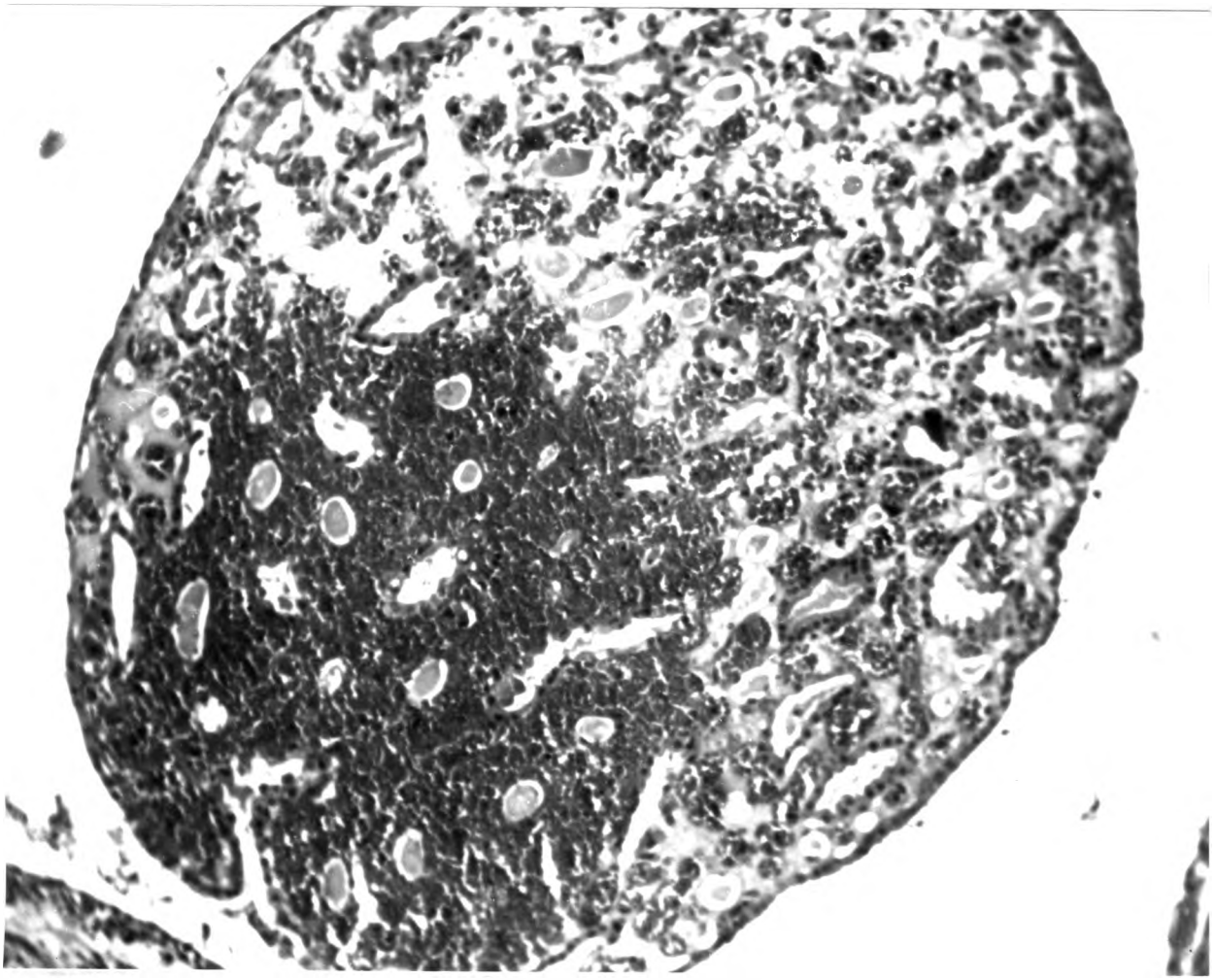

Fig. 18. There is a diffuse but well localised area of haemorrhage in the interstitial tissue of the renal papilla of this rat fed phenacetin for 9 months.

H. and E. X 600

Fig. 19

Haemorrhage into the renal papilla of a rat fed A.P.C. tablets for 8 months. Note that the tip of the renal papilla is markedly distended with blood and there is necrosis of tubules

H. and E.
X 800

lesion which was borderline was excluded. Occasionally the haemorrhage was found adjacent to the transitional epithelial border of the papilla and extending into the pelvis of the kidney (fig. 20). Although there were very occasional thrombi in the vessels in the juxtamedullary zone, the thrombi did not occur in association with the haemorrhage into the renal papilla.

2. Necrosis of the renal papilla

The size of the necrotic areas varied greatly, being either limited to small focal areas of necrosis (fig. 21) (fig. 22), or to an area of necrosis which included the tip of the renal papilla (fig. 23) or finally there was complete necrosis of the whole of the renal papilla (fig. 24). In the smaller areas there was mild coagulative necrosis of the epithelium of the collecting tubules with surrounding polymorphonuclear leucocytes (fig. 22). In the larger areas the necrotic tissue showed an infarct-like appearance. There was total eosinophilic coagulative necrosis in which could be seen neither bacteria nor inflammatory cells (fig. 24). This was surrounded by a zone of demarcation between viable and non-viable areas (fig. 23,24). The zone of demarcation was made up of numerous polymorphonuclear leucocytes, nongranular cells and occasional gram negative bacilli. The surround-

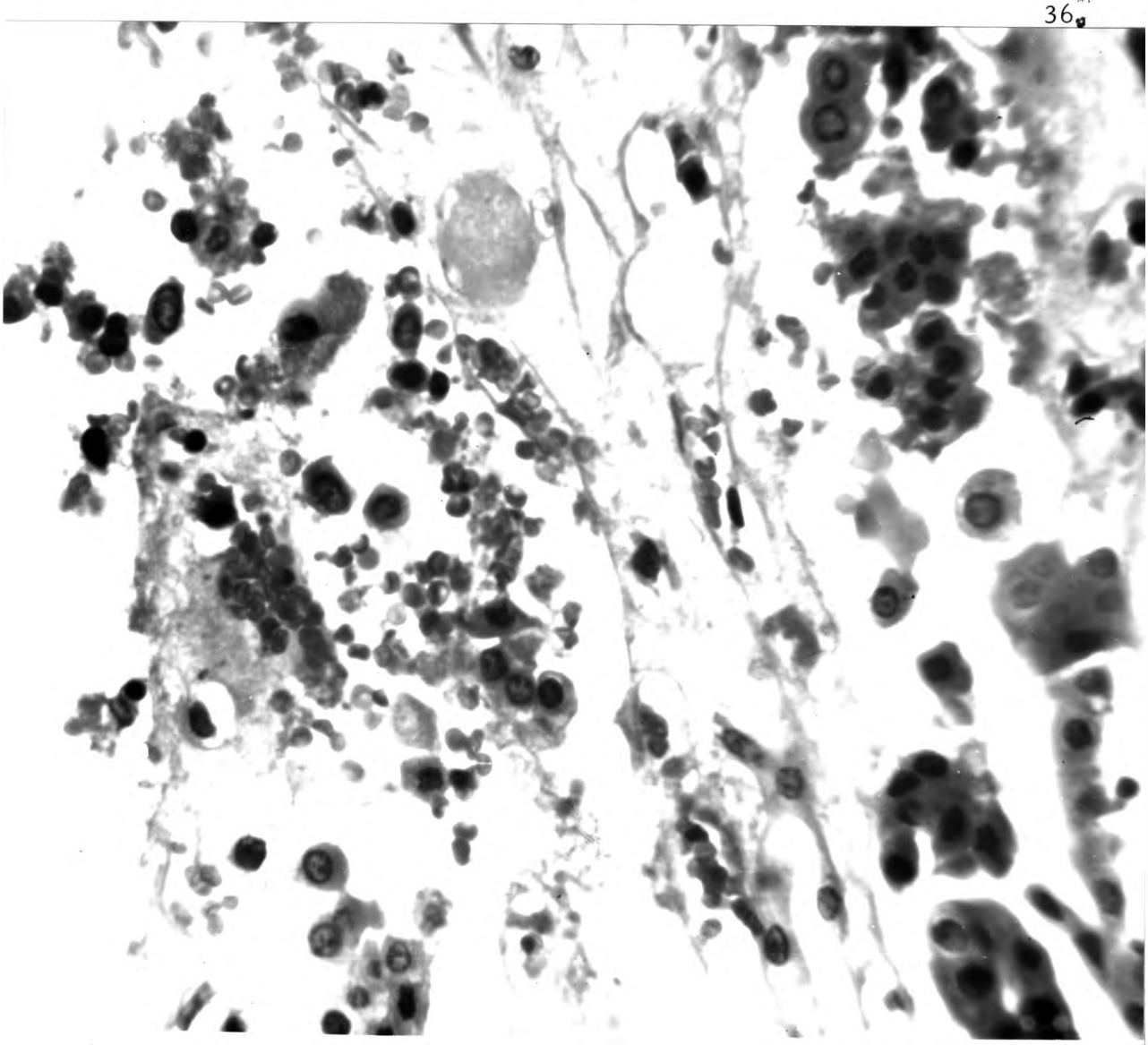

Fig. 20. High power view of a haemorrhage into the renal papilla breaking through into the pelvis in a rat fed A.P.C. tablets for 10 months.

H. and E. X 2,500

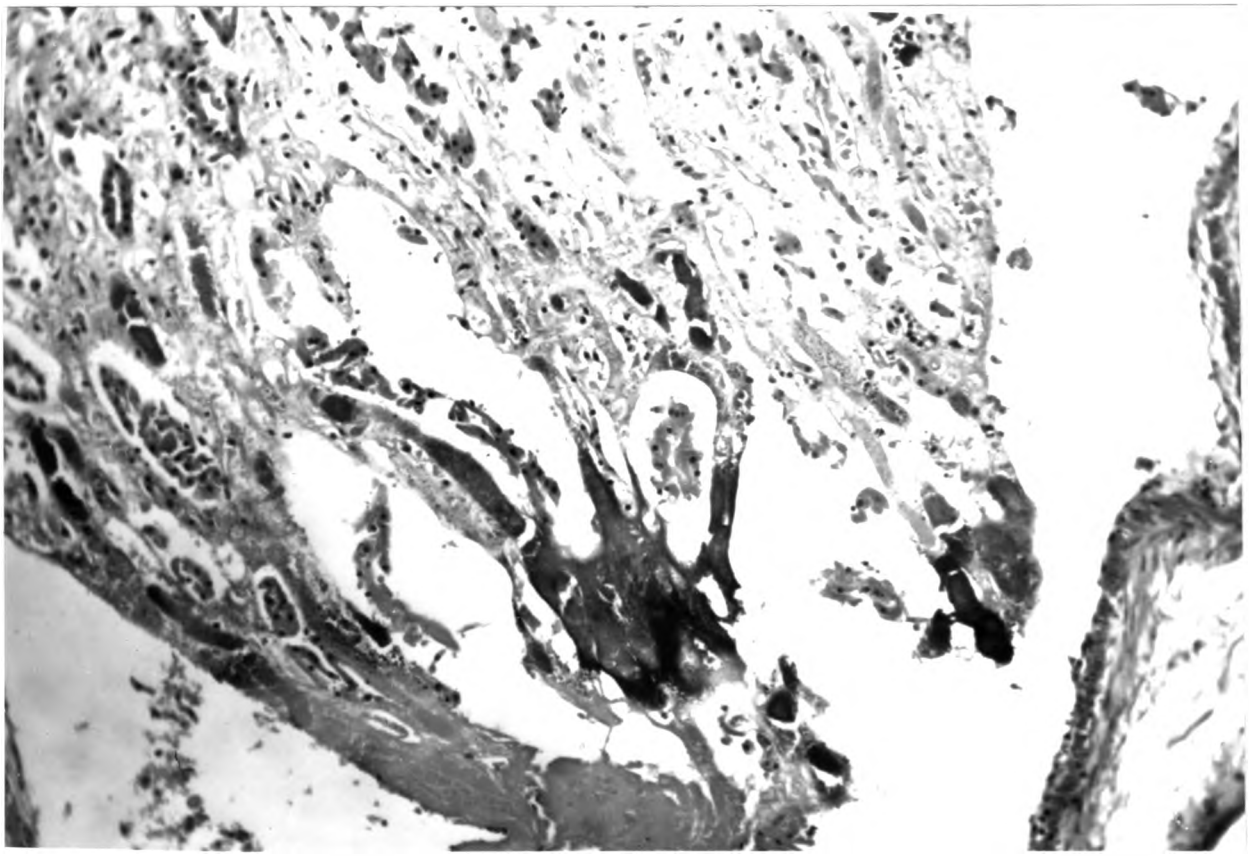

Fig. 21 Note the presence of a small focal area of necrosis of the tip of the renal papilla. This rat was fed A.P.C. tablets for 10 months.

H. and E. X 400

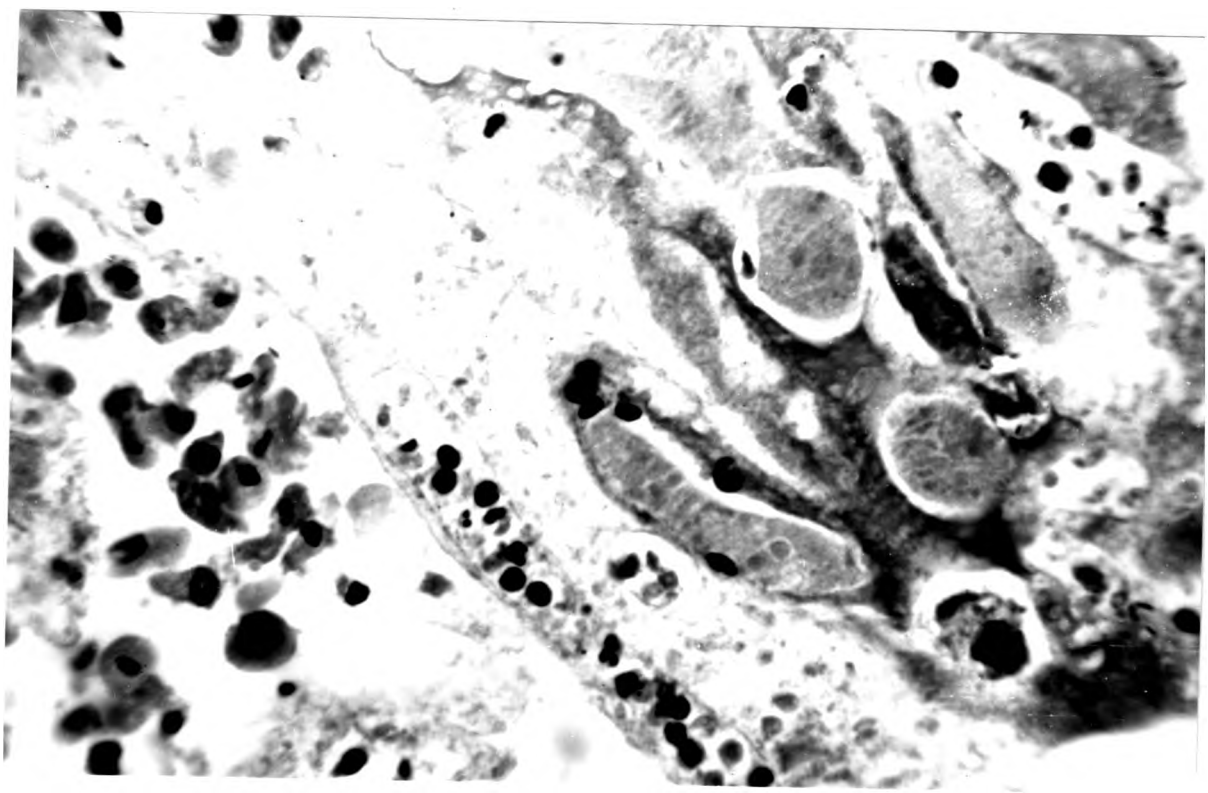

Fig. 22. High power view of a focal area of necrosis showing the vital reaction of polymorphonuclear leucocytes emigrating through the wall of a vessel.

H. and E. X 1600

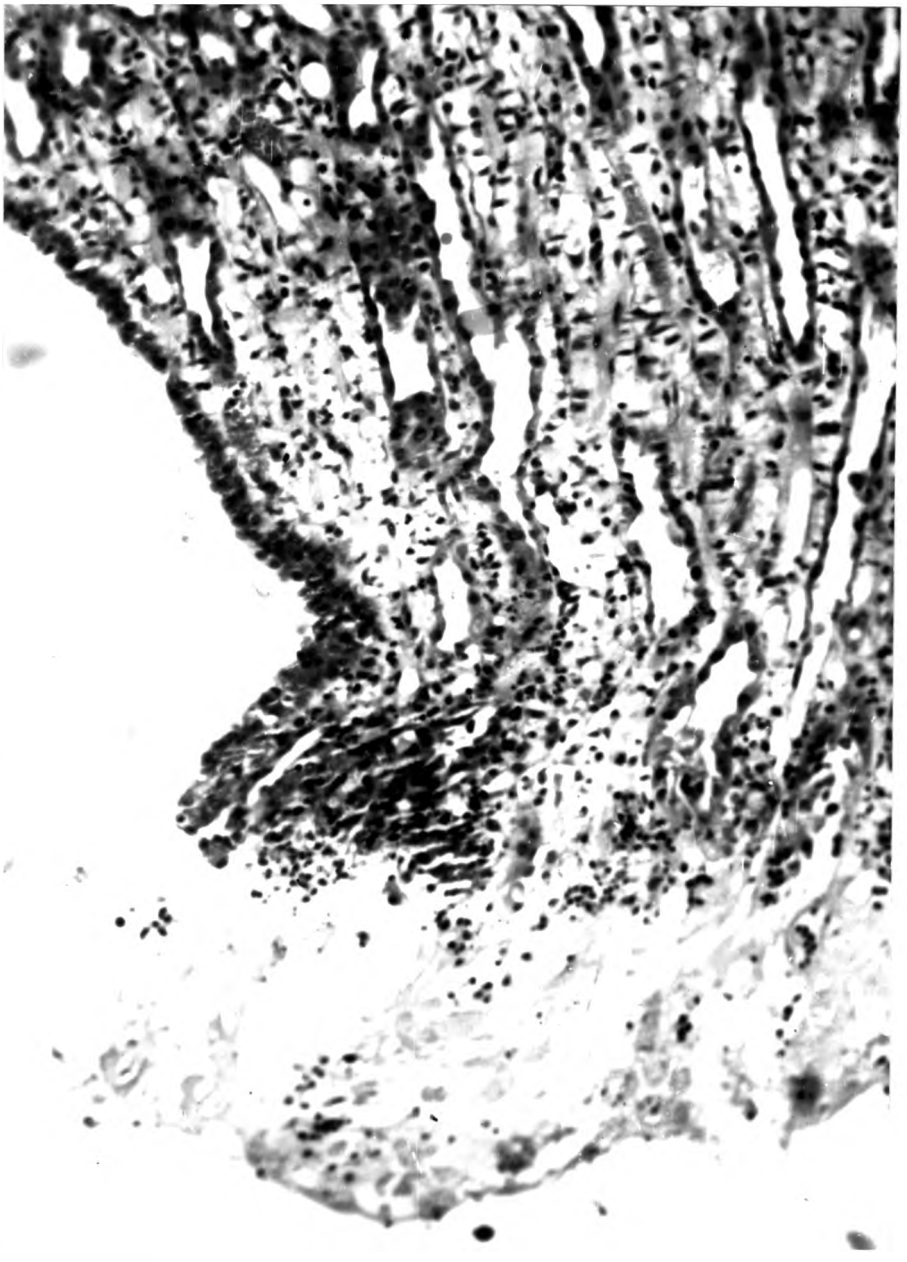

Fig. 23 Section of a renal papilla of a rat fed A.P.C. tablets for 8 months showing necrosis of the tip of the papilla. There is a well marked zone of demarcation consisting of polymorphonuclear leucocytes, nongranular cells and occasional gram negative bacilli between the viable and nonviable zones.

H. and E. X 500

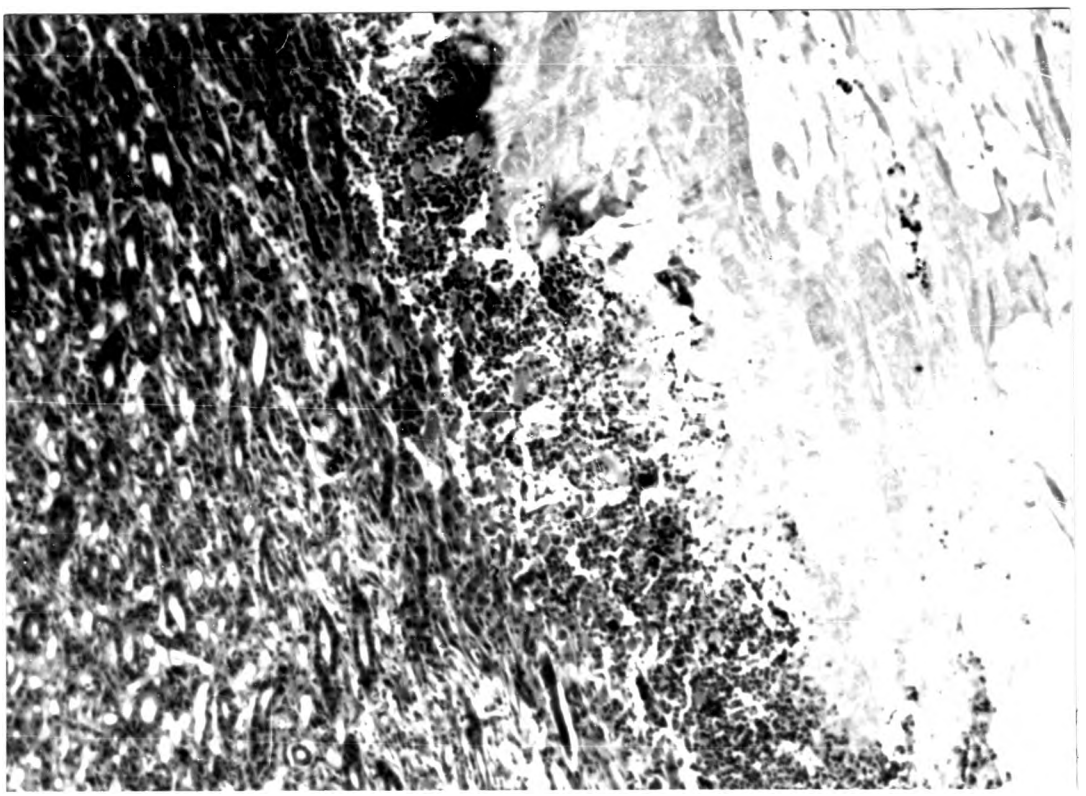

Fig. 24. Total papillary necrosis in a rat fed A.P.C. tablets for a period of one year. Note the presence of the large area of coagulative necrosis and the inflammatory cell infiltration in the zone of demarcation.

H. and E X 240

ing medullary tissue showed some increase in interstitial fibrosis and active hyperaemia of the blood vessels. There was a sharp separation of these necrotic papillae from the medulla and the cortex of the kidney, and the two latter areas showed only minimal cortical lesions and no acute inflammatory change.

The transitional epithelium covering the necrotic papilla in most of the cases had undergone necrosis (fig. 23)

b. Analysis of the results

An analysis of the lesions that occurred in the renal papilla can be seen in the histogram (fig. 25). The control animals showed normal renal papilla. Both the phenacetin and AP.C. fed rats showed haemorrhage and necrosis of the renal papilla. However, more frequent and severe changes were observed in the A.P.C. fed rats.

