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The Reversible Nephrotoxic Effects of Biphenyl

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During the course of chronic toxicity studies on biphenyl administered to rats in the daily diet (Ambrose *et al.*, 1960), urine samples were collected from control and experimental animals for study of the metabolic fate of biphenyl. It was noted that rats on dietary levels of 0.5 and 1.0% biphenyl exhibited polyuria and that increased volumes of solids settled out of the urines after standing overnight. The volumes of urines and solids were proportional roughly to the level of biphenyl fed (Booth *et al.*, 1956). The purpose of this work was to study in more detail the relationship between biphenyl intake and polyuria. In addition, biphenyl kidney damage and reversibility thereof, as determined by microscopic examination, was compared with biphenyl dosage and urine abnormalities.

METHODS

All urine measurements were made on urine samples collected overnight during the 16-hour period from 5:00 P.M. to 9:00 A.M. During this time the rats, in groups of five, had free access to food and water. Special metabolism cages were used in order to keep the feces and urine separated. To determine the volume of precipitate excreted, 4 ml of a 3% aqueous sulfosalicylic acid solution was added to a 2-ml aliquot of the freshly voided urine in a 12-ml calibrated centrifuge tube. The resulting precipitate was packed by centrifugation before the volume was read. The total volume of precipitate excreted by five rats was

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calculated by relating the solids volume of the aliquot to the total volume of urine.

The basal diet used the following composition, in per cent: degerminated yellow cornmeal 73, crude casein 10, linseed oil cake meal 10, cod-liver oil 3, ground alfalfa 2, bone ash 1.5, and sodium chloride 0.5. The effects of prolonged ingestion of biphenyl on growth, food intake, reproduction, and mortality were reported earlier (Ambrose *et al.*, 1960).

RESULTS

Urine Studies

The precipitate occurring upon the addition of a 3% solution of sulfosalicylic acid to the fresh urine of rats fed biphenyl was initially presumed to be protein. However, it was noted that when the urine was stored in the refrigerator, a fairly large volume of solids settled out after 24 to 48 hours without the addition of sulfosalicylic acid. Furthermore, determination of the nitrogen content of the sulfosalicylic acid precipitate indicated it was not protein. Examination of the precipitate and fractionation by solvent partition showed the presence of two major components. One fraction was identified crystallographically as *p*-hydroxybiphenyl. Solubility characteristics and a positive naphthoresorcinol test for glucuronic acid after hydrolysis established the other component as the glucuronide of *p*-hydroxybiphenyl. These results are in agreement with those reported by West *et al.*, 1956. The glucuronide of *p*-hydroxybiphenyl was synthesized (Gee *et al.*, 1957) and found by paper chromatography to be similar to the isolated urinary glucuronide, including phosphorescence under ultraviolet (UV) light, R_f values in two dimensions, and formation of a yellowish color after spraying with alkaline diazotized sulfanilic acid.

In a preliminary experiment, five adult rats were started on the basal diet containing 1% biphenyl to determine the time of onset, the magnitude of increase with respect to time, and the reversibility of the urine volumes and precipitated solids content. No evidence of polyuria was noted during the first 4 days. The urine volume on the 4th day was 7 ml, and the volume of sulfosalicylic acid precipitate after centrifuging was 0.56 ml. Urine collections were made on the 8th, 18th, and 20th days. These collections showed progressive increases in urine volume, urine turbidity, and volume of precipitate, being 32 ml and 2.24 ml, respectively, on the 20th day. On the 26th day the volumes of urine and precipitate were still high compared with the original values; and in addition, a positive

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benzidine test was obtained, suggesting the presence of blood in the urine. The rats were then placed on the basal diet without added biphenyl, and observations on the volumes of urine and precipitate were made on the 28th, 32nd, 35th, and 54th days. The observations showed a pronounced trend back to normal volumes. The data are shown graphically in Fig. 1.

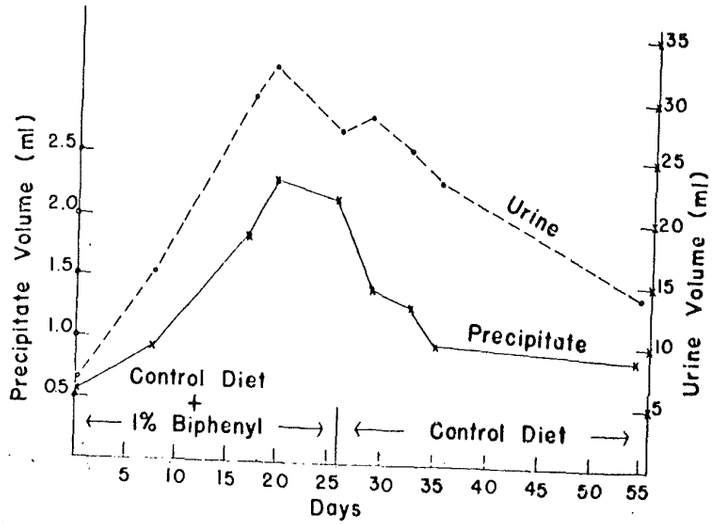

Fig. 1. Effect of 1.0% dietary biphenyl on volumes of urine and urinary precipitate of male albino rats.

Urine collections from groups of either male or female rats on diets containing 0.5% biphenyl produced effects comparable to those observed on 1.0% biphenyl. None of these effects were observed on diets containing 0.1% biphenyl.

These observations raised questions regarding kidney damage associated with polyuria, time of onset of kidney damage, minimum dosage of biphenyl required, relation of extent of kidney damage to exposure time, and reversibility of kidney injury. To answer these questions a large scale experiment was designed. Forty-two rats of each sex were placed on each of four diets consisting of the basal ration containing 0.0, 0.1, 0.25, and 0.5% biphenyl. Urine collections were made periodically from groups of five rats of each sex on the control diet and on each of the levels of biphenyl. At the end of 30, 60, and 120 days five rats of each sex on each diet were sacrificed. Sections of kidney stained with hematoxylin-eosin were prepared for histopathologic examination. After 165 days on the diet containing 0.5% biphenyl, 10 rats of each sex were returned to the control diet. Five rats of each sex were sacrificed for histopathologic study of the kidneys after being on the control diet for periods of 30 and 60 days. During these periods urine collections were made from time to time for measurement of the volumes of urine and sulfosalicylic acid precipitate.

On the 27th and 28th days rats fed a dietary level of 0.5% biphenyl showed a definite increase in volumes of urine and precipitate as compared with the controls. These effects were much less marked for rats fed a 0.25% level of biphenyl and were absent at the 0.1% level. At the end of 60 and 120 days the increases in volumes of urine, turbidity, and precipitate were progressively more striking for rats (both sexes) on a a dietary level of 0.5% biphenyl but were of questionable significance on the lower dietary levels. Measurement of urine and precipitate volumes were made 10, 30, and 65 days after rats fed 0.5% biphenyl for 165 days were returned to the control diet. By the 10th day the urine and precipitate volumes had decreased markedly and on the 30th day were practically normal.

Histopathologic Studies

At the end of 30 days the kidneys of male and female rats fed 0.1% and 0.25% levels of biphenyl showed no recognizable changes as compared with kidneys of control animals. The kidneys of one of five male rats fed 0.5% biphenyl showed several small cysts and dilated tubules in the medulla and inner cortex. Kidneys of the other four males were not distinctly altered. Kidneys of two of five female rats on the same dosage of biphenyl showed mild local tubular dilation with some epithelial flattening, which may represent an extremely early change. No abnormalities were found in kidneys of the other three female rats.

Rats sacrificed after 60 days on the control and biphenyl-containing diets showed histopathologic kidney changes similar to those noted in rats sacrificed at the end of 30 days but were distinctly more frequent and more extensive. The kidneys of all female rats and three of five male rats fed 0.5% biphenyl showed distinct changes. One of five female rats on 0.25% biphenyl showed a single prominent kidney lesion.

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Histopathologic study of kidneys of rats fed the various levels of biphenyl for 120 days revealed changes somewhat more prominent than at the end of 60 days. All kidneys from both male and female rats fed 0.5% biphenyl showed distinct focal tubular dilation (Fig. 2) although only a few tubules were involved in four of the female rats. Kidneys of male rats showed several zones of involvement, several millimeters in



FIG. 2. Section of a kidney from a rat which received 0.5% biphenyl in the diet for 120 days. A localized zone of irregular tubular dilation occupies the center of the field. Several large segments of dilated tubules contain blood. Inflammation is minimal. Magnification: ×50.

diameter, clearly affecting a number of tubules. Adjacent to the dilated tubules there were some small atrophic tubules, a little cellular fibrous tissue, and a few lymphocytes. None of the kidneys of rats receiving 0.25% biphenyl showed dilated tubules. One kidney on this dosage level had a small calculus in the pelvis, and another had a small calcareous deposit in a renal pyramid. No abnormalities were found in the kidneys of rats fed 0.1% biphenyl.