2. EXPERIMENT II

The method of assessment of the cortical and papillary lesions and the grading of the cortical lesions was the same as that described for Experiment I. In view of the smaller numbers of rats in the various groups in this experiment compared with Experiment I,

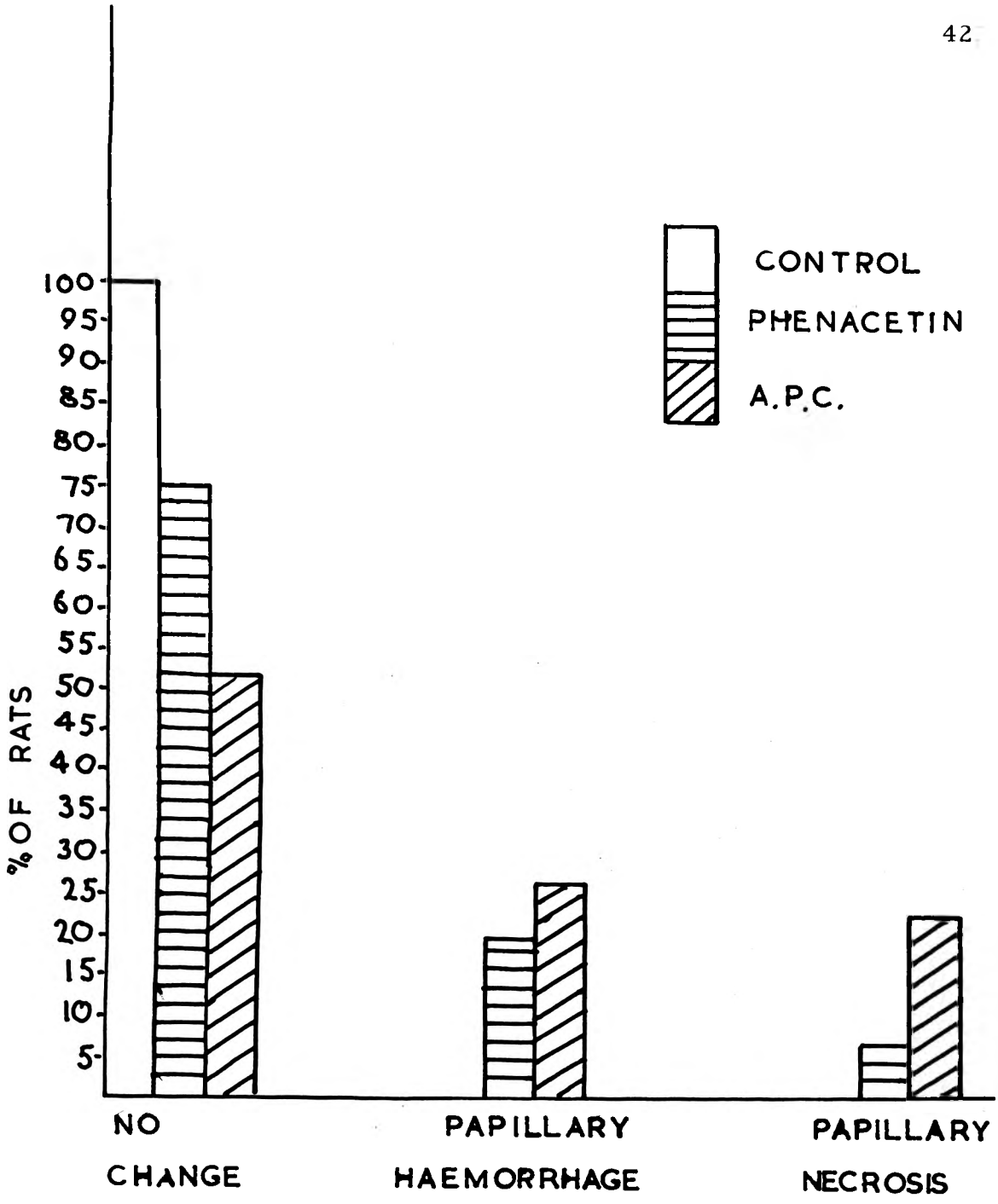

Fig. 25. Histogram showing the lesions occurring in the renal papilla in Experiment I.

all results have been tabulated, rather than expressed as percentages.

i. Analysis of Cortical Results

The results were rather similar to those in Experiment I. Once again phenacetin appeared to be associated with the development of severe grade cortical lesions (Table 1.). In this experiment phenacetin in combination with aspirin or in combination with caffeine has resulted in an increased severity of all four types of cortical changes. However, the aspirin caffeine group were associated with only minimal cortical changes, probably within the range of the spontaneously occurring cortical changes. The control rats for this experiment were those used in Experiment I.

ii. Analysis of Renal Papillary Lesions

Table 2 shows that renal papillary haemorrhage has occurred in one rat from each experimental group, on the other hand papillary necrosis was only present in a phenacetin/aspirin rat. This latter animal showed total necrosis of the renal papilla (fig. 26) surrounded by a zone of demarcation consisting of polymorphonuclear leucocytes and lymphocytes (fig. 27). Bacteria were not observed in this

TABLE I

Analysis of the renal cortical lesions occurring in Experiment II

Drug Fed	No. of Rats	Cortical Lesions															
		I.S. Fibrosis				Thickened Basement Membrane				Colloid Casts				Non-granular infiltrate			
		0	+	++	+++	0	+	++	+++	0	+	++	+++	0	+	++	+++
Phenacetin/Caffeine	11	7	1	1	2	2	4	2	3	3	4	2	2	6	2	2	1
Phenacetin/Aspirin	12	5	2	2	3	2	4	3	3	3	6	2	1	5	6	1	0
Aspirin/Caffeine	9	6	3	0	0	4	5	0	0	7	2	0	0	4	5	0	0
Control	22	15	6	1	0	8	11	3	0	9	12	1	0	11	10	1	0

TABLE 2

Analysis of the renal papillary lesions occurring in Experiment II

Drug Fed	Number of Rats	Papillary Lesions	
		Haemorrhage	Necrosis
Phenacetin/ caffeine	11	1	0
Phenacetin/ aspirin	12	1	1
Aspirin/ caffeine	9	1	0

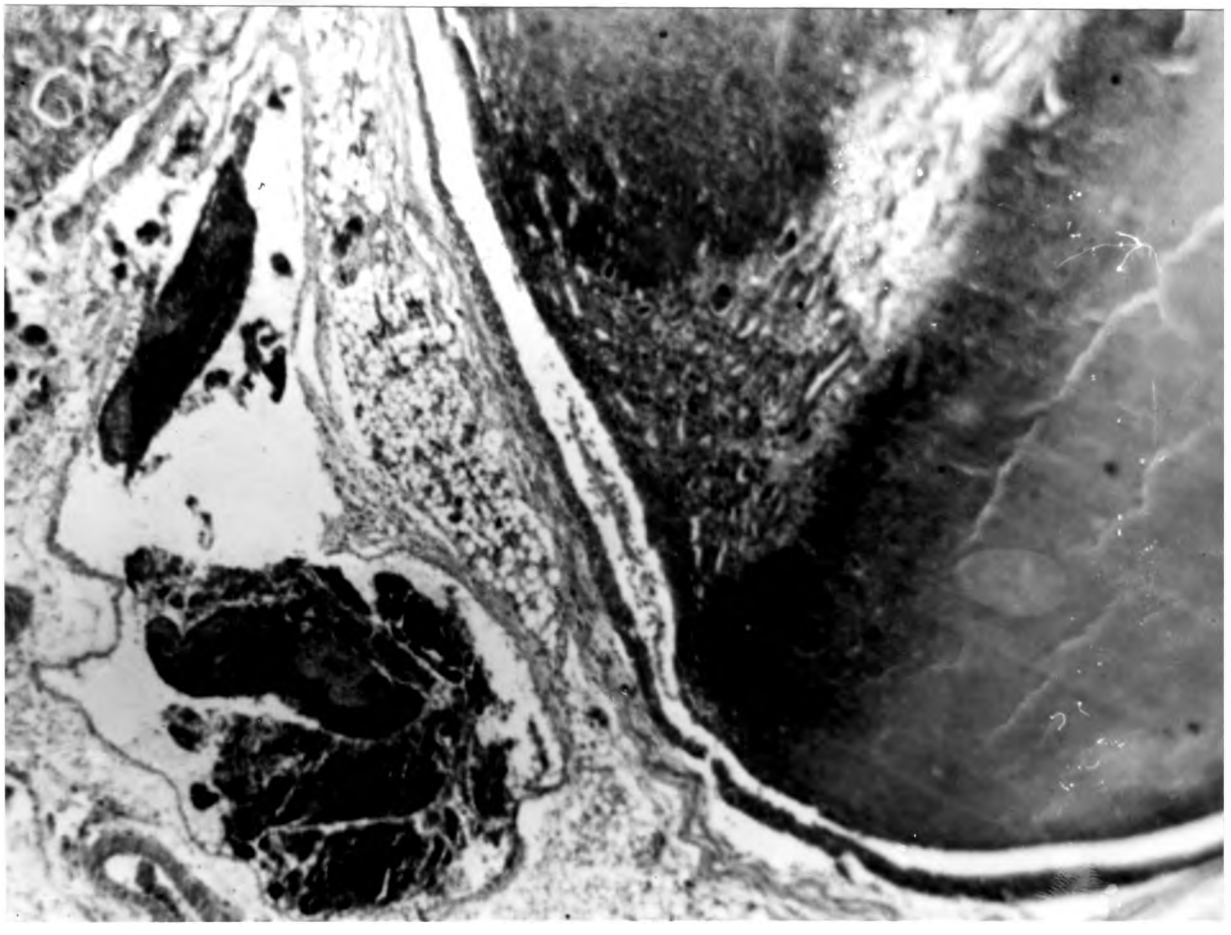

Fig. 26. Low power view of two large areas of infarct-like total papillary necrosis in a rat fed phenacetin/aspirin containing powders for 13 months. Note the viable tissue between the areas of coagulative necrosis. There is a thrombus in the renal vein.

H. and E. X 240.

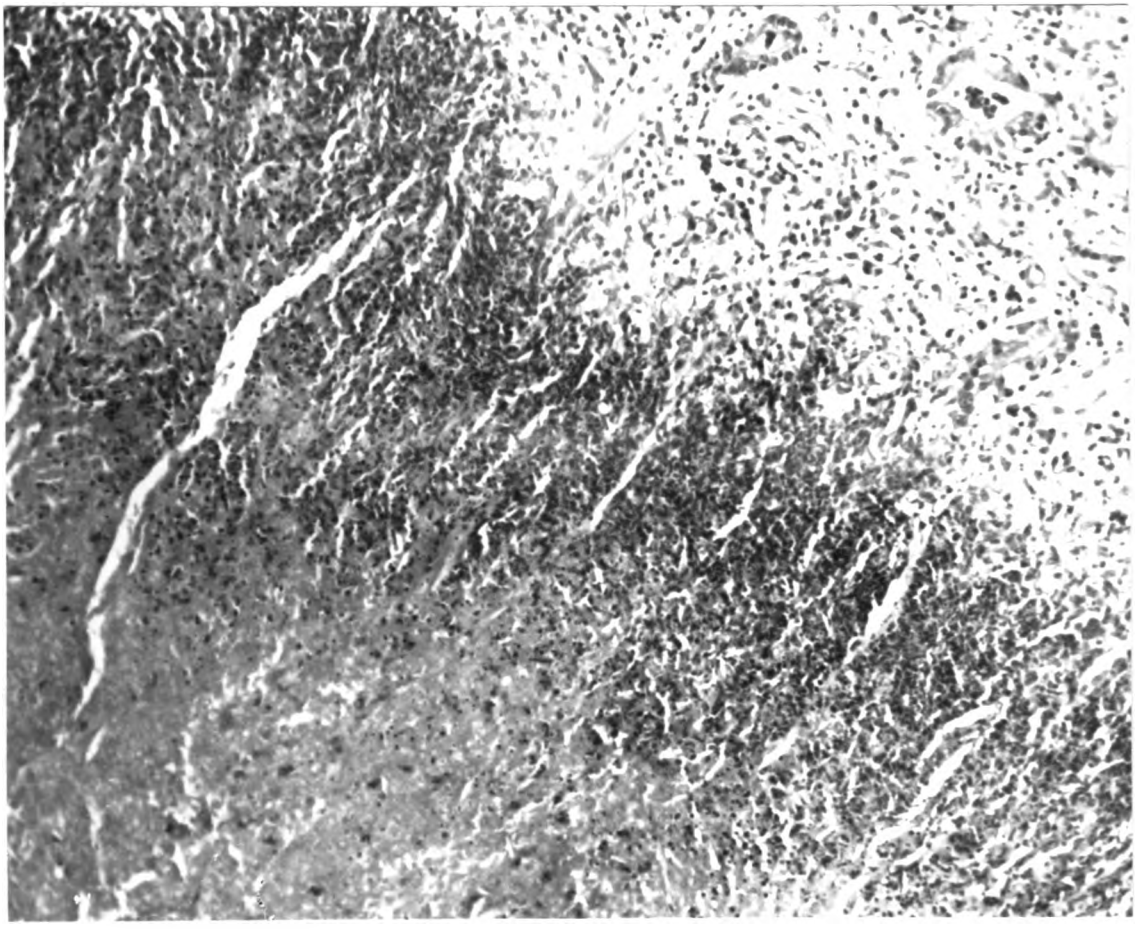

Fig. 27. Zone of demarcation surrounding renal papillary necrosis of rat in Fig. 26. Note the presence of polymorphonuclear leucocytes and nongranular cells.

H. and E. X 600

necrotic papilla. The area of necrosis was divided into two by a strip of viable tissue and the whole appearance resembled an infarct. There was a fairly recent fibrin thrombus with enmeshed red cells and polymorphonuclear leucocytes in the lumen of a large renal vein (Fig. 26). The intimal surface of this vessel appeared to be relatively intact. The features are consistent with an infarct of the renal papilla of this rat.

The results of the changes in the renal papilla of the rats in this Experiment II resemble those seen in Experiment I, but on a smaller scale. It would appear once again that the most nephrotoxic combination is that of phenacetin and aspirin.

CHAPTER IV

REVIEW OF THE METHODS AND THE RESULTS OF EXPERIMENTALLY INDUCED PHENACETIN NEPHRITIS AND COMPARISON WITH CHANGES OCCURRING IN THE PRESENT EXPERIMENTS

1. THE METHODS OF INDUCING LESIONS

i. Role of concommitant infection and spontaneously occurring renal lesions

A number of attempts have been made to reproduce experimentally the renal cortical and papillary lesions of human phenacetin nephritis. In most of these phenacetin and its breakdown products N-acetyl-para-aminophenol and p-phenetidin have been fed to animals in excessive quantities. The methods employed and the results obtained are tabulated in Table 3. It may be noted from this table that apart from the work of Eisalo and Talanti [46] feeding of these drugs alone failed to produce renal lesions. However, renal changes occurred when animals, first prepared with phenacetin, were given intravascular injections of E. coli or staphylococci. Angervall et al. [36,37] fed phenacetin and N.A.P.A. to albino rats for five months. After the animals had been inoculated intracardially with E. coli and following massage of the left kidney, they found that there was no significant difference between the severity of the renal lesions in the group with or without drug intake. However, the incidence of right kidney nephritis, calculated on the number of rats in each group with left kidney nephritis was significantly higher in the phenacetin and N.A.P.A.

TABLE 3

Analysis of the Experimental Attempts to Induce Phenacetin Nephritis

Name of Workers	Animals Used	Drugs Used	Duration of Experiment	Concomm. Infection	Results in Cortex	Results in Papilla
Studer, Zbinden [33] (1955)	Rats	Saridone	$6\frac{1}{2}$ mths	-	-	-
Tholen, Voegtli, Renschler, Schaeffer [50] (1956)	Mice	Saridone	$11\frac{1}{2}$ mths	possible subclinical K. muris	Interstitial nephritis	-
Pletscher, Studer, Miescher [34] (1958)	Rabbits Dogs	Acetophenetidine N.A.P.A., p. Phenetidine	22 - 40 wks	-	No interstitial nephritis	-
Studer, Zbinden, Scharer, Fust [57] (1958)	Rats	Saridone	20 -50 days	Staphylococcus haemolyticus Schoch I.V.I.	Interstitial nephritis in controls and experimental groups.	-
Studer, Zbinden, Fust [35] (1958)	Rats	Acetylsalicylic acid Saridone	5 weeks	Staphylococcus aureus haemolyticus Schoch I.V.I.	Interstitial nephritis	Abscesses in renal papillae in Saridone, Aspirin & controls
Miescher, Schnyder, Krech [37] (1958)	Rabbits	Saridone	7 mths	E. coli I.V.I.	Interstitial nephritis	-
Zollinger [1] (1959)	Rabbits	Saridone	1 year	-	-	-
Eisalo & Talanti [46] (1961)	Rats	Phenacetin N.A.P.A.	1 mth	No infection, but bacteriologic studies not done	Interstitial nephritis, proximal convoluted tubule degeneration	-
Keller, Gottier, Reubi [49] (1961)	Rabbits	Saridone	8 - 18 mths	E. Coli I.V.I.	Interstitial nephritis in controls and in experimental animals.	-
Angervall, Lehmann, Lincoln [36,47] (1962)	Rats	Phenacetin N.A.P.A.	5 mths	E. Coli intracardiac	Interstitial nephritis	-
Clausen [48] (1962)	Rabbits	Acetylsalicylic Phenacetin	11 mths	E. Coli I.V.	Interstitial nephritis	-

group than in the controls. Miescher et al.[8] succeeded in inducing interstitial nephritis in rabbits by combining phenacetin administration with intravenous injection of E. coli, whereas the injection of E. coli alone, did not affect the kidneys. The results of Miescher et al.[8] were reduplicated by Clausen[48]. On the other hand the injection of organisms by some workers led to no increase in interstitial nephritis. Keller et al.[49] could show no evidence of a harmful effect on the kidney, neither in animals given phenacetin alone for months on end, nor in those subsequently injected with E. coli. They found no significantly greater lesions in these groups than in control groups.

Keller et al.[49] have intimated that the presence of interstitial infiltrates in the renal cortex of animals occurs often, so that any such finding in an experiment specifically designed to induce interstitial nephritis is thwart with danger. Such findings must be assessed with the greatest reserve. Tholen et al.[50] feel that the most likely cause for such naturally occurring lesions is infective. They were of the opinion that mice used by them for phenacetin feeding experiments were prone to infection with K. muris. Clausen[48] too, suggests that the rat and mouse are animals susceptible to spontaneous urinary

infection, and Keller et al.[49] state that interstitial infiltrates are found in completely normal rabbits. Eisalo and Talanti[46] who produced renal disease in rats with phenacetin alone, state that it was not possible to show anything indicative of infection in their animals and as far as is known, no infection had occurred in their rat strain in the previous few years. But they admit that bacteriological cultures were not performed from their material and renal infection cannot be excluded.

The cause of the spontaneously occurring renal lesions in the rats in the present experiment is unknown. As discussed in Chapter III (page10) the lesions consisted of thickening of tubular basement membranes with tubular atrophy, nongranular cell infiltration into the interstitial tissue with mild increase in interstitial fibrosis and dilated tubules with colloid casts. These changes fulfil the histological criteria for the diagnosis of chronic pyelonephritis[51]. Yet despite the fact that histological sections of the kidneys were studied at different stages throughout the experiment, no evidence was forthcoming of any acute inflammatory change which may have proceeded to a chronic pyelonephritis. Further the lesions in the present experiment,

resemble those described by Saxton and Kimball [45] in the ageing rat (See Chapter III p. 31). Saxton and Kimball [45] feel that the lesion commences as cast formation in the collecting tubules and is then followed by cast formation in the medullary and cortical tubules and dilatation of the glomerular capsule. Atrophy of the tubular epithelium occurs in association with large casts, accompanied by increased fibrous tissue and slight lymphocytic infiltration about the involved tubules and glomeruli. They feel that because there is no inflammation, it is a nephrosis "any degeneration of the kidney without inflammation" [52] Occasionally, however, the vascular changes and cellular infiltrate may predominate, when the lesion can no longer be called a nephrosis. However, they do not draw much attention to the role of infection in the development of these lesions.

On the other hand Mallory et al. [53] state that the chronic stage of experimental healed pyelonephritis consists of tubules with colloid casts, cortical fibrosis with focal lymphocytic infiltration and disappearance of glomeruli and tubules, a description which is almost identical to findings in the above condition . Thus the puzzling situation arises in the kidney of the rat where two possible pathogenetic mechanisms seem to lead to similar histopathological pictures. This

picture very closely resembles that of human chronic pyelonephritis.

A similar confusing situation exists in the relationship between acute renal infection and the development of chronic pyelonephritis in man, a situation which prompted Kark [54] at a Symposium on the Biology of Pyelonephritis to say:-

> " One wonders how often cases of chronic pyelonephritis start off as a result of infection complicating a minor developmental disturbance in the kidney which smoulders to become chronic pyelonephritis. Today's discussion of animal experiments would lead one to believe that all cases of chronic pyelonephritis seem to start off on the basis of an acute disease. I wonder whether this is really true in the clinical situation"

At the same symposium in a summation Paul Beeson [55] stated -

> " a point which I am sure is going to come up over and over again in the next two and a half days, is whether all this talk about infection has anything really to do with chronic pyelonephritis"

He continues by saying -

> " There are impressive reasons for arguing that the entity known as acute pyelonephritis may not be the one that is a forerunner of the disease that we call chronic pyelonephritis".

Thus, at the present time, it is not possible to be sure what the exact cause is of the renal cortical lesions found in the

laboratory rats in the present experiment. The question arises as to whether the lesions, whatever causes them, are produced more severely, frequently and earlier by the possible damaging effects of phenacetin. As can be seen from the discussion in Chapter III the drawing of conclusions is difficult. The general impression, however, is that phenacetin and A.P.C. can result in a "naturally" occurring renal lesion being more severe and occurring earlier than in control animals.

This reinforcement of spontaneously occurring renal lesions by phenacetin has been demonstrated experimentally by giving phenacetin together with intravascular injections of organisms with or without renal massage to damage the kidneys [8,56,57]. Zollinger [1] feels that the experiments of phenacetin ingestion and intravenous innoculation of bacteria, do not solve the problem of phenacetin abuse and chronic interstitial nephritis. However, they do appear to show that the kidneys of animals fed phenacetin are more susceptible to kidney damaging agents.

ii. Comparison of drugs fed experimentally and the resultant cortical and papillary lesions.

Nearly all phenacetin entering the human organism is metabolised. Most of it is excreted into the urine as conjugated N.A.P.A. (Table 4) and a small proportion converted to p-phenetidin[58].

In view of all the early reports on "phenacetin nephritis" focussing all the attention on the importance of phenacetin in producing lesions, most of the experimental work has dealt with the feeding of this drug or its breakdown products of N.A.P.A. and p-phenetidin to animals. However, there has been some inconsistency in the duration of feeding and in the amounts of the drugs necessary to produce cortical and papillary lesions.

a. Cortical Lesions

There does not appear to be a strict correlation between the amount of phenacetin fed and the development of cortical lesions. Studer et al.[57] found that in rats total doses of approximately 1,500 mgms of phenacetin fed over a period of 3 to 7 weeks in the presence of intravenous injections of staphylococci could not produce significant lesions in their experimental groups. On the other hand in another almost identical experiment Studer et al.[35] produced interstitial nephritis on injection intravenously of staphylococci and with feeding

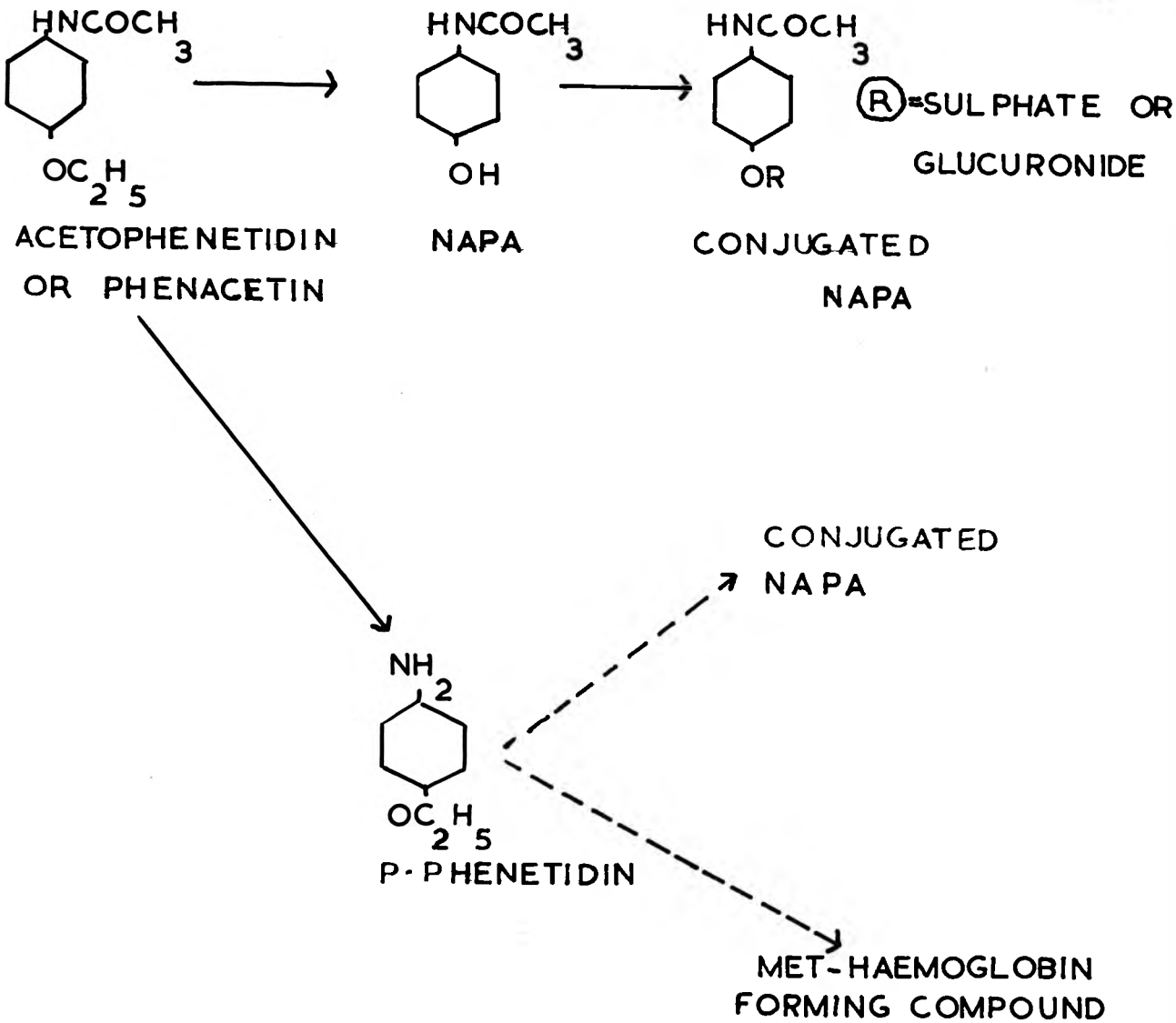

Table 4: Metabolism and breakdown of phenacetin

total doses of 1,300 mgms of phenacetin over a period of 5 weeks. High doses of phenacetin for longer periods were also given. Angervall et al.[36,47] fed rats phenacetin for 5 months. One week prior to termination of the experiment the animals were injected intravenously with E. coli. Their total intake of phenacetin was ± 11,300 mgms. Interstitial nephritis was produced. The scattergrams (Fig. 14 - 16) in Chapter III indicate that in the present experiment lesions of tubular atrophy with basement membrane thickening, interstitial fibrosis and dilated tubules with colloid casts and which were of +++ severity occurred in the renal cortex with doses of pure phenacetin varying between 5,400 mgms to 15,600 mgms. These doses of phenacetin had been fed for 6 months to 14 months respectively.

The feeding of phenacetin to rabbits also led to variable results. Doses of 180,000 mgms fed to rabbits in a year gave no kidney changes[1], whereas others using similar doses of 173,000 mgms of phenacetin fed over a period of 7 months produced interstitial nephritis[8]. However, the additional innoculation of E. Coli in the latter experiment was probably responsible for this difference. On the other hand Keller et

al. [49] fed total doses of 120,000 mgms to 250,000 mgms of phenacetin to rabbits over periods of 8 months to 18 months and produced no lesions. Acetylsalicylic acid was fed by a few workers. It was always fed alone and not in combination with phenacetin. Studer [35] found that rats prepared with acetylsalicylic acid and then injected with organisms developed interstitial nephritis, showing that aspirin without phenacetin can damage the kidney. The dose of aspirin given to each rat was 2,625 mgms over a period of 5 weeks. In the present experiment aspirin was not fed alone but rather in combinations with phenacetin and caffeine. In both Experiment I where aspirin was fed together with phenacetin and caffeine as A.P.C. tablets and in Experiment II where it was combined with phenacetin as aspirin/phenacetin +++ severe cortical lesions were produced (figs. 14-17 andTable 1). The dose of aspirin fed to each rat was approximately 9,000 mgms in one year. However, when aspirin was combined with caffeine as aspirin/caffeine and 7,500 mgms of aspirin was fed in 10 months, no +++ severe lesions were produced (Table 1). It would appear therefore that aspirin must either be combined with phenacetin or be given simultaneously with innoculation of organisms to produce cortical lesions.

Thus it would appear that in view of the variability of the dose of the drugs causing the lesions that there is an individual susceptibility to the condition of phenacetin nephritis. In addition the combination of phenacetin with aspirin appears to have the most nephrotoxic effect.

b. Papillary Lesions

This variation in the amount of phenacetin necessary to produce cortical lesions can also be observed in the amounts which cause renal papillary damage. As can be seen in Table 3, the production of papillary lesions has met with little success by other workers. Apart from Studer et al.[35] who injected staphylococci into rats prepared with saridone and acetylsalicylic acid and found abscesses in the renal papillae of these two groups and in the controls, no other workers describe papillary necrosis.

In the present experiment, necrosis and haemorrhage of the renal papilla of the experimental groups was found as early as 7 3/4 months of feeding of A.P.C. tablets. At this stage each rat had consumed 3,900 mgms of phenacetin (Fig. 28). The papillary lesions were found to occur throughout the duration of the experiment, up to the 14th month of feeding with A.P.C., when 7,000 mgms of phenacetin had been ingested. As

	HAEMORRHAGE	NECROSIS
PHENACETIN	△	▲
A.P.C.	○	⌀

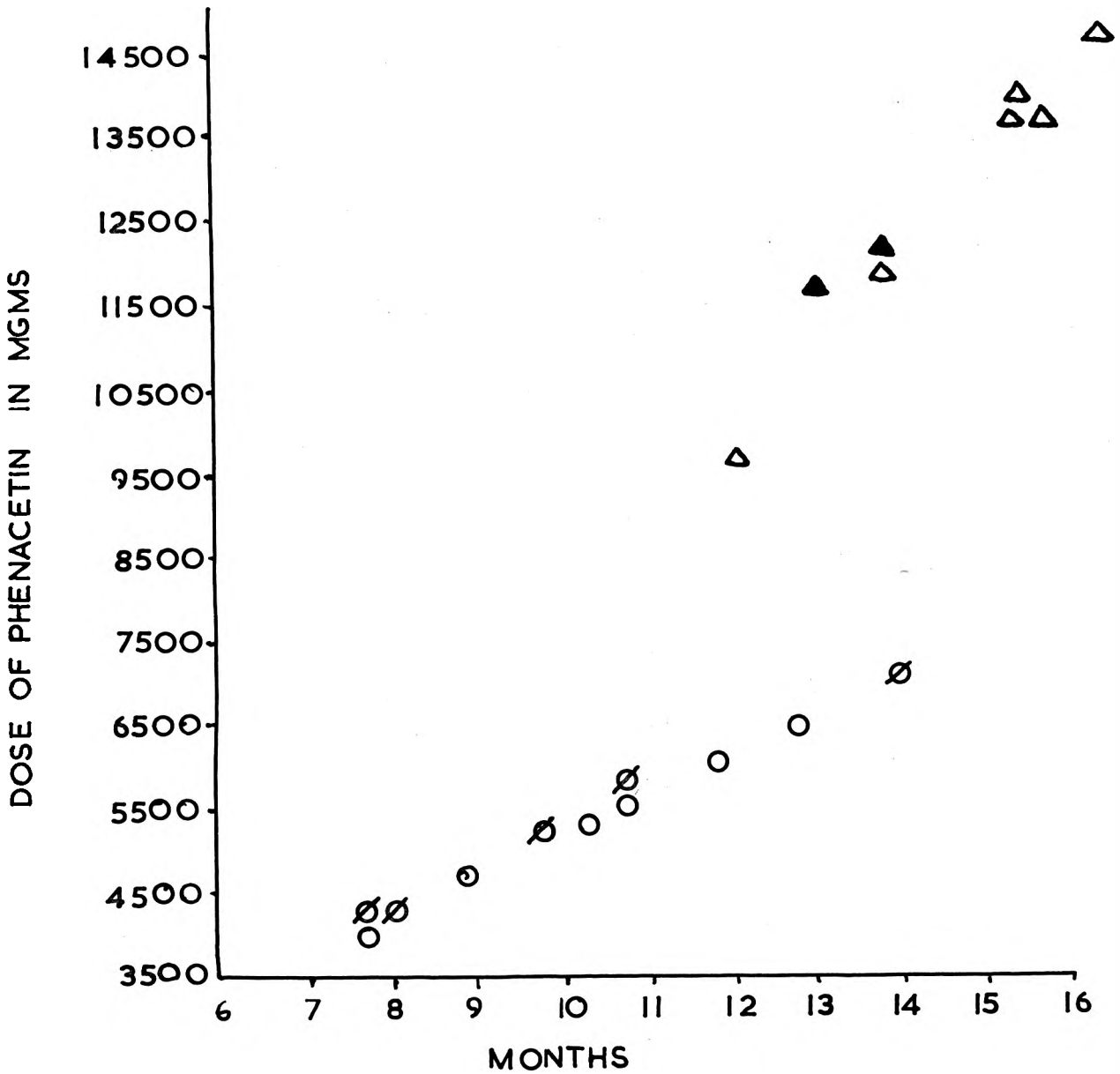

Fig. 28. Time relationship between the development of renal papillary lesions and the dose of phenacetin ingested.

opposed to the early onset of papillary lesion in the A.P.C. group, in the pure phenacetin fed animals the papillary lesions commenced at 11 months when 9,900 mgms of phenacetin had been fed and extended to 16 months at which time the rats had ingested 14,900 mgms of phenacetin (fig. 28). These findings would tend to show that the amount of phenacetin fed is not all important. Despite the greater doses of phenacetin consumed by the pure phenacetin group, the renal papillary lesions occurred with greater severity and increased frequency and with less phenacetin intake in the A.P.C. animals. This suggests that the combination of phenacetin/aspirin is most significant in being responsible for the papillary damage.

These observations are borne out further by the results in Experiment II, where combinations of phenacetin/caffeine, phenacetin/aspirin and aspirin/caffeine were fed. As can be seen from Table 2 papillary lesions occur with slightly greater frequency and severity in the phenacetin/aspirin group of rats. The rat with papillary necrosis died at 13 months having ingested approximately 6,600 mgms of phenacetin, that with papillary haemorrhage at $13\frac{1}{2}$ months having ingested 7,000 mgms of phenacetin. The phenacetin/caffeine animal with

papillary haemorrhage was killed at 14 months and had consumed 12,600 mgms of phenacetin.

The reasons for the failure of other workers to produce papillary lesions in rats, may be that sufficiently high doses of phenacetin have not been fed or that the all important combination of phenacetin and aspirin has not been used. Papillary haemorrhage and necrosis may be produced by feeding smaller doses of phenacetin when the latter drug is combined with aspirin.

2. COMPARISON WITH THE HISTOLOGICAL FINDINGS INDUCED BY OTHER WORKERS

i. Cortical Changes

Most workers have found that after feeding phenacetin the commonest change in the kidney is an interstitial nephritis. This consisted predominantely of nongranular cells. Angervall et al.[36,37] observed focal lesions occurring in the cortex, subcapsularly, at the cortico-medullary border or in the medulla. They consisted of gross inflammatory infiltrates of nongranular cells and also large numbers of polymorphonuclear leucocytes. The cortical lesions of some rats were fibrotic. Dilated and atrophic tubules contained only leucocytes whilst

other tubules also contained a fluid rich in proteins. Eisalo and Talanti [46] produced a subcortical, intensive tubular degeneration and an inflammatory infiltration into the interstitial connective tissue. The tubular damage was predominantly in the proximal tubules, most of which were completely destroyed in these areas, whereas the distal tubules appeared perfectly normal microscopically. The inflammatory cell infiltrate was relatively diffuse but a certain tendency to form radiating or spherical infiltrates was nevertheless observable. The majority of the inflammatory cells were lymphocytes, in addition to which there were abundant mature plasma cells and some large monocytes. Granulocytes were not present. There was no older fibrosis.

Of the few cortical changes described as occurring in the present experiment, the one produced to the least extent was that of nongranular cell infiltration. The changes of thickening of basement membrane with tubular atrophy, interstitial fibrosis and dilated tubules with colloid casts may be seen to occur more frequently than nongranular cell infiltration in the ++, +++ grade of severity (figs. 14 - 17). The reason for the difference between this experiment and that of others is probably related to the innoculation of organisms which was not done in

the present experiment.

ii. Papillary Lesions

Papillary changes have not been produced as frequently as cortical changes in phenacetin feeding experiments. Apart from Studer et al.[35] most workers have reported normal renal papillae. Angervall et al.[36] reported that in two phenacetin fed rats profuse calcified deposits occurred along the cortico-medullary border. It is interesting that one rat fed A.P.C. tablets for 7 months in the present experiment, showed foci of calcification in the renal papilla.

Studer et al.[35] observed that when rats prepared with saridone and acetylsalicylic acid were given intravenous innoculation of staphylococcus aureus haemolyticus Schoch, some of the rats died during the experiment. These animals showed vast kidney damage and foci of tissue necrosis particularly at the border of cortex and medulla and at the papilla. There were, in addition, dense infiltrates of polymorphonuclear leucocytes. The description of the histological findings of these workers seems to resemble that of abscess formation. As compared with this, the features of papillary necrosis in the present experiment were not associated with abundant polymorphonuclear

leucocyte infiltration, but appeared rather as lesions resembling infarcts.

It is interesting that in the experiment of Studer et al.[35] three out of twenty control rats receiving only the injection of staphylococci died. The papillary necrotic lesions were observed in these animals, as well as in three out of twenty phenacetin fed rats and thirteen of twenty acetylsalicylic acid fed rats. These results show that the production of papillary necrosis was induced mainly by inoculation of the organisms. This is different to the present experiment where necrosis occurred without inoculation of organisms. There is, however, a similarity between the present experiments and the work of Studer et al.[35] and that is that the presence of aspirin does seem to potentiate papillary damage as was observed in their experiment and in the finding in the present experiment of marked papillary damage in the presence of the combination of phenacetin and aspirin.

CHAPTER V

COMPARISON BETWEEN THE PATHOLOGICAL CHANGES IN HUMAN AND EXPERIMENTALLY INDUCED PHENACETIN NEPHRITIS

Thus far the discussion has centered around the possibility that phenacetin can weaken the defences of the kidney making it more susceptible to a spontaneously occurring lesion. Whether this lesion is inflammatory or whether obstructive is not clear. The question arises as to whether phenacetin can cause a specific lesion without necessarily incurring the complication of some spontaneously occurring disease. In man there is much argument as to whether chronic interstitial nephritis induced by phenacetin is a definite lesion,[7,8,9,10] or whether it is part of a chronic pyelonephritis [29,30,31,32].

1. The pathology of human phenacetin nephritis.

The following is the pathology of 8 cases of phenacetin nephritis studied personally. The ages of the patients, their sex, the amount of phenacetin ingested and period of consumption have been tabulated in Table 5.

Two of the patients were examined at autopsy, in the remaining 6 cases the material was obtained by renal biopsy. Both the autopsy and renal biopsy material was fixed in 10% formol saline and sections cut and stained with haematoxylin and eosin. In addition pigment present in this material was investigated as outlined in Chapter II page 8 . The biopsy material of Case 5 was examined electron-

TABLE 5

Comparison of Human Cases of Phenacetin Nephritis

Case No.	Age in Years	Sex	Amount of Phenacetin Ingested in Kg.	Period of Consumption in Years
1.	39	Male	7	$3\frac{1}{2}$
2.	46	Male	10 - 14	6
3.	68	Male	10	3
4.	30	Male	5	4
5.	54	Female	15 - 20	7
6.	61	Male	13	20
7.	50	Female	8	10
8.	32	Female	7	5

microscopically in addition. The method employed was the same as that described in Chapter II page 9.

Case 1.

In this renal biopsy the glomeruli showed minor changes. There was glomerular tamponade, an increase of P.A.S. positive fluid in Bowman's space, and foci of golden brown pigment in the epithelium of Bowman's capsule and in the glomerular capillaries.

The epithelial cells of the proximal convoluted tubules showed marked changes. There was eosinophilic degeneration of the tubular epithelium, the cells being swollen and granular and their free margins irregular. Frank necrosis of some proximal tubular epithelial cells was observed with evidence of nuclear pyknosis, karyorrhexis and karyolysis. Desquamated tubular epithelial cells were seen lying at lower levels of the nephron, in the loops of Henle and distal convoluted tubules. Occasional groups of cells in the descending loops of Henle showed large vacuoles in the cytoplasm.

The architecture of the tubular system was grossly disorganised by an increase in the interstitial tissue. This consisted mainly of an increase in fibrous tissue with very occasional nongranular cells.

All segments of the tubular part of the nephron showed marked thickening and hyalinisation of the basement membrane, which consisted of collagen as demonstrated by the Mason's stain. The thickened membrane was associated with atrophy of the tubular epithelium. There was also an increase in golden brown granules of pigment in these tubular epithelial cells. The pigment showed negative results for iron, haemoglobin and bile. It stained blue-green with the reduced ferricyanide method, and had a yellow-orange autofluorescence. It was negative to Sudan Black B and feebly P.A.S. positive. These findings indicated a highly oxidised lipofuscin. The blood vessels throughout the biopsy were normal histologically.

Case 2.

In this renal biopsy the glomeruli appeared relatively normal although there was some periglomerular fibrosis present. The tubules were diminished in number and were architecturally disorganised being separated from one another by an increase in interstitial tissue (fig. 29). There was marked hyaline thickening of the basement membranes of the tubules (fig. 30). This change was more marked in the distal tubules where the epithelial cells were atrophied and contained a golden brown pigment in the cytoplasm which was similar in type to

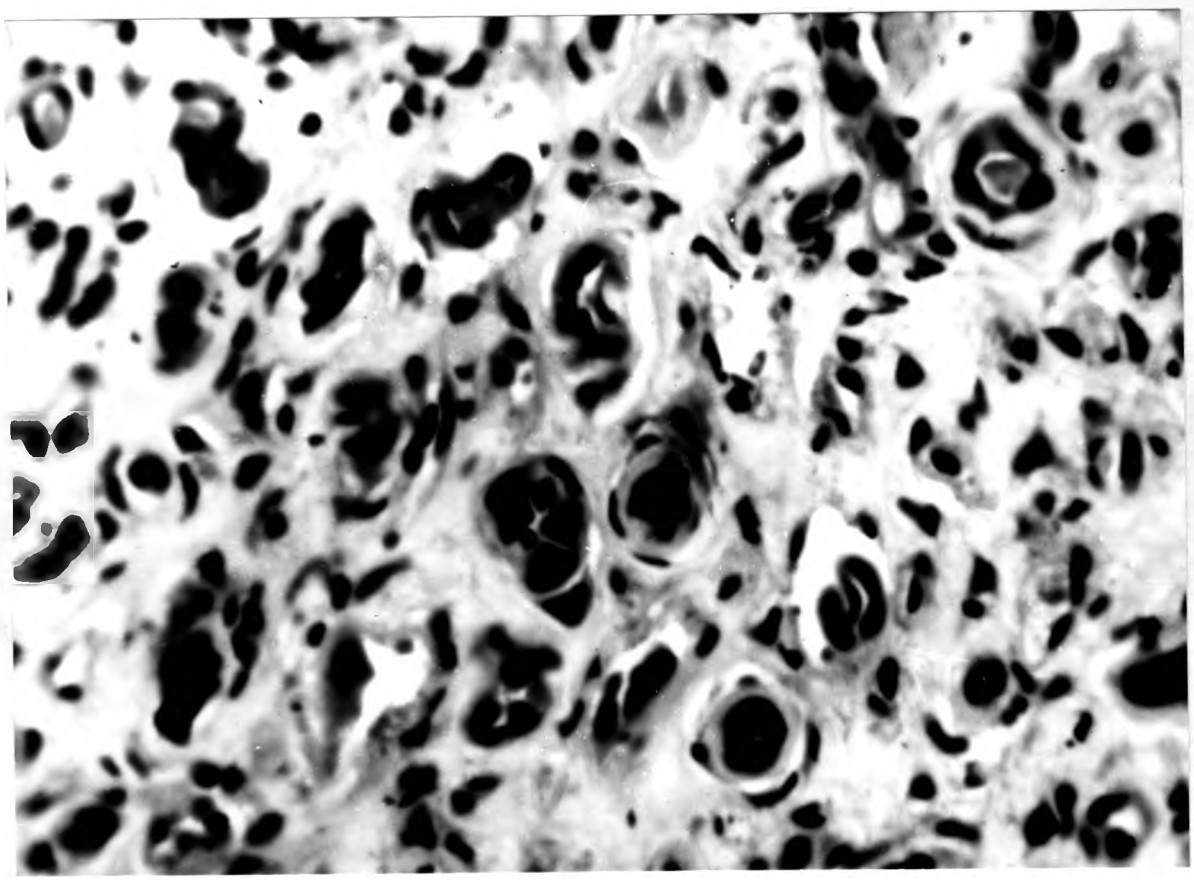

Fig. 29 (Case 2) Section through the renal cortex showing marked interstitial fibrosis and separation of the tubules. Only minimal nongranular cell infiltration is present.

H. and E X 600

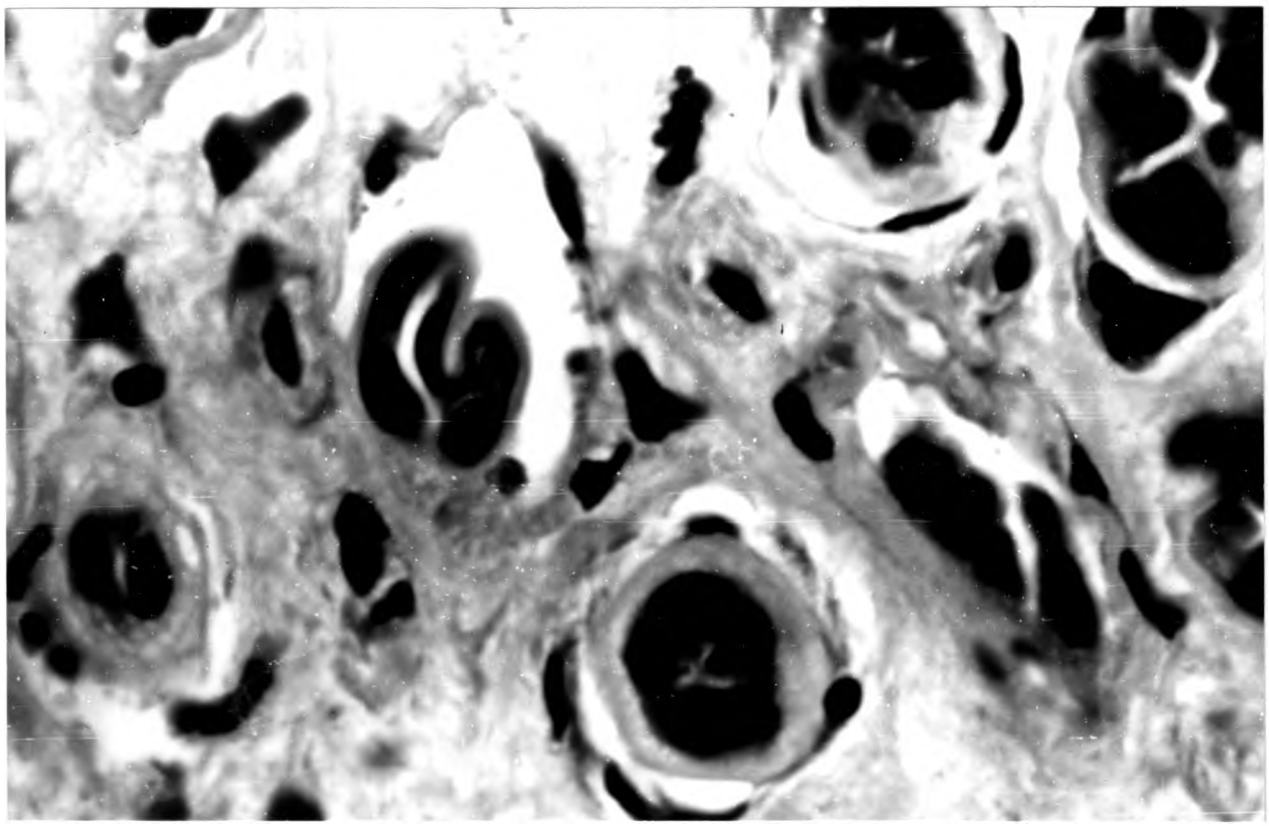

Fig. 30 (Case 2). Section through the cortex of the kidney. There is marked thickening of basement membranes of distal convoluted tubules with atrophy of tubular epithelial cells. Note also the increase in interstitial fibrous tissue.

H. and E X 3,600

that described in Case 1. Small foci of tubular regeneration were present. Most of the tubules showed a marked reduction of their lumina, but a few were dilated and contained colloid casts. The increase in the interstitial tissue was mainly due to fibrous tissue, however, there was one small focus of nongranular cells present. The vessels were normal.

Case 3.

In this renal biopsy there was marked thickening of the basement membrane of both the proximal and distal tubules together with an increase in interstitial fibrous tissue. This was accompanied by atrophy of the tubular epithelial cells. Golden brown pigment was again present in some of the cells. The vessels showed marked aterio-sclerotic changes. The renal papilla present had undergone necrosis and was associated with a nongranular cell infiltrate.

Progress: Despite antibiotic therapy this patient did not improve, the blood urea rose to 80 mgms percent and the patient died of bronchopneumonia.

Autopsy Findings: The significant findings were confined to kidney and liver.

Macroscopical Findings: The kidneys were slightly diminished in size, the left weighing 140 grams and the right weighing 120 grams. The capsules of each kidney stripped with difficulty and the surfaces had slightly irregular contours and were pale. The cortex of both kidneys was reduced in thickness. On section the palor of the cortices contrasted with the congested medulla. The renal papillae of both kidneys showed marked necrosis and ulceration into the renal pelves (fig. 31). The pelvis was slightly dilated and congested. The two ureters contained necrotic material which had sloughed off from the necrosed papillae.

Histological Findings: Sections were prepared from both the right and left kidneys and were stained as described in Case 1. The majority of the glomeruli were normal but occasional ones were hyalinised and a few showed dilatation of Bowman's space with increased fluid. Although numerous glomeruli were examined, only one showed periglomerular fibrosis. There was marked arteriosclerotic changes of the interlobular and intralobular vessels with proliferation and hyalinisation of the intima and media. An occasional afferent arteriole showed arteriosclerotic changes and was hyalinised. The epithelial cells of proximal convoluted tubules were swollen and vacuolated and in areas appeared to be desquamating into the lumen. The epithelial cells of the loops of Henle and distal convoluted tubules were also swollen. In other areas there was a

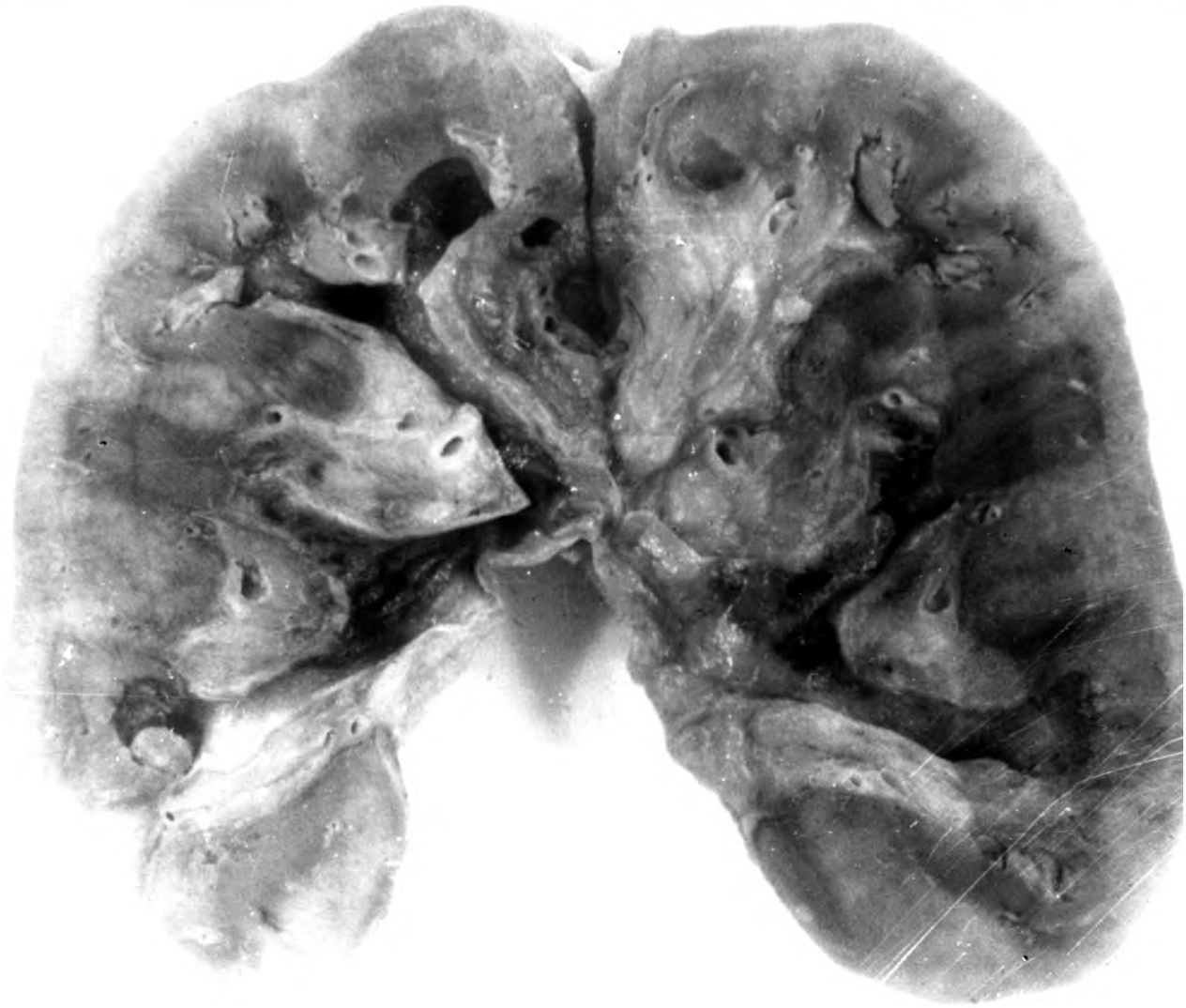

Fig. 31.(Case 3). Gross view of the necrotic papillae.

disarrangement of the normal tubular pattern with an increase in interstitial fibrous tissue and focal non-granular cell infiltrate. In these areas the tubular basement membrane was markedly thickened and hyalinised and associated with this the tubular epithelium was atrophic. Golden brown pigment was seen in the cytoplasm of the tubular cells. Colloid casts were present in only an occasional tubule. The collecting tubules towards the pelvis contained a basophilic granular debris which consisted of degenerate polymorphonuclear leucocytes. The papillae were necrotic and surrounding the necrosed tubules was a nongranular and polymorphonuclear leucocyte infiltration (fig. 32). The pigment was shown to be a highly oxidised lipofuscin, as confirmed by the positive ferricyanide reduction test, while all other pigment stains were negative.

Sections of the liver showed a marked increase of a granular golden brown pigment within the parenchymal cells. The pigment was similar in all respects to that described in the kidney.

Case 4.

In this renal biopsy the glomeruli varied markedly, some appearing histologically normal, while others were totally hyalinised. There was mild periglomerular fibrosis. The architecture of the

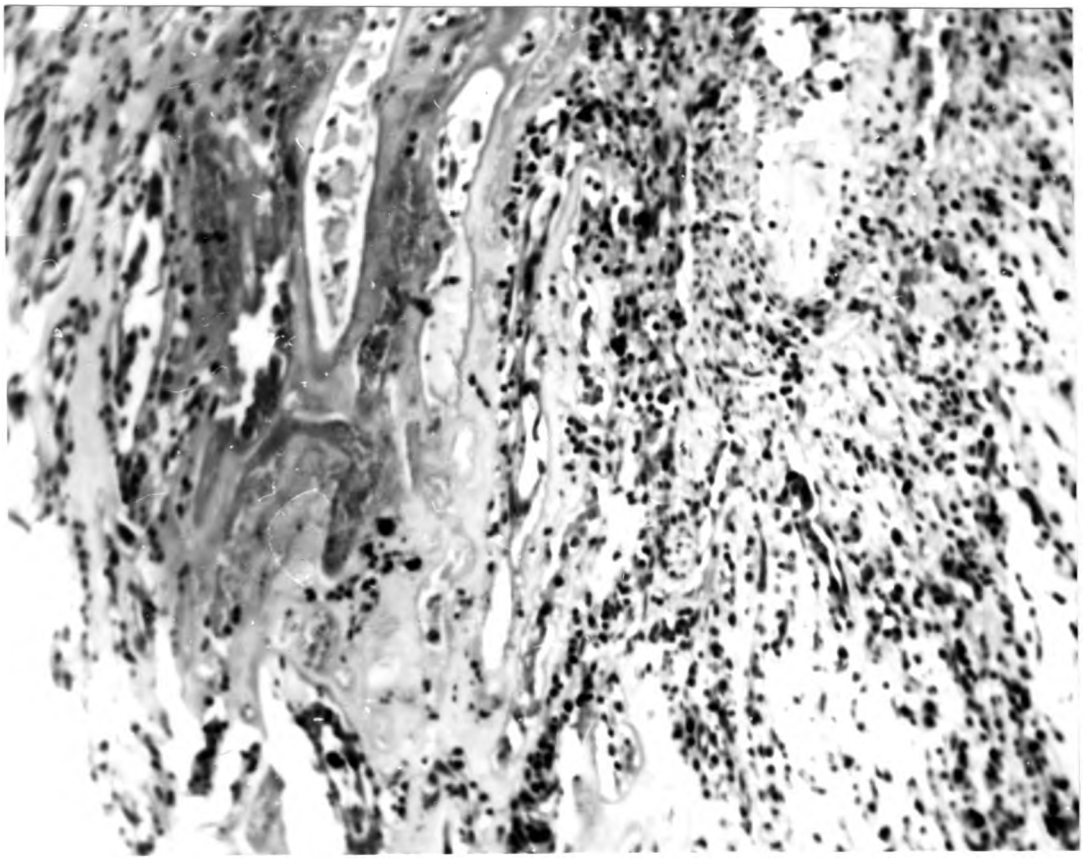

Fig. 32(Case 3). Section through the renal papilla showing total necrosis with surrounding inflammatory cell infiltrate. There is an increase in interstitial fibrous tissue.

H. and E X 400

tubular system was markedly disorganised, by an increase in interstitial tissue consisting mainly of fibrous tissue devoid of nongranular cell infiltrate. The tubules showed marked hyaline thickening of the basement membrane. This was associated with atrophy of the tubular epithelial cells and with reduction in size of the lumen of the tubules. Some of these tubular epithelial cells contained a golden-brown pigment which on identification showed similar characteristics to those described in the previous cases. Many proximal convoluted tubules had vacuolated cytoplasm and desquamation of the lining cells into the lumen of the tubule had occurred. There was an area of necrotising papillitis present. The arterioles showed hyalinisation and an occasional artery was arteriosclerotic.

Liver Biopsy: There was a diffuse finely granular golden brown pigment present within the hepatic parenchymal cells. This pigment was a highly oxidised lipofuscin similar in all respects to that seen scattered in the renal tubule epithelial cells.

Case 5.

In this renal biopsy the majority of the glomeruli seen were histologically normal. One glomerulus showed marked periglomerular fibrosis and another was markedly hyalinised. The epithelium of the

proximal tubules were vacuolated and the luminal margin poorly preserved. The basement membrane of these tubules was markedly thickened and hyalinised and in relation to this change the tubular epithelium was atrophic (fig. 33). The epithelium contained a granular reddish brown pigment which again proved to be a highly oxidised lipofuscin. The distal convoluted tubules showed similar changes of atrophy, basement membrane thickening and pigmentation (fig. 34). Only occasional distal convoluted tubules contained colloid casts. The tubular architecture was disorganised by an increase in the interstitial tissue, which consisted of fibrous tissue. There was no nongranular cell infiltrate and the vessels throughout were normal.

Electron Microscopy: The glomeruli studied showed the presence of normal basement membranes, podocytes and endothelial cells. The main changes were seen in the tubules and in particular in the distal convoluted tubules. The basement membranes were markedly thickened measuring 0.5 to 2.5 u. In parts the thickened membrane was homogenous while in others it was split (fig. 35). Between the individual fibres of this split membrane there were granules measuring 500° A. in diameter (fig. 36). Some granules were present in the interstitial tissue. The osmiophilic plasma membrane of the distal convoluted

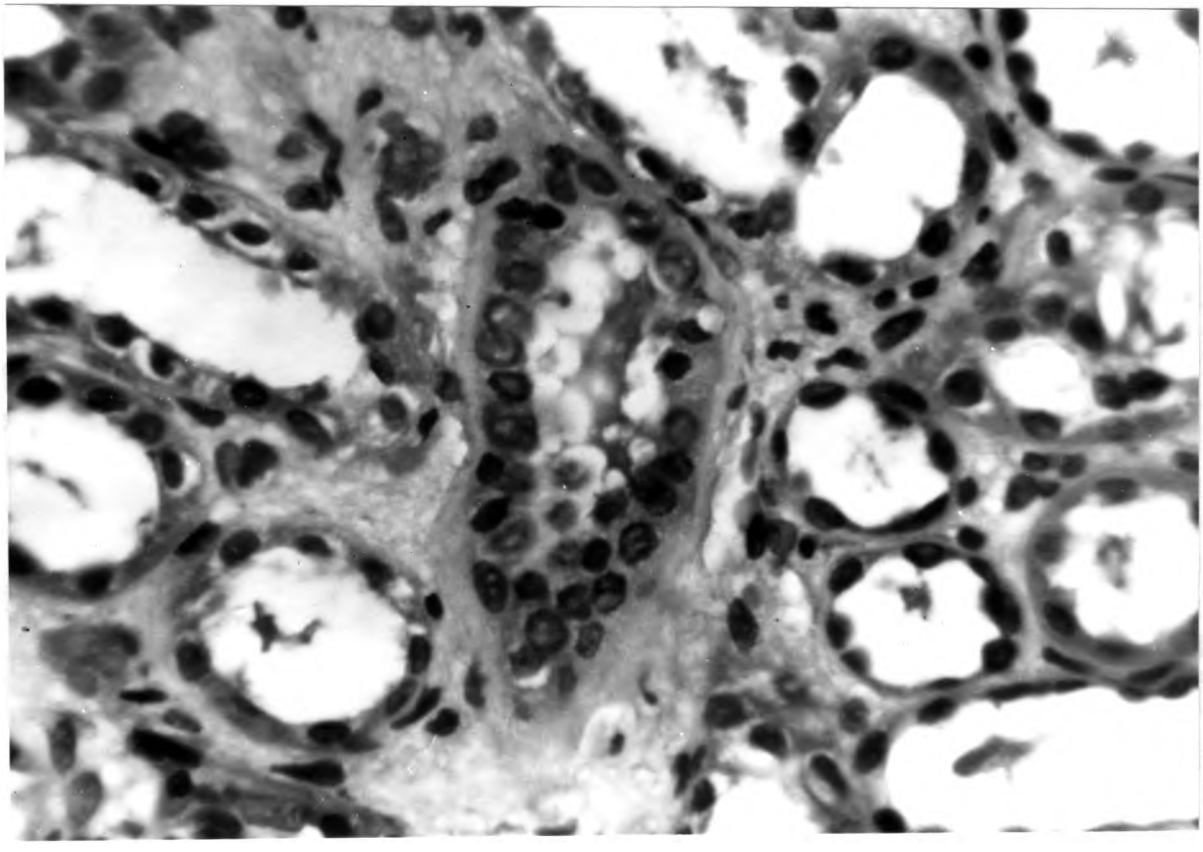

Fig. 33 (Case 5) Section through cortex of kidney showing the markedly thickened basement membranes of proximal and distal convoluted tubules with epithelial cell atrophy and degeneration.

H. and E X 2,4000

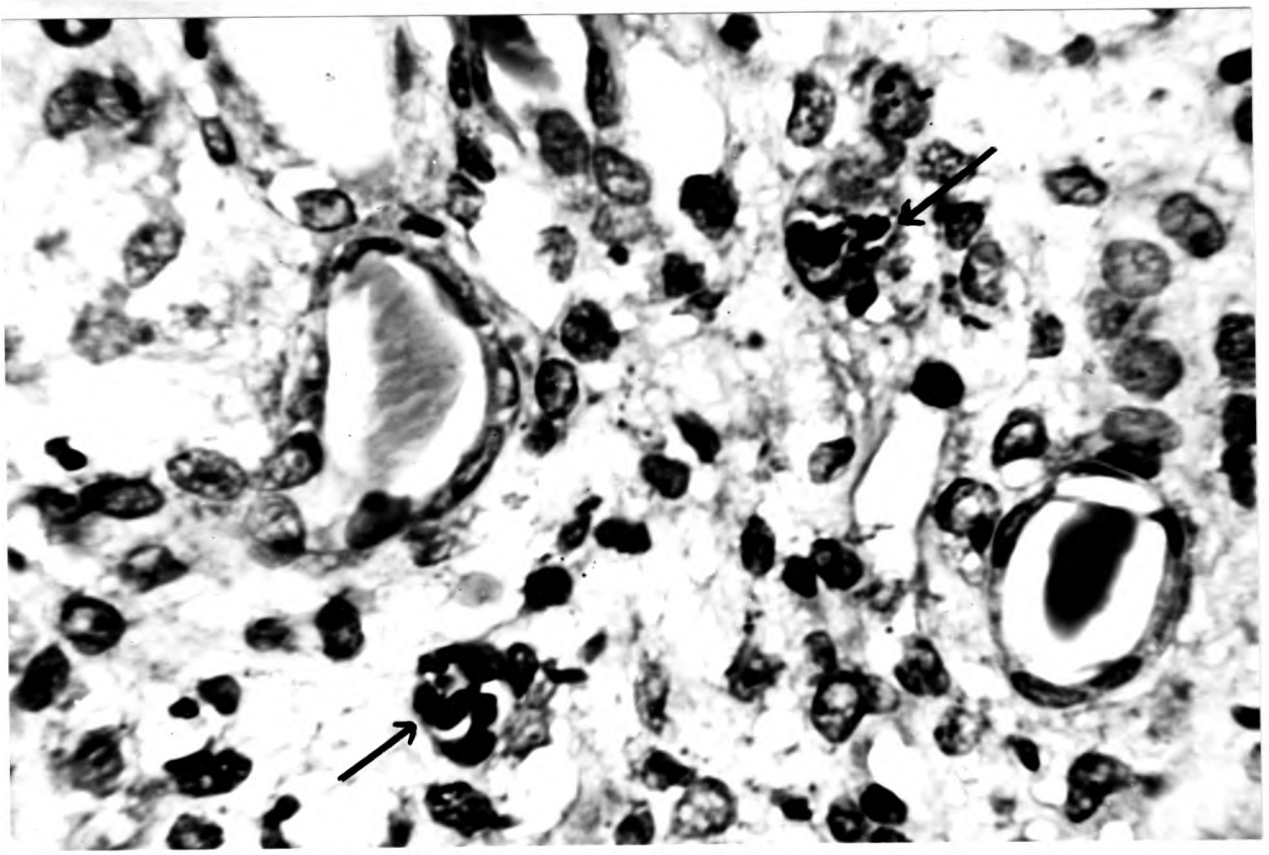

Fig. 34 (Case 5) Section through cortex of kidney. Note the presence of lipofuscin pigment granules in the cytoplasm of tubular epithelial cells. There is also interstitial fibrosis present. (The arrows indicate the pigment granules)

Reduced ferricyanide X 2,400.

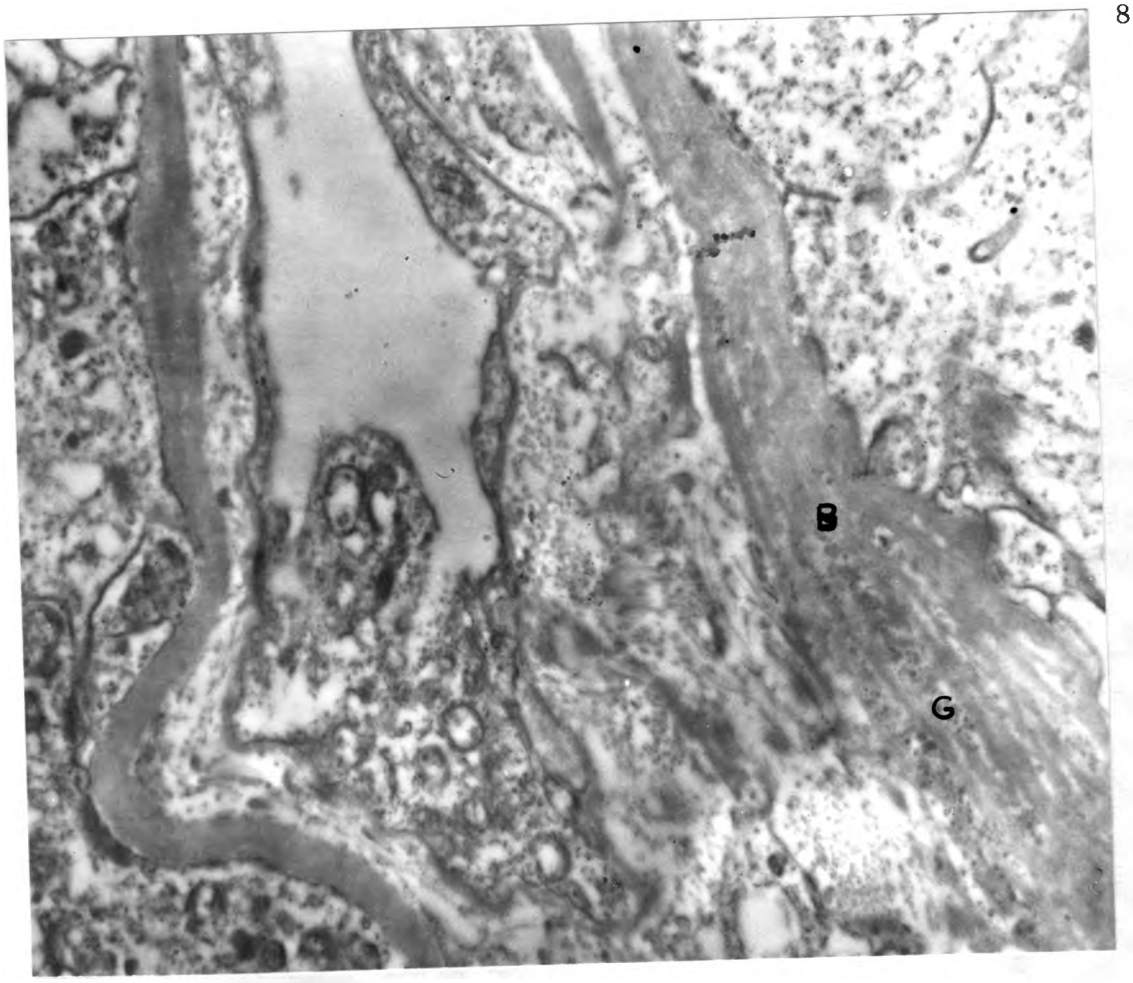

Fig. 35 (Case 5) Section through distal convoluted tubules. The basement membrane (B) is thickened and split. Small electron dense granules (G) measuring approximately 500° A may be observed between the split fibres.

Magnification X 12,500

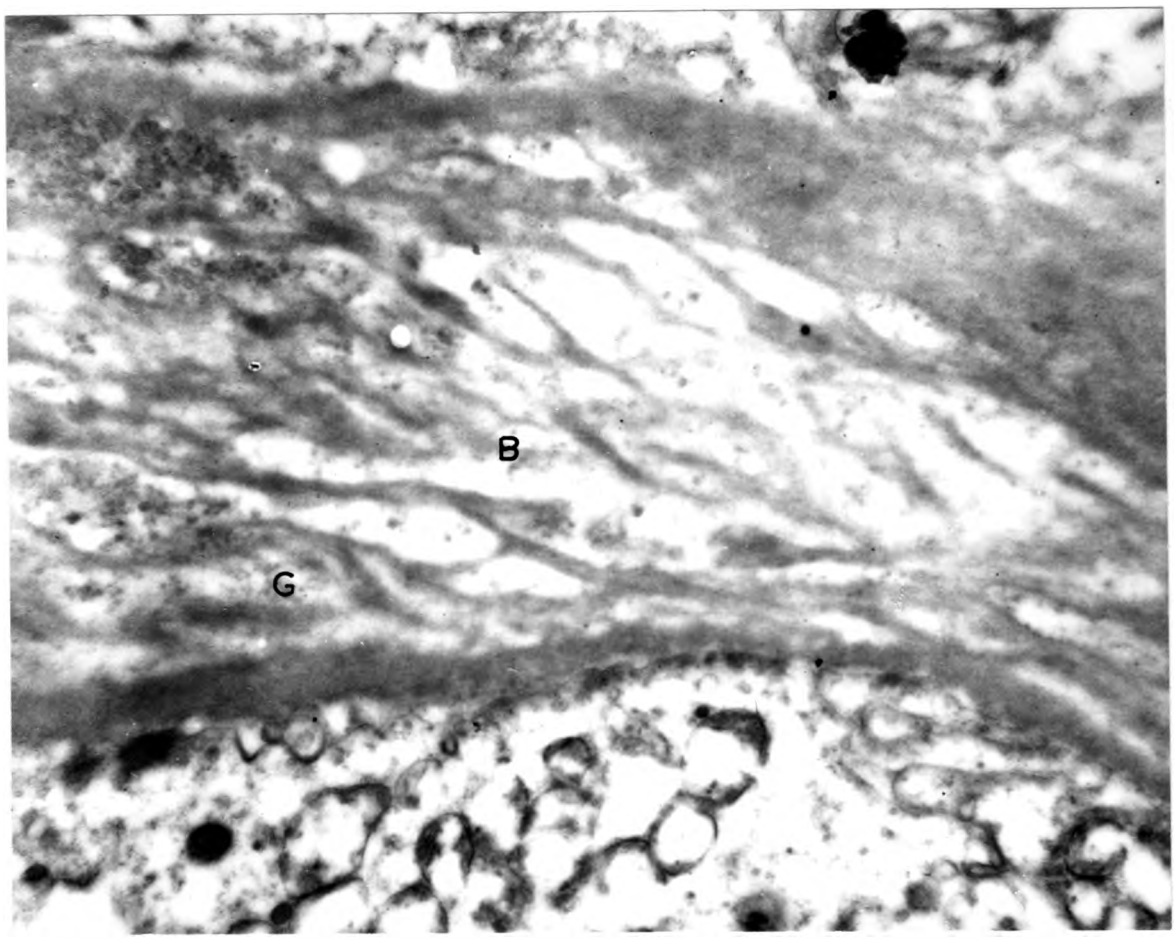

Fig. 36 (Case 5) Thickened and split basement membrane (B) of distal convoluted tubule. There are aggregates of electron dense granules between the split fibres (G).

Magnification X 20,000

epithelial cells was disrupted. The mitochondria appeared swollen and lying adjacent to them were large finely granular bodies measuring approximately 0.7 u in diameter (fig. 37). It is possible that these granules represent the lipofuscin pigment seen on light microscopy.

Fibroblastic nuclei were observed in the interstitial tissue and fibres present in this site showed the periodicity of collagen.

Liver Biopsy: The hepatic parenchymal cells contained a diffuse granular golden brown pigment with the tinctorial properties of a highly oxidised lipofuscin (fig. 38). Electron microscopy showed lipofuscin granules (fig. 39).

Case 6.

Some areas of this renal biopsy showed normal histological features, in others there was distortion of the renal architecture due mainly to fibrosis in the interstitial tissue. Associated with this change was marked tubular atrophy, making the distinction between proximal and distal tubules difficult. The basement membrane of the atrophic tubules was markedly thickened and the cytoplasm of the epithelial cells grossly reduced. In these cells there was a granular golden pigment. Some of the distal tubules and collecting tubules showed the presence of a vacuolation of the cytoplasm. There was no definite

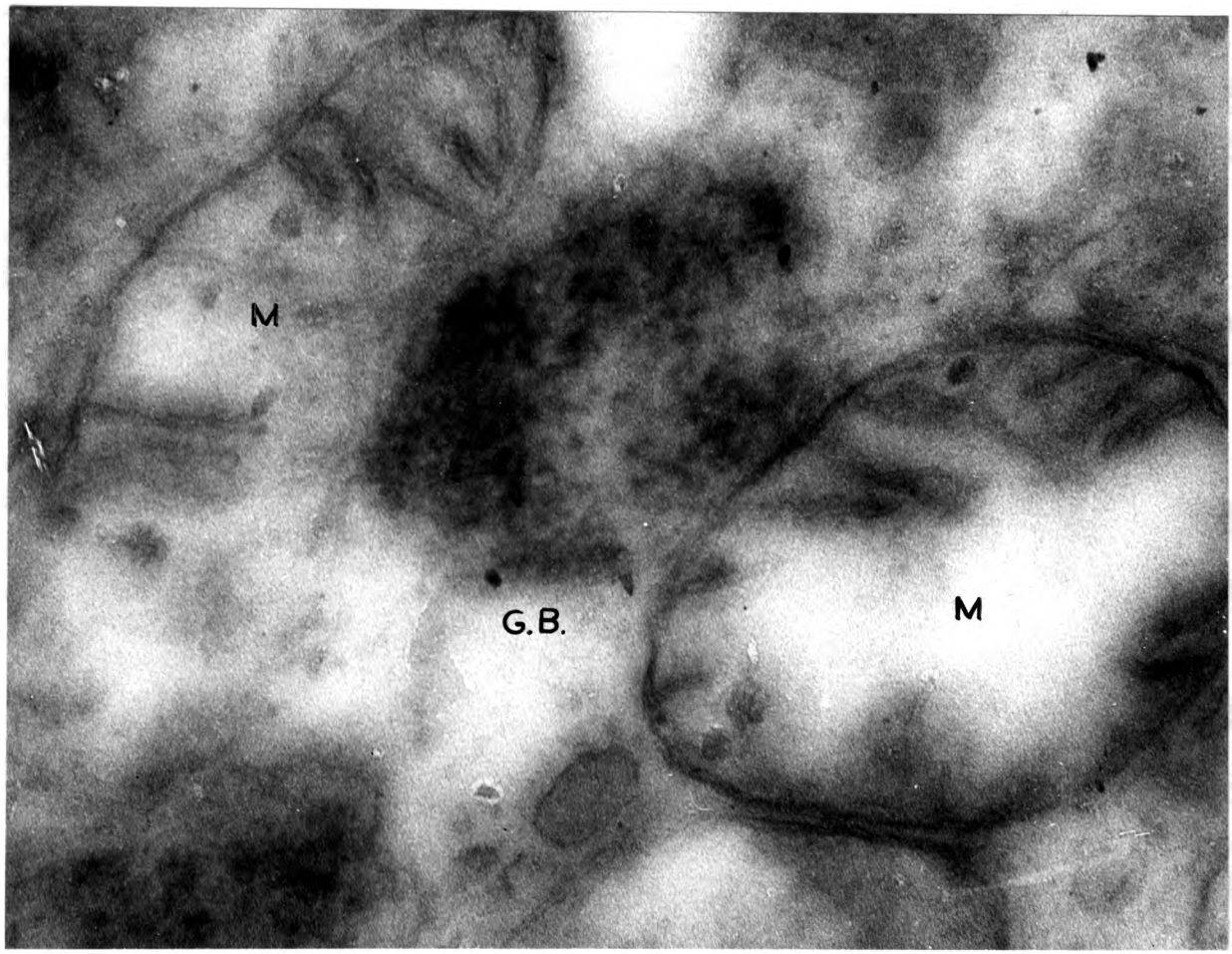

Fig.37 (Case 5) Note the large granular body (G.B) (measuring 0.7 u) adjacent to the mitochondria (M) in the cytoplasm of a distal convoluted tubule.

Magnification X 100,000

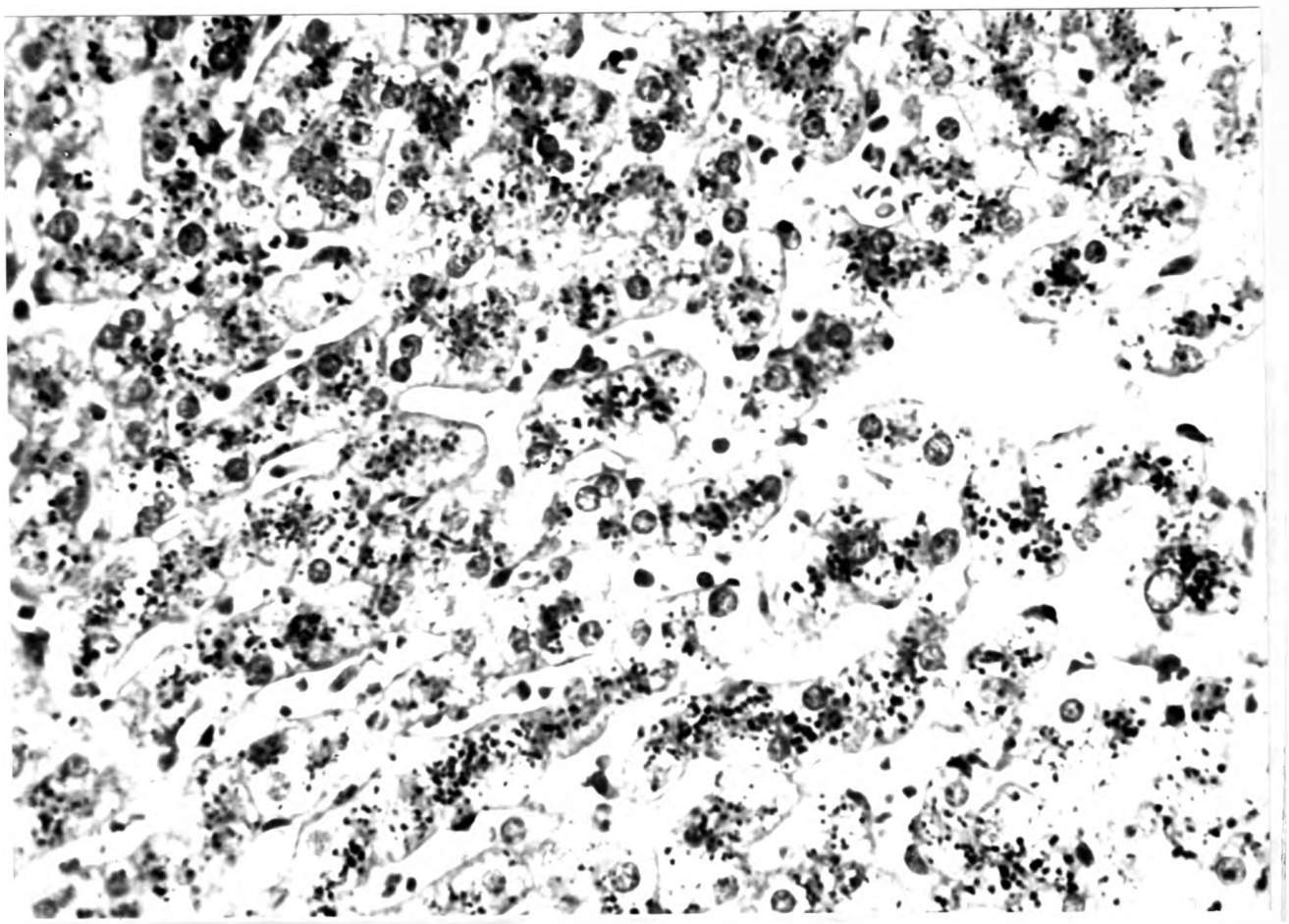

Fig. 38 (Case 5) Section of the liver showing darkly staining granules of lipofuscin in the cytoplasm of hepatic parenchymal cells.

Reduced ferricyanide X 480

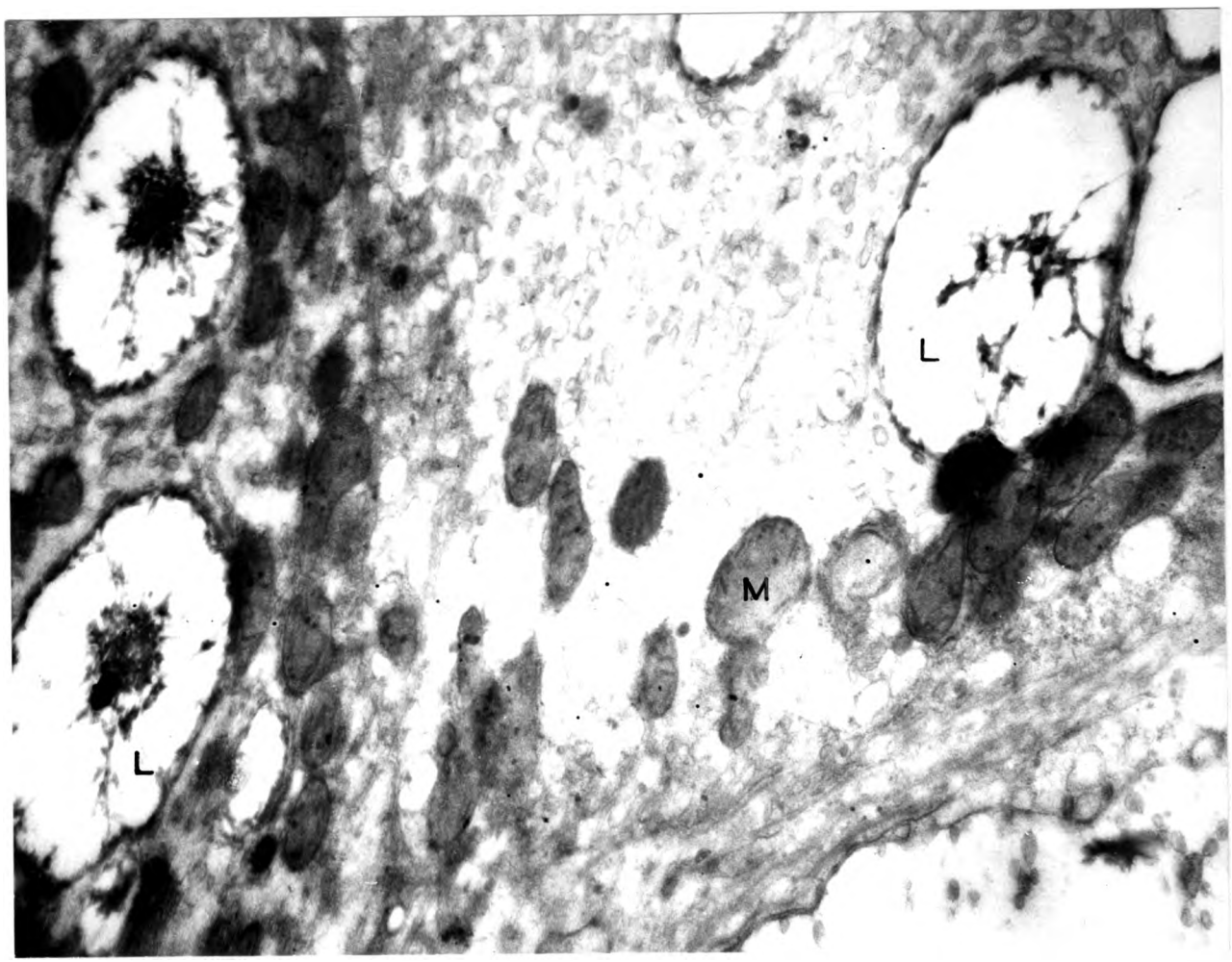

Fig. 39 (Case 5) Electron micrograph of a hepatic parenchymal cell showing numerous mitochondria (M), and large lipofuscin granules (L) with centre electron dense bodies and threadlike attachment to the periphery.

Magnification X 8,000

evidence of inflammatory cell infiltration in the biopsy material studied. One large vessel present showed arteriosclerotic changes with hyalinisation of the intima. The glomeruli were normal.

Liver Biopsy: A similar pigment to that described in the previous cases was present in the hepatic cells.

Case 7.

Sections of this renal biopsy showed the presence of relatively healthy glomeruli. The tubular system appears intact. However, there was some regeneration of distal convoluted tubules present. The tubular basement membranes showed minimal increase in thickening. There was, however, a marked amount of granular golden pigment in the cytoplasm of tubular epithelial cells and in particular of the collecting tubules and distal tubules. Investigation of this pigment indicated a highly oxidised lipofuscin. The interstitial tissue of the kidney was not increased and there was no cellular infiltration present.

Case 8.

The renal biopsy of this patient showed evidence of marked interstitial fibrosis. In addition the basement membranes of the distal tubules was hyalinised, thickened and associated with atrophy of tubular

epithelial cells. In the cytoplasm of some of these cells there was a golden brown pigment which tinctorially proved to be a highly oxidised lipofuscin. There was only moderate nongranular cell infiltration present.

Progress: The patient was uraemic, did not improve on therapy and died.

Autopsy Findings: The significant findings were confined to kidney and liver.

Macroscopic Findings: Both kidneys were grossly reduced in size (fig. 40). The right kidney weighed 70 g and the left 75 g. The capsule stripped with difficulty and the surface was granular. The cortex was markedly reduced in thickness and the medulla and papilla appeared to be shortened and rather flattened and irregular in shape. The pelvis was congested.

Histological Findings: The histological examination of kidney tissue largely confirmed the biopsy findings. The striking feature once again was the marked interstitial fibrosis (fig. 41) with the severely hyalinised and thickened tubular basement membranes of the distal convoluted tubules (fig. 42). The tubular cells were also atrophic and granules of golden brown pigment were present in these cells. The interstitial

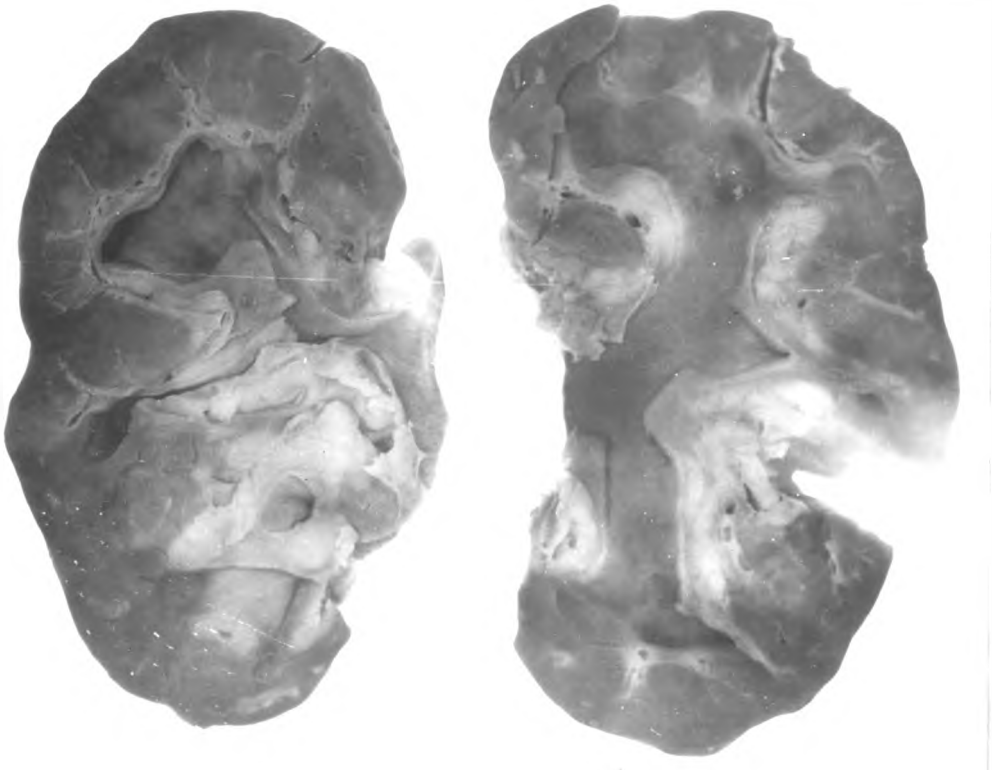

Fig. 40 (Case 8) Gross view of the kidney showing the irregularity of the surface and general reduction in size of cortex and medulla.

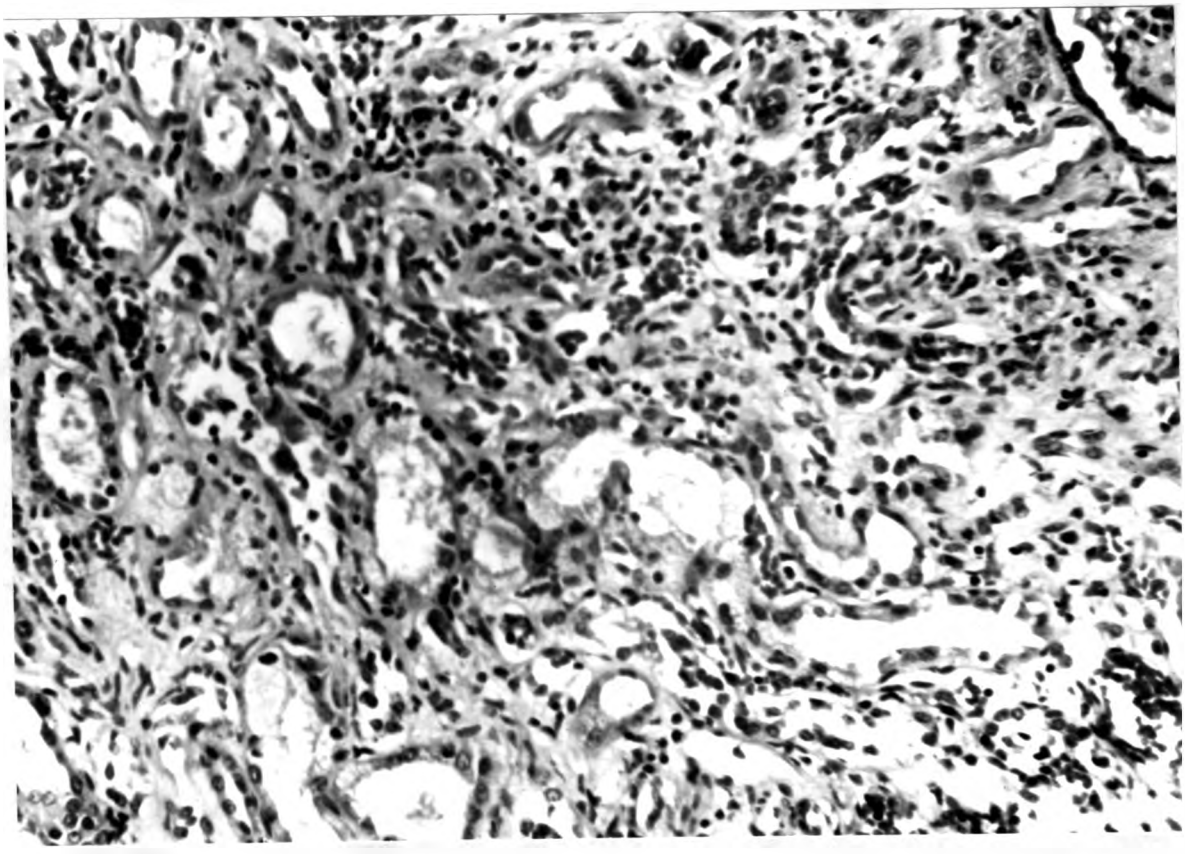

Fig. 41 (Case 8) Note the loss in normal architecture with separation of renal tubules by a marked increase in interstitial fibrous tissue. There is also a little nongranular cell infiltration into the interstitial tissue.

H. and E X 480

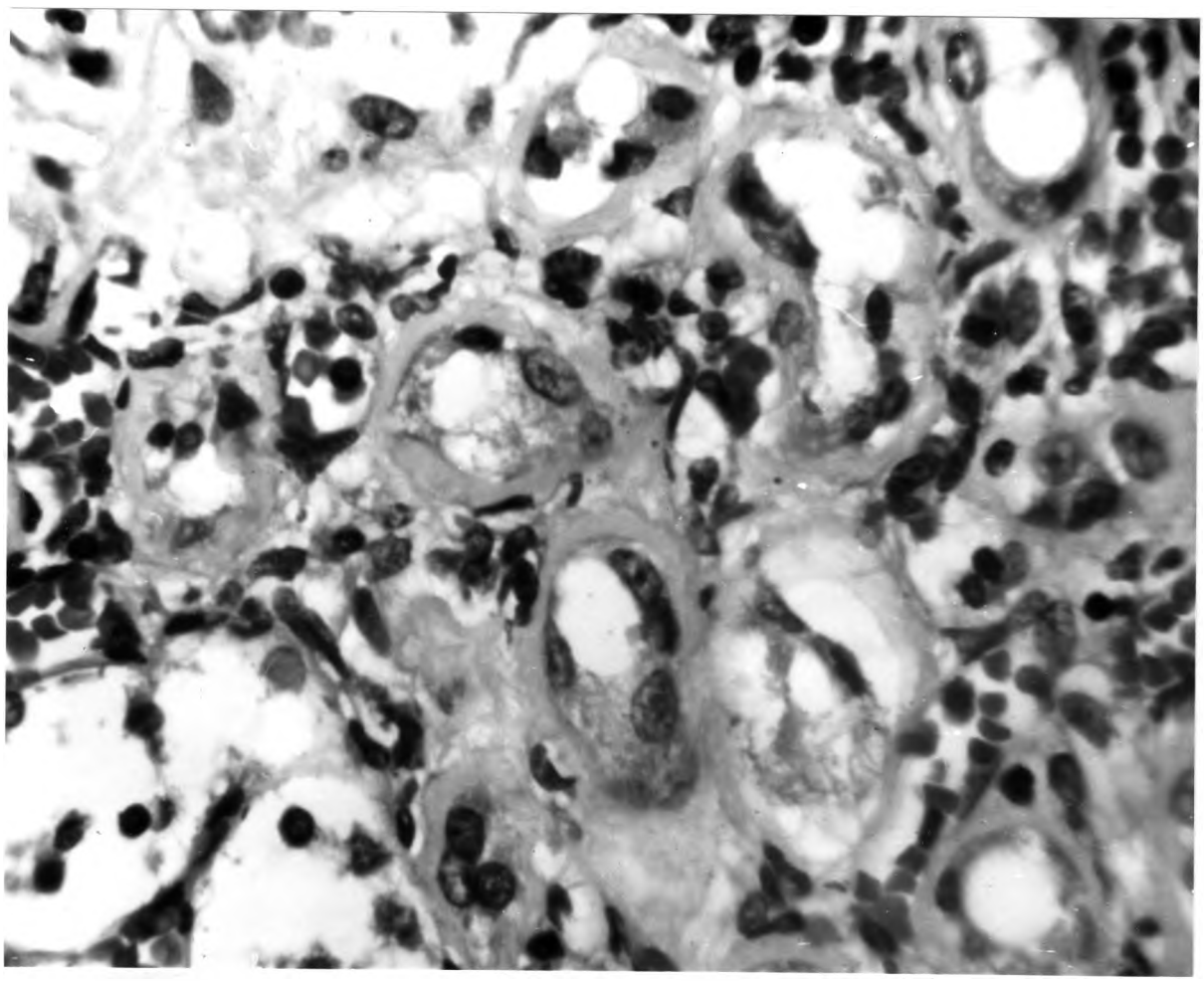

Fig. 42(Case 8) High power view to show the marked thickening of tubular basement membranes with atrophy of tubular epithelial cells.

H and E. X 2,400

tissue was the site of only mild nongranular cell infiltrate. Some of the glomeruli were more cellular than normal and showed occasional glomeruli with fine periglomerular fibrosis.

The liver on section showed the presence of increased amounts of lipofuscin pigment in the parenchymal cells.

2. Comparison between the histopathological changes in the cortex in human and experimentally induced phenacetin nephritis.

The main changes seen in these cases of phenacetin nephritis were:-

1. Thickening of the basement membrane of distal convoluted tubules with atrophy of tubular epithelial cells.
2. Lipofuscin pigment in tubular epithelial cells.
3. Interstitial fibrosis.
4. Only scattered nongranular cell infiltrate.
5. Occasional colloid casts and periglomerular fibrosis.

When taken individually the changes outlined above are not specific for chronic interstitial nephritis of phenacetin. They may be present in

almost any vascular or inflammatory renal disorder. However, it is the frequency of the lesions and the severity of the changes, which helps to make the diagnosis of phenacetin nephritis. The cosmopolitan nature of these lesions is responsible for many workers feeling that phenacetin nephritis is not specific , but merely chronic pyelonephritis. In the following Table 6 the findings in fifty cases diagnosed at autopsy on histology as chronic pyelonephritis have been compared with the findings of the human phenacetin kidneys. As can be seen from this table, although most of the lesions occurring in the one disease are also found in the other, using the degree of severity of the lesions and the frequency of the lesions the two conditions can be separated. In particular the marked interstitial fibrosis and tubular atrophy with basement membrane thickening and the constant finding of lipofuscin pigment, help to differentiate between phenacetin nephritis and chronic pyelonephritis. Another feature of importance is the relative infrequency of a nongranular cell infiltrate in the interstitial tissue of the kidneys of those patients with phenacetin nephritis. Vascular changes are also less marked and less frequent in the phenacetin nephritis compared with the chronic pyelonephritic kidneys.

Most of the changes found in human phenacetin nephritis are found

TABLE 6.

Comparison of the Histological Features of Phenacetin Induced Renal Disease and Chronic Pyelonephritis

Pathological Changes	Phenacetin Nephritis (8)			Chronic Pyelonephritis (50)		
	No. of Cases	%	Severity	No. of Cases	%	Severity
Interstitial cell infiltrate	4	50	+	48	96	+++
Interstitial fibrosis	7	88	+++	50	100	++
Tubular atrophy and basement membrane thickening	7	88	+++	28	56	+
Tubular pigment	8	100	+++	24	48	+
Periglomerular fibrosis	4	50	+	26	52	++
Cell casts	1	13	+	5	10	+
Vascular changes	3	38	+	40	80	++
Tubular dilatation with thyroid-like tissue	4	50	+	30	60	++

\+ : Mild
++ : Moderate
+++ : Severe

in the experimental and the control groups of rats in the present experiment. As summarised in Chapter III, it is only the increased severity of the four lesions of interstitial fibrosis, basement membrane thickening with tubular atrophy, dilated tubules with colloid casts and non-granular cell infiltration which make for a possible diagnosis of experimentally induced phenacetin nephritis in the rats in this experiment. It is not the development of a new lesion or a lesion different to the spontaneously occurring cortical changes in rats. Furthermore the interstitial fibrosis in the experimental material was never very dense, on the other hand, the nongranular cell infiltration and dilated tubules with colloid casts was much more severe in those rats diagnosed as experimentally induced phenacetin nephritis than in the human cases. The lipofuscin pigment so characteristic of human phenacetin nephritis was only occasionally seen in the experimental animals. Thus it would appear that in human phenacetin nephritis the lesion is more specific than in experimental phenacetin nephritis where it is probably an acceleration and increase in severity of a "spontaneously" occurring renal disease.

Not only is there some lack of correlation between the above human and experimental cases of phenacetin nephritis, but there is also

a lack of uniformity about the changes in the various experimentally induced phenacetin nephritis (Chapter IV p 64) and the reports on the findings in human phenacetin nephritis also show much variation.

Reynolds and Edmondson [27] give a good review of the pathological changes. Diffuse interstitial fibrosis and round cell infiltration has been described by most authors [31, 59, 7]. Tubular changes are common with degenerative changes in the epithelium and also the presence of pigment and iron deposition. The nature of this pigment is not stated, whether or not it is lipofuscin is both speculative and interesting in view of the findings in the above reported cases. In most cases glomerular changes have been minimal, however, in some there have been moderate numbers of hyalinised glomeruli. Reynolds and Edmondson [27] found that the basic lesion in all patients studied was a chronic fibrosing interstitial nephritis that resulted in increasing atrophy of the tubular system, particularly in the medulla. This lesion has been given many names by authors, such as primary chronic interstitial nephritis [5], chronic tubular nephritis [7], sclerosing tubular nephritis [26]. Reynolds and Edmondson prefer the term "phenacetin nephritis" because of the fact that the sclerosing change in the kidney appears to be a fairly specific one that is related to phenacetin over-

dosage. They feel they have not seen identical changes in other renal diseases. A perusal of Table 6 will show that similar changes do occur in chronic pyelonephritis, although they are more severe in phenacetin nephritis. Reynolds and Edmondson's main emphasis is placed on the presence of a lesion which was not observed in the human cases described above, namely medullary fibrosis with profound change in the peritubular capillaries. They feel that the changes in one case of a focal lesion of cortical fibrosis and atrophy with nongranular cell infiltration and sclerosis of interstitial tissue resemble the changes seen in the animals of Eisalo and Talanti [46]. Further, according to Reynolds and Edmondson iron in the cells beyond the proximal convoluted tubule is of help in diagnosis as few cases with contraction of kidneys have iron. However, an examination of some of the cases of chronic pyelonephritis show haemosiderin pigmentation, and it would appear from the above human cases that perhaps lipofuscin is of greater diagnostic significance.

Finally, the lipofuscin pigment was also observed in the livers of the patients with phenacetin nephritis. In order to assess the significance of this the livers of the patients with chronic pyelonephritis

were compared with those of the phenacetin cases. The results may be seen in Table 7. As can be seen from this there is a greater frequency and also severity of the lipofuscin pigment in the livers of those patients with phenacetin nephritis than in those with chronic pyelonephritis. This is a further point of differentiation between these two diseases.

Lipofuscin pigment was not observed in the livers of experimental animals with phenacetin nephritis.

TABLE 7

Analysis of liver pigment in cases of chronic pyelonephritis and phenacetin habitues

	No pigment	Iron	Lipofuscin	Density of lipofuscin pigment			
				+	++	+++	++++
Chronic pyelonephritis (26)	5	7	14	5	6	2	1
Phenacetin patients (5)	-	-	5	-	-	-	5

The pigment was graded according to its density in each quarter of a high power microscopic field as follows:-

+	Minimal
++	Mild
+++	Moderate
++++	Severe

CHAPTER VI

PATHOGENESIS OF PHENACETIN NEPHRITIS

I. THE DRUG RESPONSIBLE FOR PHENACETIN NEPHRITIS

From the summary of the findings in Chapter III it would appear that the important combinations of the drugs responsible for renal lesions are in decreasing order of severity aspirin, phenacetin and caffeine; phenacetin and aspirin; phenacetin alone; and phenacetin and caffeine. The aspirin caffeine fed rats showed minimal cortical and papillary changes. The following is a pharmacological and toxicological study of the possible relationship between these drugs and the development of renal disease.

1. Phenacetin

i. Pharmacology

Phenacetin or acetophenetidin is derived from aniline as is acetanilid, and aceto-aminophen. They are coal tar derivatives. As detailed in Table 4 the one breakdown product of phenacetin is N.A.P.A. which is responsible for the therapeutic effect of phenacetin. Phenacetin is absorbed from the gastro-intestinal tract and reaches highest titres in 2 hours and disappears in 5 to 7 hours. It concentrates in the tissues and is widely distributed. Phenacetin is degraded in the liver to N.A.P.A. and N.A.P.A. is excreted in the urine to the extent of approximately 4% and approximately 85% in conjugation with glycuronic acid and

sulphuric acid.

The drug can accumulate in the body and become toxic so that only 6 doses of 300 mgms and at 3 hour intervals should be taken without a drugless interval.

ii. Toxicological effects

a). Effect on enzymes

Eisalo and Talanti [46] found that phenacetin and N.A.P.A. when fed for one month, could reduce the activity of certain enzymes present in the renal tubules. The enzymes studied were succinic dehydrogenase, acid phosphatase, leucine aminopeptidase. The activity of these enzymes disappeared in the same areas in the renal cortex where they found nongranular cell infiltration. Thus it would appear that phenacetin can damage the enzymes of renal tubular cells. This may be due to an oxidation inhibiting effect. Staub [60] studied the oxygen utilisation of guinea pig renal cortex and its ability to take up phenolsulphonphthalein after being treated with phenacetin, N.A.P.A., p-phenetidin and aminopyrine. All these substances appear to have an oxidation inhibiting effect. The reserves o f energy of the proximal convoluted tubules were

therefore reduced. An additional factor such as infection may lead to chronic possibly incurable renal damage.

b. Methaemoglobin

In the pathway of breakdown of phenacetin, very small amounts of an unidentified compound are produced which oxidises haemoglobin to methaemoglobin in the red blood cell [61]. The methaemoglobin can affect the enzyme mechanisms of red cells [62]. The methaemoglobin produced may have a deleterious effect on the function of tubular cells. Gillman et al. [63] have shown that the renal tubular epithelium is extremely susceptible to agents which combine with or oxidise sulphydryl groups, and Harrison et al. [64] have demonstrated that methaemoglobin may act as oxidant and thus may possibly catalyse the oxidation of sulphydryl groups in the renal epithelial cells. Moreover, Bing [65] has shown that in acidotic dogs methaemoglobin solutions produce renal injuries characterised by hydropic degeneration in the proximal convoluted tubules.

Although in one of the first cases of human phenacetin nephritis studied personally, a haemoglobin pigment was observed in the renal tubules, this finding could not be repeated in other cases [22]. However,

it is possible that the tubular damage sometimes observed is due to the above mechanism.

c. Reduction in red cell life span

Pletcher [37] fed N.A.P.A., acetophenetidine and p-phenetidine to rabbits and dogs and showed that the red cell life span after high oral doses for 22 to 40 weeks was decreased. The most significant changes occurred with p-phenetidine, but interstitial nephritis did not occur. Miescher and Pletcher [66] in a clinical investigation state that it has been shown that Saridone, a phenacetin containing analgesic drug, induces a diminution of the red cell survival time. In animal experiments it has been confirmed that phenacetin provokes a shortening of red cell survival time. The other components of Saridone were without effect on the longevity of the red cell.

This pathological haemolytic anaemia may be responsible for renal damage by anoxia as suggested by Angervall et al. [36] The administration of haem pigments, especially haemoglobin from various sources, to the experimental animal has been the subject of considerable study. In general it has failed to produce serious renal lesions and for this reason haemoglobin has been said to be

non-toxic [65]. But renal lesions have been produced when large amounts of haemoglobin were administered [67]. However, Lalich and Schwartz [68] found that just as important as the level of haemoglobin, is prior dehydration and fasting. Both of these conditions lead to more severe renal damage [69] and particularly if combined with acid producing diets [68]. De Gowin et al. [70] demonstrated that an acid urine increased renal damage considerably. Thus possibly when A.P.C. tablets are ingested a similar chemical environment may occur; the phenacetin being responsible for release of haemoglobin and the acetylsalicylic acid for the acid urine. This may help to explain the greater severity of lesions in the experimental group of rats fed phenacetin and aspirin combination as opposed to those fed phenacetin alone.

As detailed above haemolysis with the resultant effects of pgiment release and possible anoxia, can damage the kidneys. However, preliminary experiments on phenylhydrazine induced haemolysis, followed by injection of E. coli bacillus, have not shown an increased susceptibility to the development of chronic pyelonephritis.

Although it is possible that anaemia induced by phenacetin may be responsible for hypoxia, Sorenson [71] found in an elevation of kidney function in patients taking phenacetin that a relationship between the degree of anaemia and the duration of phenacetin intake has not been proved. In addition Marti [72] found that in people taking phenacetin there was no direct relationship between the severity of the anaemia and the amount of phenacetin ingested.

d. Impurities

It has been suggested that a common contaminant, acetic-4-chloranilide, formed during the manufacture of phenacetin from 4-nitrochlorbenzol, may be the toxic substance responsible [73]. However, phenacetin made in accordance with the British Pharmacopoeia contains minimal amounts of acetic-4-chloranilide. The amount of this contaminant present in phenacetin varies between 0.01 to 0.03% which is minimal and probably non-nephrotoxic.

e. Evidence from Renal Functions

In human subjects with presumed phenacetin nephritis, administration of 3 g of phenacetin daily for 5 days caused only slight changes in the urinary sediment (as judged by the Addis Count) unless traces

of acetic-4-chloranilide were present [73]. Sorenson [71] used a 3 day endogenous creatinine clearance, to assess renal function when a group of patients were challenged with phenacetin. He found in an analysis of 780 patients, 265 of whom suffered from rheumatoid arthritis and 515 patients had other diseases and served to act as a control; that neither in the control group nor in the rheumatoid arthritis is reduction of kidney function caused by phenacetin. Nor does phenacetin seem to aggravate an already reduced kidney function. However, these were short term experiments and phenacetin probably damages the kidneys gradually and over a prolonged time period.

2. Aspirin

i. Pharmacology

As opposed to phenacetin, aspirin is slowly eliminated from the body. Small doses are excreted in 15 to 30 hours compared to 5 to 7 hours in the case of phenacetin; high doses in 6 to 10 days. The diffusable portion of the salicylate is equally distributed throughout the body water.

Small amounts may actually be recovered from the kidneys. The process of excretion has four phases, i.e. filtration at the

glomerulus, tubular reabsorption, tubular conjugation and tubular excretion [61].

ii. Toxicity

The role played by acetylsalicylic acid in relationship to body water and protein metabolism and weight loss will be discussed in Chapter VIII.

a) Effects on Potassium

The chronic effect of hypopotassaemia may result in a renal histopathological picture simulating that of chronic pyelonephritis [74]. Beckman [61] states that aspirin promotes a potassium loss from isolated diaphragm. Whether this loss of potassium occurs elsewhere and is sufficient to give rise to hypopotassaemia and thereby to produce the histological picture of chronic pyelonephritis is purely speculative.

b) Effect on renal function

Harrow [38] states that the drug assumed to be the specific drug causing renal damage is phenacetin. This is based upon the evidence that it has been associated with most of the cases reported from Europe. However, the acetylsalicylic acid in tremendous doses in

AP.C. tablets has caused renal damage in animals, and in adults moderate doses have caused increase in cellular elements in the urine. Provocation experiments showed an increase of the urinary sediment as determined by the Addis Count, with administration of aspirin (4 g per day), phenylbutazone (0.8 g per day) acetanilide (0.75 g per day) or phenacetin (3 g per day) [76,73]. Schreiner [39] states that aspirin is nephrotoxic in large quantities, producing albuminuria and haematuria.

Acetylsalicylic acid may increase uric acid clearance [75]. Patients with a salicylism often have urate crystalluria which is a potential mechanism for induction of secondary tubular damage and ascending pyelonephritis so that renal function may be impaired by Aspirin.

c. Bactericidal activity.

Although salicylic acid is bactericidal in vitro, it cannot cannot accumulate in tissue in sufficient quantities to be bactericidal there during life. Aspirin therefore does not protect the kidney against secondary infection.

d. Impairment of excretion

Aspirin may accumulate in the kidney after kidney damage has occurred, as the excretion by a diseased kidney is impaired[61]. This accumulation may reach toxic levels so that a vicious cycle may result.

Summary of the effects of aspirin

The question arises as to whether aspirin alone is sufficient to damage the kidney or is it the combination with the nephrotoxic effects of phenacetin which are important. As discussed previously in Chapter III, in the present experiment the most severe renal changes resulted when phenacetin and aspirin were combined. The changes produced by aspirin/caffeine on the renal cortex were minimal, although papillary haemorrhage did occur. It would appear from the present experiments therefore, that the combined nephro-toxicity of phenacetin with aspirin produces the most damaging effects. However, the nephrotoxic effects of aspirin alone cannot be overlooked.

In Chapter IV it was discussed how Studer[35] and also Clausen[48] produced severe renal damage in animals by feeding aspirin together with intravenous inoculation of organisms.

As opposed to this, long term consumption of aspirin in man, has not been associated with renal damage. Harrow [38] notes that renal papillary necrosis did not occur in patients receiving 6.5 gr. acetylsalicylic acid tablets per day for long periods to prevent kidney stones. In man then it may be the combined effect of phenacetin and aspirin which is the nephrotoxic agent.

3. Caffeine

As far as is known there are no physiological or toxic effects of caffeine on the kidney and it probably plays no part in the disease entity "phenacetin nephritis".

CHAPTER VII

THE PATHOGENESIS OF PHENACETIN NEPHRITIS

(Continued)

II. THE PATHOGENESIS OF THE RENAL CORTICAL LESIONS IN PHENACETIN NEPHRITIS.

At the present time, the exact pathogenesis of the cortical lesions is an extremely difficult and confused subject. It is argued firstly that it is the tubular damage that is the primary change in phenacetin nephritis, and that all other changes are secondary. Others feel that the interstitial tissue is the primary target for the nephrotoxic effect of phenacetin. The role played by infection is also under question.

1. Role of Tubular Damage

In Chapter VI various harmful effects of phenacetin and aspirin on the kidney have been outlined. Many of these nephrotoxic properties act specifically upon the tubular epithelial cells. It is possible that this effect may result in abnormalities in the function of these tubular cells. Certainly Eisalo and Talanti [46] have shown loss of enzyme activity in tubular cells of rats fed phenacetin and N.A.P.A. Light microscopy and electronmicroscopy have shown that tubular damage occurs specifically [23]. In human cases of phenacetin nephritis an increase in lipofuscin pigment has been demonstrated [23]. This pigment had collected in the tubular

epithelial cells. In the present experiments there was always a fair amount of iron pigment in the tubular epithelial cells of the rats. In addition, lipofuscin was also present in these experimental animals and in the control group. The greatest amount of pigment was observed in the phenacetin fed rats. The significance of this pigment is not clear. It has recently been suggested that lipofuscin pigments are altered lysozymes [77]. Lysozymes are packets of hydrolytic enzymes containing at least 8 enzymes capable of hydrolysing certain chemical compounds within the cell and are also known to play an important role in cell autolysis after ligation of the vasculature of the kidney [77]. It is thus possible that the presence of the particularly large amounts of lipofuscin pigments seen in the renal tubules may be an indication of damaged enzymes in the renal cells, induced by excess ingestion of phenacetin. Thus it is possible that the primary change is upon the tubular cells and this is followed by changes in the interstitial tissue. It is interesting that one of the human cases studied showed accumulation of lipofuscin pigment as the only change with no increase in interstitial fibrosis. This then may be one of the earliest changes in phenacetin nephritis. Many of the rats too, fed phenacetin showed pigmentation with minimal other lesions.

2. Interstitial Fibrosis and Basement Membrane Changes

Electron microscopic study of one human case of phenacetin nephritis has revealed small granules measuring (± 500° A) between the split membranes of distal convoluted tubules. Similar changes were observed in electron microscopic studies of one A.P.C. fed rat (fig. 43). Whether these granules are breakdown products of phenacetin or aspirin, phenacetin and caffeine is not known. However, it can be suggested that as these granules seem to be related to the splitting of the basement membrane, so they may be related to fibrillogenesis in the interstitial tissue of the kidney. Reynolds and Edmondson [27] state that the renal changes may best be considered as a chemical nephritis, and feel that interstitial fibrosis follows on this. They suggest that it is possible that the peritubular fibrosis is related to the absorption of some breakdown product of phenacetin that is present in the glomerular filtrate over long continued periods. This could selectively damage the connective tissue as it is absorbed during the concentrate of the filtrate. The granules seen on electron-microscopy may correspond to this breakdown product of phenacetin. Reynolds and Edmondson [27] suggest that under such circumstances the breakdown product may be present in highest concentration

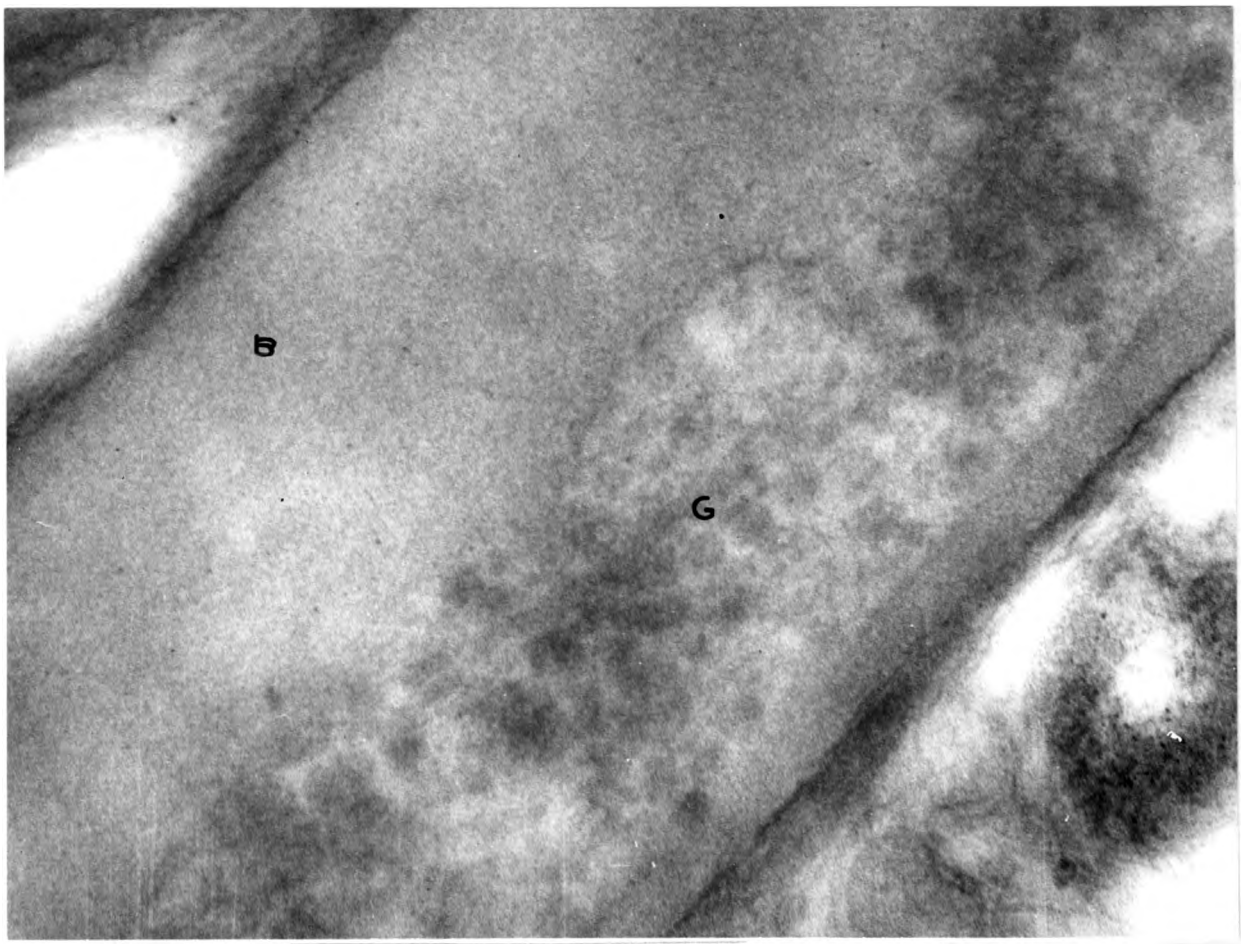

Fig. 43 Note the markedly thickened basement membrane (B) of the distal tubules of this rat fed A.P.C. tablets for 8 months. There appears to be a change in the normal regular electron dense membrane and at this site a granular appearance may be observed (G)

Magnification X 100,000

where the urine is most highly concentrated. The fluid in the interstitial tissue contains the greatest concentration of solute (i.e. in the papillae of the kidney). Although they observed marked medullary fibrosis, this does not explain the selective foci of atrophic changes seen in the cortex of the kidney. Further in this present experiment and in the human cases of phenacetin nephritis the interstitial tissue of the papilla has not been a prominent site of interstitial fibrosis. In view of the electron microscopic findings showing the granules in the basement membrane of distal convoluted tubules, perhaps the granules are reabsorbed at this site and not only in the collecting tubules of the papilla.

Many workers feel that the primary change in phenacetin nephritis is an interstitial tissue change [27]. Reynolds and Edmondson state that in human cases it cannot be stated with complete assurance that tubular atrophy may not have occurred first and that the interstitial fibrosis was a secondary phenomenon. However, in one of their cases the severity of the interstitial fibrosis and destruction of the capillaries suggest that this was the primary change.

From personal observations of both human and experimental

phenacetin nephritis, it would appear that the pathogenesis lies in a combination of both a primary tubular and primary interstitial change. Alteration in the quality of the interstitial tissue may play a further part in damaging the tubules, by preventing diffusions of the normal interstitial fluid to these cells.

3. Relationship to Chronic Pyelonephritis.

The pathological changes in human and experimental phenacetin nephritis resembles those of chronic pyelonephritis. However, as shown in Table 6 (Chapter V), there are differences which help to distinguish between these two diseases. Reynolds and Edmondson [27] are of the same opinion as they feel that the paucity of cellular infiltrate and the generalised nature of the medullary fibrosis argue against pyelonephritis being a pathogenetic factor. They also feel that the usual diagnostic findings of chronic pyelonephritis are not present. The periglomerular fibrosis, colloid casts and alternating bands of scarred and normal cortex were either minimal or absent. What is more, chronic pyelonephritis is a disease notoriously difficult to diagnose and there are many conditions which simulate the picture of this condition. Ischaemic lesions [78, 78(a)] can resemble chronic pyelonephritis and chronic hypopotassaemia [74] may mimick

the histological features of this disease. Perhaps to this list may be added phenacetin nephritis. In each of these lesions to make a definite diagnosis the histological features must be considered together with the history, clinical, biochemical and bacteriological examinations. A similar situation may exist with chronic phenacetin nephritis.

A complicating factor in the diagnosis of phenacetin nephritis is that any scarred kidney can become secondarily infected and present a picture in the late stages almost indistinguishable from the histological features of chronic pyelonephritis [56,58]. Reynolds and Edmondson [27] feel that the kidney damaged by phenacetin is prone to bacterial infection that may accelerate the disease process or even precipitate papillary necrosis.

Von Colombi [79] stated that bacteria are more commonly found in the urine of patients with chronic pyelonephritis than chronic interstitial nephritis. In his experience chronic interstitial nephritis was primarily abacterial. Larsen and Moller [7] reported that 17 out of 21 patients whose total consumption of phenacetin was 7 Kg or more suffered from frequent urinary tract infections, but this did not occur until phenacetin had been taken for many years and they regarded the infection as secondary.

To produce phenacetin nephritis in animal experiments, the kidney must be prepared first with phenacetin and this followed by intravascular inoculation of organisms. Then the organisms acting upon the kidney which is damaged by the phenacetin causes an interstitial nephritis. Perhaps generally speaking phenacetin merely weakens the defences of the kidney and nephrotoxic agents may then easily damage it. Infection may be the most important of a whole host of "kidney damaging" agents; others being vascular, dietary or obstructive mechanisms. The initial histopathological picture may be constant, but in the late stages due to this host of nephrotoxic agents, the picture may be very variable.

CHAPTER VIII

PATHOGENESIS OF PHENACETIN NEPHRITIS

(Continued)

III. THE PATHOGENESIS OF THE RENAL PAPILLARY CHANGES IN PHENACETIN NEPHRITIS.

Renal papillary necrosis is a property which could be regarded as a special feature of the renal lesions observed in connection with phenacetin [5,31]. At the present time, the only report of experimentally induced renal papillary necrosis in animals in association with phenacetin is that of Studer et al. [35]. They found that inoculation of staphylococci intravenously, produced papillary necrosis and abscesses in animals fed phenacetin, acetylsalicylic acid and in controls who received the injections of staphylococci only. The renal papillary lesions produced by feeding phenacetin and tablets of aspirin, phenacetin and caffeine to rats in Experiment I, and phenacetin/caffeine and phenacetin/aspirin and aspirin/caffeine in Experiment II without inoculation of organisms are therefore unique.

Renal papillary necrosis has been induced experimentally in a number of different ways. Many pathways have been suggested in the pathogenesis of these lesions based both upon the pathological changes and the manner in which the necrosis was produced. Lauler et al. [80] suggest three methods of producing papillary

necrosis in a review dealing with this subject.

A comparison of the pathological changes of these experimentally induced papillary necrosis, and the papillary lesions produced by the feeding of excessive quantities of phenacetin and A.P.C. as in the present experiment, may provide a possible pathogenetic mechanism of the renal lesions in the latter group.

1. Methods of experimentally inducing renal papillary necrosis and comparison with the changes found in the present experiments.

i. Artificial obstruction of the urinary tract and superimposing on this an increased bacteraemia with consequent pyelonephritis.

Muirhead, Vanatta and Grollman [81] found that when ureters are ligated in dogs 60% develop papillary haemorrhages, 32% coagulative or suppurative papillary necrosis and 36% diffuse pyelonephritis. In the present experiment haemorrhage into renal papillae was observed in 28% of the experimental animals and papillary necrosis in 14%. However, there was no cause present for ureteral obstruction and hence no cause for an increase in intrapelvic pressure in these animals.

Attempts to experimentally produce papillary necrosis by

producing renal infection with urinary tract obstruction have not always been successful. Edmondson et al.[82] tied off the ureter in depancreatised rats. The animals developed a pyonephrosis (whereas only hydronephrosis developed in the non-diabetic rats), but papillary necrosis was not found. Mallory, Crane and Edwards[53] in their study dealing with experimental pyelonephritis in rabbits do not describe the lesion of papillary necrosis, although Robbins, Mallory and Kinney[83] refer to its occurrence in such experimental work.

Harrow[28] states that the role of infection and urinary tract obstruction in the production of papillary necrosis has been documented by Lauler et al.[80]. The role of infection in causing renal papillary necrosis in human cases of phenacetin nephritis is unsettled. Harrows[28] human cases of papillary necrosis showed no bacterial infection, Hultengren[12] found 17 out of 98 cases of renal papillary necrosis with sterile urine. Moreover renal papillary necrosis due to trauma, renal vein thrombosis and hypotension occurred without urinary tract infection[84,85,86,87].

In the present experimentally induced papillary lesions with

phenacetin and A.P.C. bacteriologic cultures were not done and although bacteria were present in the areas of necrosis of most of the papillae, they were present in only small numbers and could therefore merely be a superimposed phenomenon accelerating the renal damage [28]. Furthermore true abscesses of the type described by Studer et al. [35] were not observed.

ii. Injection of toxins

Certain specific chemical poisons have successfully produced papillary necrosis in the experimental animal. Levaditi [88] produced the lesion in rabbits, guinea pigs and mice by subacute poisoning with vinylamin. Refino [89] produced lesions in rabbits and guinea pigs, but not rats or mice by administration of tetrahydroquinoline and its methyl esters. The mode of action of these chemicals is probably through a vascular spasm. Such an effect on vessels has not been shown in the case of phenacetin or acetylsalicylic acid.

iii. By metabolic means

Burr and Burr [90] in 1929 described a deficiency disease in rats fed a diet which was virtually fat free. Rats fed such a diet from the day of weaning develop (1) a lesion of the tail characterised by

scaliness, inflammation, swelling and later necrosis of the tip. (2) redness, swelling and scaliness of the feet, (3) dandruff and loss of body hair, (4) cessation of growth (5) bloody urine. The animals maintain a plateau of the weight curve for weeks and months then declined in weight and died. At autopsy 5 of the 8 animals had abnormal kidneys.

The renal lesions were studied in some detail by Borland and Jackson in 1931[91]. A group of 21 rats on the fat deficient diet were autopsied. The kidneys were large and pale and coarsely granular. In 8 of the 21 animals the papillae appeared largely necrotic and much of certain papillae might be sloughed off into the pelvis.

Microscopically the glomeruli were normal. Certain cortical tubular cells were the site of deposition of calcium. The lumina of these tubules often contained debris which was also calcified. This was present in 15 of 21 rats. There were no inflammatory infiltrates. Widespread degenerative changes in cortical tubules occurred in 8 cases. Sudan III stain showed increase in intracellular fat or lipoid in 18 cases. Occasionally there was dilatation of some of the

cortical tubules suggesting obstruction to the flow of urine in the papillary ducts. In the medulla degenerate changes were found in the cells of the papillary ducts. Sudan III showed free fat in the cells and in the interstitial tissue. Fatty casts were present. Higher up in the pyramid casts of fatty albuminous material appeared in degenerate ducts and as the necrotic area was approached this material, as well as the degenerative tissue, became intermingled with deposits of calcium. In 2 of these 8 cases bacteria and a few polymorphonuclear leucocytes were present in the necrotic area, but in the remaining kidneys no inflammation was found.

Thus characteristic renal lesions were present in rats fed on a diet free of fat but otherwise adequate. The most striking lesion was calcification of tubules and necrotic areas in the renal medulla with disintegration of the apex of the pyramid in some. The addition of lard to the diet prevented the renal lesion or cured it to a large extent.

It is interesting that despite the fact that no fat studies were done some of the experimental animals in the present experiment were in a poor state of nutrition. This could be seen mainly in the animals

fed A.P.C. tablets and the combination of phenacetin and aspirin.

The animals were weighed at the commencement of the experiment and after 8 months of feeding the drug (Table 8).

Considering that the average weight of the rats at the commencement of the experiment was350 grams, the control animals here only gained 25 grams. On the other hand the pure phenacetin group had gained 85 grams, whereas theA.P.C. group had lost 10 grams in weight. The presence of an only slight increase in weight of the controls could have been due to the fact that they were only given oats once a week.

These findings are borne out at the autopsy of the rats where it was observed that the phenacetin animals had large subcutaneous and retro-peritoneal fat depots whereas the A.P.C. animals showed marked loss of depot fats and were obviously undernourished. Not only was this poor state of nutrition visible in the loss of fat stores, but also in the loss of hair of the body and ulceration of the tails. the cause of the loss of weight and poor nutrition in the A.P.C. group of rats is unknown, however, it would appear to be related to a decreased intake of oats. The dish containing the drug plus oats

TABLE 8

Average weight of rats at eight months of feeding of the drugs.

	Average Weight in Grams
Phenacetin	435.00
Aspirin/Phenacetin/Caffeine	340.0
Phenacetin/Caffeine	417.3
Phenacetin/Aspirin	387.0
Aspirin/Caffeine	403.0
Control	375.0

was consumed twice as fast in the case of phenacetin fed rats compared with the A.P.C. group. It is interesting that the phenacetin /aspirin rats only gained 12 grams over the controls, whereas the phenacetin/ceffeine showed a weight gain of 42 grams. This would appear to indicate that the aspirin/phenacetin combination is one which leads to a poor intake of food, and a poor state of nutrition.

These findings of a weight gain in the phenacetin group do not agree with the findings of other workers. Angervall et al.[36] noted that the admixture of phenacetin and N.A.P.A. did not effect the dietary intake of the rats, the males perhaps showing a slight tendency to inhibition of bodily growth. Clausen[48] fed phenacetin alone and acetylsalicylic acid to rabbits and noted that in both groups there was a slight gain in weight compared with the controls receiving a placebo tablet. Eisalo and Talanti[46] noted that rats fed phenacetin showed a poor appetite for the diet in which the drug was mixed, but after some days their appetite returned. Some weight loss was observed in both groups during the test period. Apart from showing a weight loss the rats remained healthy throughout.

The cause of the weight loss in the A.P.C. group is possibly due

to a lesser intake of oats. However, it is possible that the aspirin in the A.P.C. combination may be responsible for an upset in general metabolism. Beckmann [61] states that in the University of Glasgow the general effect of salicylate administration has been shown to reduce the volume of total body water as a result of a negative nitrogen balance resulting from diminution in both cellular and plasma protein. This could be responsible for loss of weight and poor nutrition of the animals. Two additional ways of accounting for the destruction of protein have been offered.

1) salicylates primarily stimulate vagal nerve endings and induce respiratory alkalosis through deep breathing, the alteration in the chemical environment of the body cells then leading to increased protein catabolism.

2) they primarily stimulate protein catabolism, the liberated nitrogenous organic compounds then stimulate afferent vagal fibres. Cochran [92] also showed an increase in the B.M.R. which possibly causes a further weight loss. Thus for two reasons animals on A.P.C. may be in a state of fat depletion. Firstly there is a decreased intake of food. This gives rise to some mild undernutrition and therefore loss of fat depots. Secondly, there is the increased catabolism of protein which causes a deficiency of fat either by

virtue of a decreased formation of fat from protein through the pathway of glucose and glycogen or by fat being used for "protein sparing". Thus although the animals are not on a fat free diet, through their decreased fat content they have a metabolic state equivalent to a fat deficient diet.

Burr and Burr[90] state that the experimental papillary necrosis produced by fat free diets is due to a deficiency of unsaturated fatty acids, and that a very small amount of these may restore normal fat metabolism. Although fat deficient animals synthesise fat, they cannot synthesise the necessary long chain unsaturated fatty acids.

Robbins and Angrist[75] studied the papillary necrosis occurring in association with acute pyelonephritis in diabetes mellitus and in non-diabetics with obstruction of the urinary tract. In both of these groups there is an upset of fat metbolism. In diabetes mellitus there is uncontrolled overproduction of fatty acids. The non-diabetic group consisted of elderly males with chronic urinary tract obstruction and infection, with anaemia and azotaemia all of which were additive in producing debility and malnutrition, with its accompanying disturbance of fat metabolism (starvation acidosis). Boyd[93] found that during fever neutral fat increases 10% but total and free cholesterol and

phospholipids fall after an initial rise. He noted that I_2 number of plasma fatty acids fell markedly after an initial rise. It may be said then, that disturbed fat metabolism is a common factor in diabetes, in non-diabetics with debility, malnutrition and sepsis; and in the experimental animals on fat free diet. The likelihood of the A.P.C. animals falling into this category is very great.

It is not possible to say at this stage whether a deficiency in unsaturated fatty acids plays a direct role in the pathogenesis of papillary necrosis or whether it plays an indirect role by inducing alterations in renal haemodynamics.

2. Pathogenesis of Renal Papillary Necrosis

As has been detailed in this Chapter, there are three very different experimental conditions under which renal papillary necrosis can be produced. The question arises as to whether one or more pathogenetic mechanisms are responsible for the papillary damage.

Robbins and Angrist [75] suggest that the sequence of events in papillary necrosis is an intensive rapid, complete infarct-like necrosis of the entire affected region followed by an inflammatory

reaction. In view of the pathological features of these papillary lesions resembling ischaemic necrosis, it is suggested that possibly vascular obstruction is behind all causes of papillary necrosis.

i. The vascular supply of the renal papilla

The renal papilla is the poor orphan of the kidney. Most cytological, cytochemical and electron microscopic studies have dealt with the glomeruli and proximal convoluted tubules and distal convoluted tubules. Very little attention has been focused on the renal papilla.

The renal papilla projects as a pendant of medullary tissue. Trueta and his coworkers [94] have described the course of the blood vessels through the kidneys. According to these workers the medullary vessels arise as a continuation of the efferent vessels from the juxta-medullary glomeruli, travel downward into the medulla in bundles and loop back into the venous arcade without the intermediation of capillaries, thus forming arterio venous shunts. Less conspicuous side branches act as nutritive channels. Baker [95] demonstrated an additional blood supply to the renal papilla by vessels arising from the interlobular arteries close to the calyx. These vessels form spirals in the loose adventitia of the walls of the minor calyces and give off

branches to the calycine mucosa, and to the vessels penetrating the papilla and anastomosing with the vasa recta. The blood supply of the renal papilla is thus rather tenuous and two anatomic features in the papilla would seem to make the vessels here susceptible to occlusion by pressure [81]. First this zone has a greater amount of loose interstitial tissue and therefore should be less compressible than the densely packed cortex, second, the U-shaped course of the shunt vessels should make the lower transverse segment (bottom of U) more susceptible to occlusion as the pressure here would be perpendicular to the axis of the vessel.

ii. Vascular obstruction as the main cause of renal papillary necrosis.

An obstruction of blood vessels with an ischaemic necrosis and secondary infection would be an attractive unifying hypothesis of the cause of papillary necrosis, which would encompass all experimental and human cases of this renal disease. Many authors have suggested that impairment of vascular supply is of aetiological importance, [5, 95, 96] Murphy and Campbell [97] actually define renal medullay necrosis as ischaemia obstruction involving the renal medulla.

a) Role of infection and urinary obstruction in giving vascular occlusion

Referring back to the study of Lauler et al.[80] the first cause of experimentally induced papillary necrosis is that associated with urinary tract obstruction and infection. Muirhead et al.[81] state that in their experiment with ligation of the ureters in dogs and resultant papillary necrosis, the increase in intra-pelvic pressure accounts for the ischaemia which ultimately causes papillary necrosis.

Urinary infection probably plays a major role in causing renal papillary necrosis through an ischaemia. The papillary necrosis occurring in diabetic patients is of the coagulation-suppuration type and resembles that occurring in obstructive uropathy with pyelonephritis. The source of the ischaemia in diabetes is at present not clear. Muirhead et al.[81] suggest that pyelonephritis associated with diabetes causes an increase in interstitial pressure which compresses vessels and gives an ischaemia. Robbins, Mallory and Kinney[83] feel that the associated pyelonephritis causes abscesses at the base of the pyramid with distal ischaemia and infection. However, Robbins and Angrist[75] although agreeing that the sequence of events in papillary necrosis is an initial rapid, complete infarct-

like necrosis, they feel that the inflammation which follows is secondary and occurs in the junction between viable and necrotic tissue. The cause of the infarct-like necrosis is thought, by them, to be related to an upset in fat metabolism and simulating the necrosis seen in rats on fat free diets [91]. They feel that there is usually no inflammatory exudate in the area of necrosis, but bacterial colonies are commonly found in the lumina of the dead tubules. With disintegration of the necrotic papilla, a diffuse overgrowth of bacteria occurs.

Bengston [11] states that prolonged consumption of phenacetin makes the kidney susceptible to infection. It can also be said that regardless of which came first, the bacteria or the phenacetin, once the kidney is diseased, excessive phenacetin consumption frequently causes renal papillary necrosis. Most workers agree with this and feel that human papillary necrosis occurs only in severely damaged kidneys after chronic ingestion of phenacetin. This would be at variance with the results in the present experiment, where papillary changes are associated with only minimal amounts of renal damage (fig. 44, 45). Further the general cortical changes are not much more severe in the animals with papillary lesions than in control

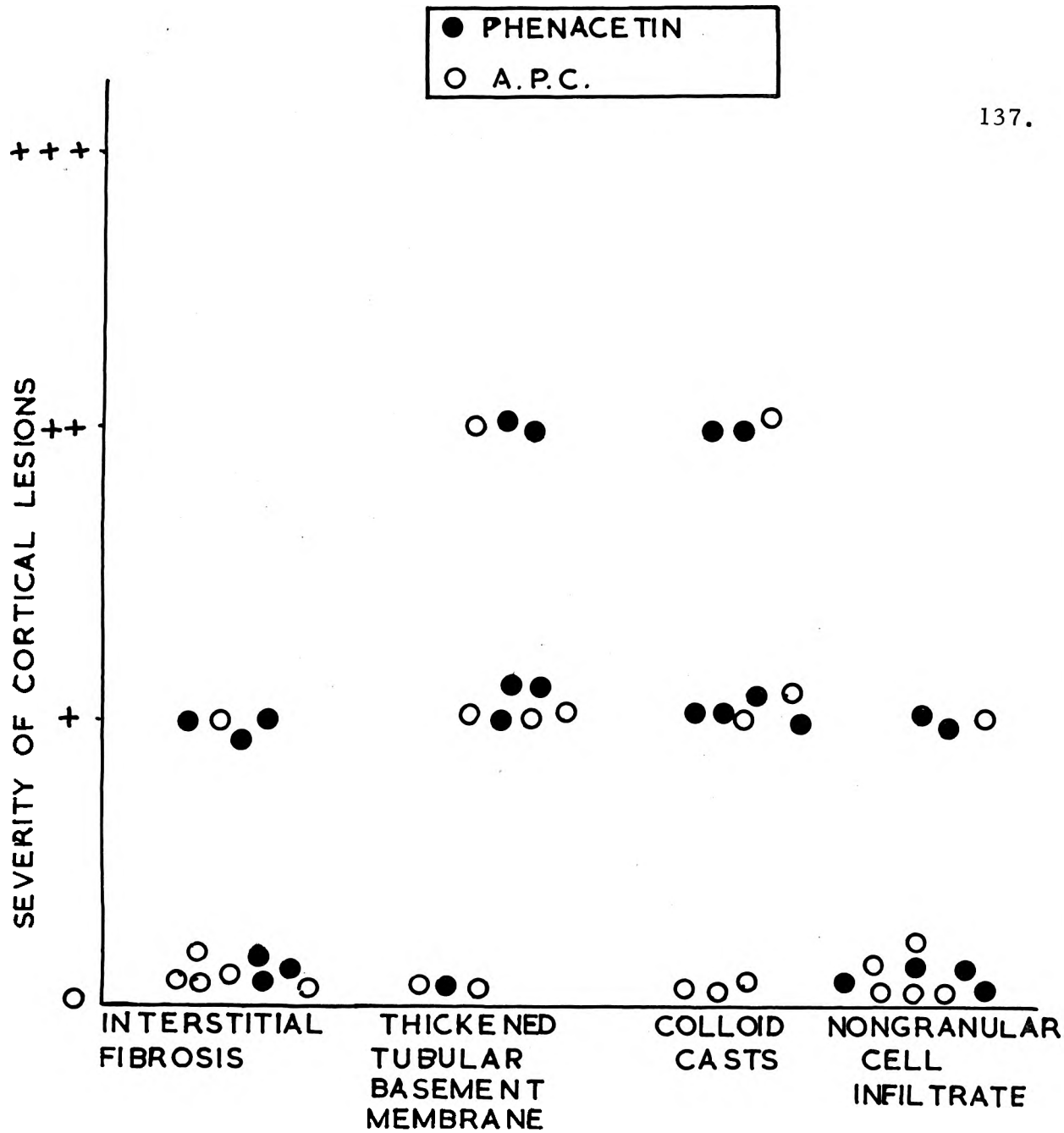

Fig. 44: Relationship between the degree of severity of cortical changes and the development of papillary haemorrhage in rats fed phenacetin and A.P.C. tablets.

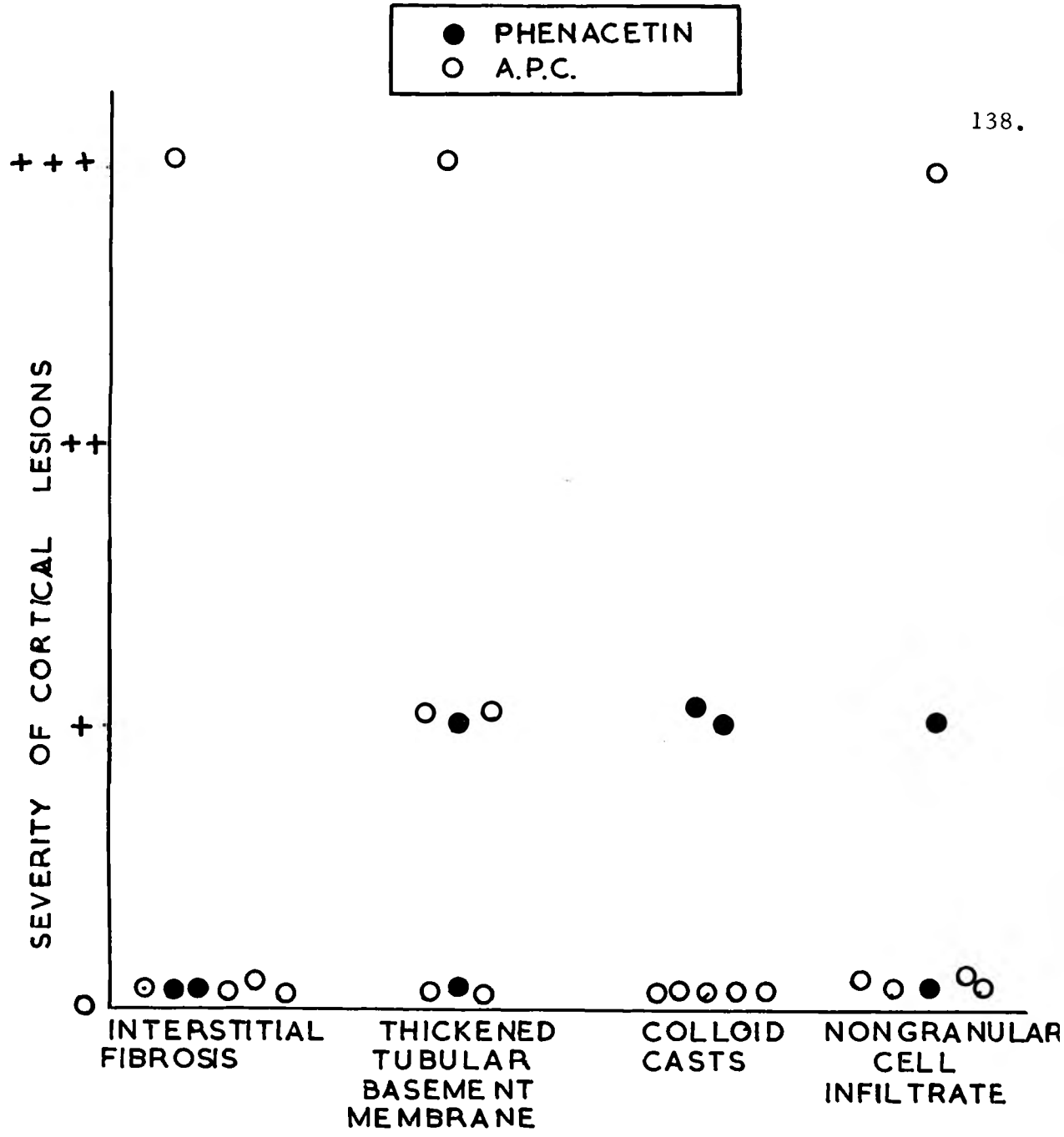

Fig. 45: Relationship between the degree of severity of cortical changes and the development of papillary necrosis in rats fed phenacetin and A.P.C. tablets.

animals (figs. 44, 45).

Eisalo and Talanti [46] failed to produce papillary lesions after feeding 3,000 mgms of phenacetin per rat for one month. They state that this is because of papillary lesions in man occurring in chronic states, whereas changes in their experimental rats are acute. In the present experiment the earliest haemorrhage and necrosis occurred after 7 3/4 months of feeding. This rat, fed on A.P.C. tablets, ingested only 3,900 mgms of phenacetin. As opposed to this papillary haemorrhage and necrosis occurred after 9,500 mgms and 14,500 mgms of pure phenacetin had been fed for 11 and 13 months respectively (see fig. 28, Chapter IV).

Angervall et al. [36] fed rats phenacetin for 5 months at 60 mgms per day, i.e. a total of 9,450 mgms per rat. Even in the presence of E. coli intravenously and massage of the kidney, necrosis of the renal papilla was not observed.

Thus it would appear from these findings that renal papillary necrosis in experimental animals on phenacetin and A.P.C. is not directly related to the presence of infection in the kidney and the infection that may be present is probably a secondary phenomenon.

b) Role of chemicals in causing vascular obstruction

The second of Lauler's suggested causes of renal papillary necrosis is that attributable to the effect of poisons. Robbins and Angrist [75] feel that the death of tissue en masse followed by a variable amount of inflammatory reaction is suggestive that the lesion is infarct-like in nature. Mandel and Popper [98] agree with this and feel that some vascular disturbance consequent upon vinylamine poisoning, must have been the cause of the medullary necrosis.

As far as is known at present neither phenacetin nor aspirin give rise to vascular spasm.

c) Possible role of abnormalities in fat metabolism in causing vascular obstruction

The third and final pathway of development of renal papillary necrosis is that of a disturbed metabolic state. In this the production of renal papillary necrosis is related to fat free diets. As already mentioned, the renal papilla and in particular the interstitial tissue has been poorly studied. For this reason a personal study of the interstitial tissue of the renal papilla was undertaken [99].

1. The interstitial tissue of the renal papilla.

Numerous specialised interstitial cells are present in the interstitial tissue of the renal papilla of the rat (fig. 46). These cells are present betweens the ducts of Bellini and sometimes lie in close proximity to blood vessels (fig. 46). The cytoplasm is rather unusual in that it contains numerous large electron dense bodies which show the characteristics of fat droplets. These fat droplets are also found to accumulate in the interstitial tissue (fig. 47). The function of these cells is not clear. Sternberg et al.[100] used a tetrazolium staining procedure on frozen section and found that these cells were arranged at right angles to the collecting ducts. Noting a certain cytological resemblance to the pericapillary Rouget cells and suggesting that they may be contractile, as has been though by some of Rouget cells, Sternberg et al., nevertheless considered the interstitial cells to be related to the collecting ducts rather than blood capillaries. They suggested that the cells might be able to constrict the lumina of the collecting ducts and thus facilitate tubular reabsorption. They may possibly be the end organs for antidiuretic hormone action.

Novikoff[101] confirms the presence of abundant interstitial cells throughout the rat renal papilla and finds the cells more numerous in the basal than the apical half. However, he disagrees with Sternberg

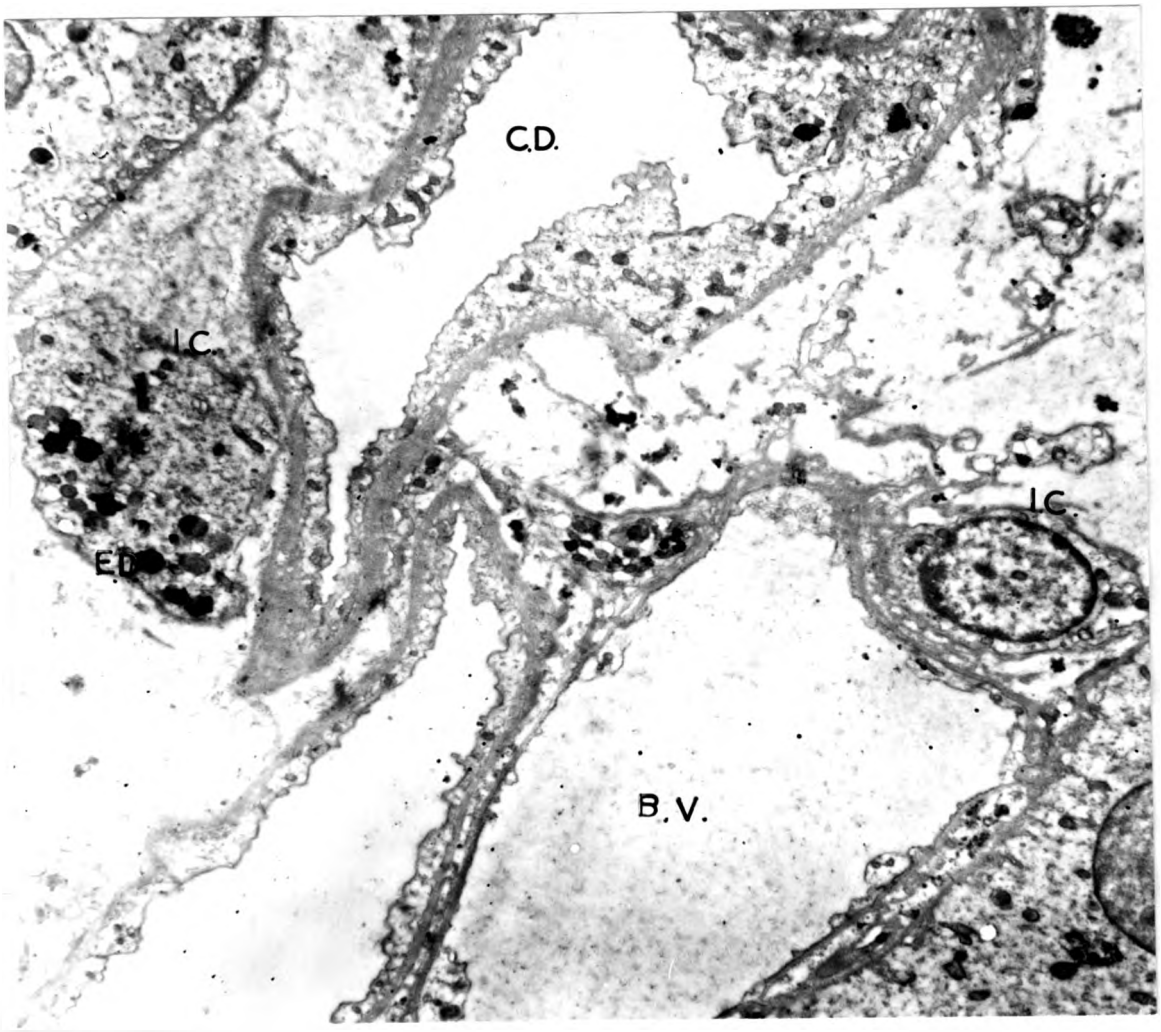

Fig. 46 Electron micrograph of the interstitial tissue of the renal papilla of a rat. Note the presence of interstitial cells (I.C.) containing electron dense bodies (E.D.) and lying in close proximity to a blood vessel (B.V.) and a collecting ductule (C.D.)

Magnification X 3,000

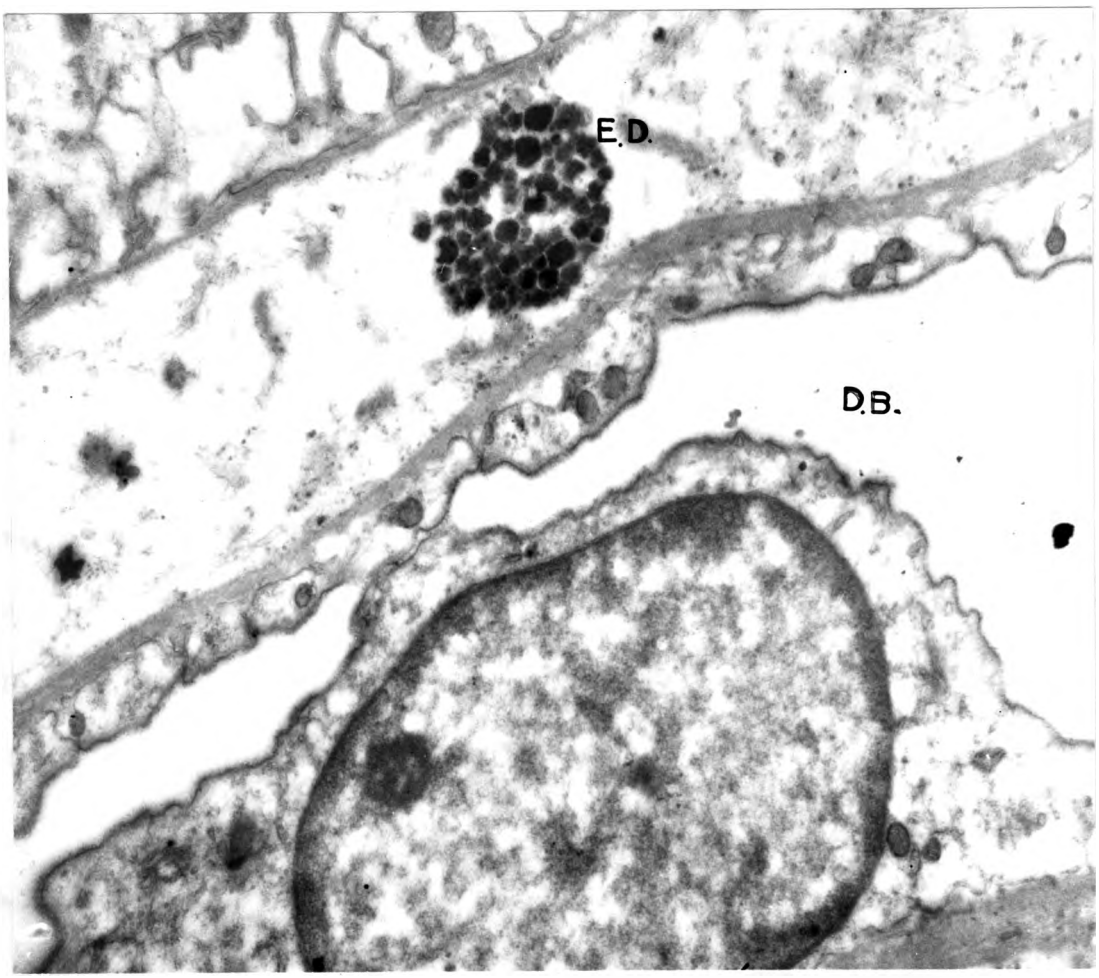

Fig. 47 Electron micrograph of electron dense lipid granules (E.D.) in the interstitial tissue of the renal papilla alongside a duct of Bellini (D.B.)

Magnification X 20,000

et al. in finding that the cells are closely related to the blood capillaries. In fact, on electron microscopy, there is often attachment of interstitial cells to the blood capillaries. On the other hand, although they may be close to the collecting ducts, and more frequently to loops of Henle, actual contact seems restricted to a few fibres resembling the basement membranes.

Novikoff states that if indeed the interstitial cells are contractile, this may constitute a means of regulating the blood flow. It may be related to the finding of Lilienfield et al. [102] that the papilla of the kidneys which had been removed from the dog and which had been frozen is "extraordinarily deficient" in erythrocytes. Longley et al. [103] also observed these interstitial cells in close contact with the blood capillaries of the vascular bundles. These interstitial cells may have other roles. Novikoff [101] suggests that they may secrete a material into the interstitium and basement membranes. An even more speculative role for these cells may be suggested by the striking numbers of large lipid droplets in their cytoplasm. Perhaps they play a part in lipogenesis, particularly as the cells in the neighbourhood contain enzymes which can take part in fat metabolism.

2. Relationship between abnormal fat diet and vascular occlusion

Borland and Jackson [91] have shown an increase in fat in the renal papilla of their rats on fat free diet. Whether the accumulation of the fat is intra or extracellular is not known. Fat strains done on the renal papilla of a few of the experimental animals in the present study showed much fat, but in view of the normally large quantities of fat in this site, it was difficult to judge an increase when compared with controls. However, it is conceivable that the fat accumulates in the interstitial tissue and compresses the capillaries thereby giving an ischaemic necrosis. Whether the accumulation of fat, if it occurs, can give a stimulus for increased contractility of interstitial cells is unknown. It is also unknown whether in man the interstitial tissue of the renal papilla has a similar structure to that in rats. A personal study of mice and guinea pigs shows similar features in the interstitial tissue to those in the rat but with less interstitial cells [99].

3. Summary of the causation of renal papillary necrosis

All pathogenetic pathways of renal papillary necrosis appear to lead in the same direction - a precarious blood supply challanged in numerous ways. Acc epting the possibility of a vascular obstruction as the main cause of renal papillary necrosis the question arises

whether the occlusion is arterial or venous.

In the course of an experiment in rats, in which ligation of renal veins and arteries and their main branches was performed, Beswick and Schatzki [104] observed that there were clear cut differences between the distribution of infarcts which resulted from occlusion of veins or of arteries. Arterial infarcts always involve the whole thickness of the cortex as well as of the medulla, whereas venous infarcts involve only the medulla with slight limited spread to the cortex. Ligating renal veins in animals gives infarction of the medulla [95, 97, 107] Marks [84] describes a case of exsanguination of an infant being followed by medullary necrosis. He suggests that the condition is probably due to renal vasospasm following hypotension. The vasospasm differed from the usual pattern by involving medullary rather than cortical arteries and arterioles. As far as is known neither phenacetin nor aspirin can cause vasospasm.

There is evidence, however, that in the present experiment, papillary damage in the rats fed phenacetin and A.P.C. was associated with vascular obstruction. In one phenacetin/aspirin fed rat who developed total papillary necrosis, there was thrombus present in the

renal vein. Although thrombi were not observed in other animals with papillary damage, the presence of haemorrhage into the renal papilla could indicate vascular occlusion. Murphy and Campbell [97] tied off the renal veins in rats and found general venous congestion and "red infarcts", Early on the red blood cells were intact. By 48 hours degeneration and necrosis of the red cells, tubular epithelial cells, and of the medulla and pyramid was evident. These findings may indicate that in the present experiment the haemorrhage may precede the extensive papillary necrosis.

The question may arise as to whether the bleeding into the renal papilla is due to the drugs. In the case of combinations containing aspirin this is possible, although unlikely because the bleeding associated with aspirin overdosage usually occurs into the gastro-intestinal tract. A bleeding tendency has not been described as being a side effect of phenacetin. Thus in both cases the haemorrhage probably indicates an obstructive vascular lesion.

Although many of the features of the extensive infarcts in the present experiment point to an obstruction of a large renal vein, as seen in the one phenacetin /aspirin animal, the possibility of

occlusion of smaller vasa recta cannot be excluded. Particularly because the areas of haemorrhage and necrosis are often focal and small and hence more likely to be due to obstruction of a smaller vessel. The cause of this could be related to accumulation of interstitial fat with compression of the vasa rectae.

A similar variation in the size of the damaged area in the renal papilla is seen in diabetes and in experimental papillary necrosis. Robbins and Angrist [75] state that in diabetes the size of the necrosis of the renal papilla varies. It may be:

1) confined to the tip of the papilla
2) confined to a small central part of the papilla
3) involve most of the papilla
4) involve all of the papilla and part of the pyramid (extending in rare cases up to the cortico-medullary junction)
5) the necrotic papilla may show a separation at the line of demarcation from the rest of the pyramid
6) the papilla may be sloughed entirely.

The cause of the papillary necrosis in diabetes is probably occlusion of small vasa rectae by interstitial oedema and in flammatory cell

infiltration or possibly accumulation of interstitial fat. This would compress the small arteries in the renal pelvis [81,82,83]. Such compression may be caused by accumulation of interstitial fat in the A.P.C. fed rats. On the other hand Beswick and Schatzki [104] feel that smaller veins are more probably occluded partially or completely. They found that tying off the renal vein completely led to total infarction of the medulla. Partial occlusion led to either:

1) total infarction of the medulla with slight cortical extension,
2) total infarction of the medulla confined to the medulla
3) focal infarcts in the pyramids
4) focal necrosis of tubular epithelial cells in the papillae.

So that it would appear that both obstruction of the renal vein by thrombus and the vasa rectae by local pressure can give rise to similar histopathological changes of haemorrhage and necrosis of varying sizes in the renal papilla.

CHAPTER IX

CONCLUSIONS

The kidney like many organs has only a limited number of ways of responding to various injurious agents. On the other hand there are fairly numerous causes of renal damage. Thus it is easy to confuse the histopathological pictures of the end stages of these nephrotoxic conditions, and very difficult if not at times impossible to separate out the aetiology and pathogenetic mechanisms of the final picture caused by kidney damaging agents. It is further conceivable that any tissue that has become scarred may be more prone to other different and new injuries thereby complicating the situation. Thus the existence and specificity of new renal diseases becomes very difficult to prove particularly when only the final picture is available for diagnosis.

There has been much debate as to whether "phenacetin nephritis" is a specific entity or merely a form of chronic pyelonephritis. However the histological picture of chronic pyelonephritis is rather nonspecific and a similar pattern of changes can probably result from causes apart from infection. As already discussed in Chapter VII (page 118) even in the hands of experienced renal pathologists the diagnosis of chronic pyelonephritis may present great difficulty and variance of opinion. It is difficult at times to separate this

condition from others. In the rats in the present experiment, a picture almost indistinguishable from the so-called "infective chronic pyelonephritis" occurred spontaneously at the age of two years. This was first reported by Saxton and Kimball[45] and later by Magee[44]. Although a total of at least 200 rat kidneys were sectioned at different ages throughout this experiment, histological evidence of acute pyelonephritis was not observed. And yet a picture supposedly identical to that of chronic pyelonephritis was found. This may have resulted from obstructive causes due to some ageing effect in the papilla or possibly due to reflux. The point of importance, however, is that the end picture which is identical to the classical description of chronic pyelonephritis, has most probably not been caused by acute infection. If this is so, can one in the case of human phenacetin nephritis, totally exclude a specific entity merely because the condition has a similar histological picture to chronic pyelonephritis another more commonly occurring renal disease, whose own pathogenesis and diagnosis even after an extensive symposium[106] is still not fully elucidated ?

One of the methods of separating pathological conditions

that simulate one another is by grading the frequency and severity of the histological features of the two diseases and by comparing them in an attempt to show a statistical difference. Using the conclusions that can be drawn from such an analysis, an attempt can be made to separate the two conditions. This has been done in the case of human chronic pyelonephritis and phenacetin nephritis as described in Chapter V (pages 95, 96). Using the criteria of tubular basement membrane thickening, marked interstitial fibrosis, lack of non-granular cell infiltrate and the presence of lipofuscin pigment human phenacetin nephritis can usually be differentiated from chronic pyelo-nephritis. Thus it may be seen that the final histological diagnosis of human phenacetin nephritis rests upon a few histological features many of which occur in chronic pyelonephritis but with a different severity and frequency.

As has already been detailed there is some debate about the specificity of phenacetin nephritis. The definitive proof of this condition as a distinct entity rests with the results of animal experiments. For this reason and because of the confusion that exists the present experimental study of feeding phenacetin, A.P.C. tablets and combination of aspirin, phanacetin and caffeine to rats was under-

taken. Although the results obtained indicate that in the case of the cortical lesions, the findings must be treated with reserve because of the presence of the "spontaneously occurring renal lesions in rats", it would appear however that phenacetin and A.P.C. tablets can cause a naturally occurring lesion to occur more frequently and severely. In the case of the findings in the renal papilla there can be no doubt about the significance of the changes produced in these experiments. The presence of papillary damage and in particular papillary necrosis has been regarded as characteristic of human phenacetin nephritis. The findings of these experiments would therefore furnish unequivocal evidence of a relationship between renal damage and in particular papillary necrosis and the excessive ingestion of phenacetin.

Schreiner [39] in an editorial dealing with the problem of the nephrotoxicity of analgesic abuse states that -

> "almost all the reports indicate that ingestion is usually in the form of mixtures varying in composition, but qualitatively similar to our "A.P.C." tablets containing aspirin, phenacetin and caffeine. Assuming that there is a specific nephritis associated with abuse of analgesics, one is immediately faced with a series of possibilities".

Schreiner poses four problems relating to the subject, three of which can probably be partly answered from this study.

1. Does nephrotoxicity stem from one of these drugs, some combination of them, or their normal metabolites?

In the present study although phenacetin alone proved to be nephrotoxic, the most severe lesions occurred when phenacetin was combined with aspirin, and in particular in the production of papillary haemorrhage and necrosis. Not only did this combination occasion more frequent papillary lesions, but the changes occurred earlier and also after smaller doses of phenacetin had been fed. The combination of aspirin and caffeine, although producing renal papillary haemorrhage, produced only minimal cortical lesions.

2. Can one drug affect the metabolism of another and produce an abnormal metabolite or delayed excretion?

Although it is known that aspirin is very slowly excreted from the body compared to phenacetin, there is no evidence that this caused a delayed excretion of phenacetin. The possibility of an abnormal metabolite being produced cannot be excluded.

3. Is the toxicity of the drugs synergistic?

As discussed above in view of the more severe renal lesions occurring in the presence of the combination of phenacetin and aspirin, it would appear that the toxicity is synergistic. The possible causes of the accentuation of the effects have been discussed in Chapter VI (page 106), the most important of which is the release of haemoglobin pigments by the phenacetin which in the acid milieu caused by the acetylsalicylic acid is responsible for renal damage. This of course does not exclude the individual effects that phenacetin and aspirin may have upon the kidney as described in Chapter VI, VII and VIII. These would act together but if present alone would cause renal damage.

4. Is there a contaminant in the manufacturing process that results in the nephrotoxicity?

This has been dealt with in Chapter VI, where it was shown that the phenacetin and A.P.C. tablets manufactured according to the British Pharmacopoeia, do not contain the contaminant acetic-4-chloranilide in sufficient doses to cause renal damage.

Further studies that could be done in relationship to the problem of phenacetin nephritis are:

a) Phenacetin and A.P.C. should be fed to younger rats than those used in the present experiment so as not to confuse any cortical changes that may be produced with the spontaneously occurring renal lesions,

b) Both the cortical and papillary changes should be examined with the electron microscope and with enzyme and histochemical techniques in order to ascertain the earliest changes occurring in phenacetin fed animals.

c) In particular the lesions in the renal papilla should be studied in order to prove or disprove the role of the interstitial cells and interstitial lipid in causing papillary haemorrhage and necrosis.

d) A metabolic and biochemical study of the animals fed phenacetin and A.P.C. tablets may indicate some of the pathways and mechanisms of renal damage. In particular a study of the effects of these drugs on fat metabolism may prove significant in relationship to papillary changes.

e) In view of the debatable role of infection in the causation of phenacetin nephritis, a bacteriological examination of the experimentally fed animals is imperative.

Thus although this study opens up many avenues of further research into drug induced renal disease, it would tend to indicate that phenacetin and in particular phenacetin in combination with aspirin is an important nephrotoxic agent.

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