Histopathologic examination of kidneys from rats returned to control diet for 30 and 60 days after being on biphenyl-containing diets for 165 days showed distinct evidence of regression of lesions. After 30 days on the control diet the lesions were distinctly less pronounced than those found in rats fed 0.5% biphenyl for 120 days. Focal tubular dilation was less marked and scars were more conspicuous, suggesting the site of regressed

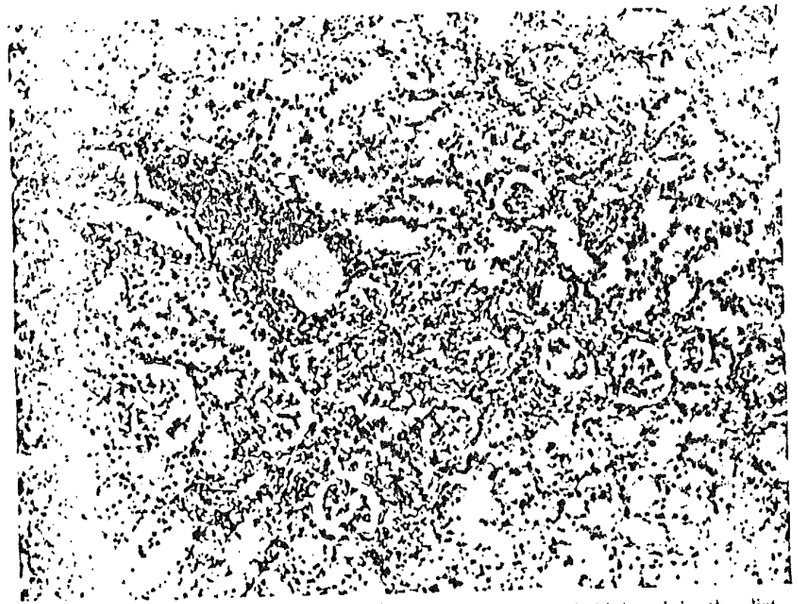

FIG. 3. Section of kidney from a rat which received 0.5% biphenyl in the diet for 165 days, followed by a control diet for 60 days. A zone of scarring in the center of the field is characterized by small, closely placed glomeruli. Atrophic tubules are obscured by fibrous tissue in which are scattered lymphocytes. Magnification: ×123.

lesions. Lymphocytes were numerous in some of the scars. Changes were seen in all the male rat kidneys. The kidneys of one female rat were not distinctly altered, those of a second showed only a few tiny, poorly defined scars, and a third showed only a mild acute pyelonephritis. Kidneys of the remaining female rats resembled those of the male rats, but the lesions were less pronounced. In general, kidneys of female rats were much less altered than those of male rats. At the end of 60

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days on the control diet, the kidneys of one of five male rats previously fed 0.5% biphenyl were normal, those of three male rats had a few inconspicuous scars, and those of the fifth rat presented a variety of more prominent scars (Fig. 3). The latter also contained several isolated cystic tubules up to 2 mm in diameter. Most of these had a peripheral rim of fibrous tissue, and they usually contained some blood and leucocytes. One of the less altered kidneys also showed two small cystic tubules. Kidneys from two of the female rats were essentially normal, while those of the remaining three female rats contained small scars and focally dilated tubules in several places.

DISCUSSION

The urinary evidence of an effect of biphenyl on the kidneys was negative at all times at a level of 0.1% biphenyl in the daily diet, and the histopathologic evidence of kidney damage was always negative. On the 0.25% level of biphenyl the urinary evidence was only slightly positive, and only a single prominent lesion was found in one kidney of a female rat upon microscopic examination at the end of 60 days. The findings at the end of 120 days on this dosage level were not appreciably different. On the 0.5% level of biphenyl intake the urinary evidence of altered kidney function was striking at the end of 30 days and became increasingly pronounced at 60 and 120 days. The presence of blood in the urine, as indicated by a positive benzidine test, occurred sporadically after 30 days. The histopathologic evidence of kidney damage was definite in only 3 of 10 rats at the end of 30 days, becoming more pronounced and extensive and involving more animals at the end of 60 days. At the end of 120 days, the histopathologic picture was somewhat more prominent than at the end of 60 days, but the difference was not great. The observations suggest that the urinary evidence is a more delicate criterion of kidney function altered by biphenyl than is microscopic examination of the kidneys. This suggestion is fortified by the fact that rats returned to control diet for periods of 30 and 60 days after maintenance on a diet containing 0.5% biphenyl for 165 days showed a regression of kidney lesions, as judged by histopathologic examination, but an even more rapid return to a normal state based on the volume of urine and solids values.

On the high levels of biphenyl intake (0.5 and 1.0%) the renal hemorrhages and calculi with basophilic staining foci are believed to be due to the irritation produced by the continuous streaming of biphenyl metabolites of very low solubility through the kidney. Polyuria and reduced food intake are likely to be defense mechanisms on the part of the animal to minimize or reduce the kidney irritation.

SUMMARY

Rats ingesting diets containing 0.5% biphenyl developed nephrotoxic effects including focal tubular dilation in approximately 60 days. Rats returned to control diet for 60 days after ingesting a dietary level of 0.5% biphenyl for 165 days showed a regression of kidney lesions with scar formation. A more sensitive criterion of the renal effects of biphenyl was polyuria, which is probably a defense mechanism response to the irritant action caused by the insoluble biphenyl metabolites passing through the kidney. No evidence of either polyuria or histopathologic damage was observed from the ingestion of 0.1% biphenyl in the diet for 120 days.

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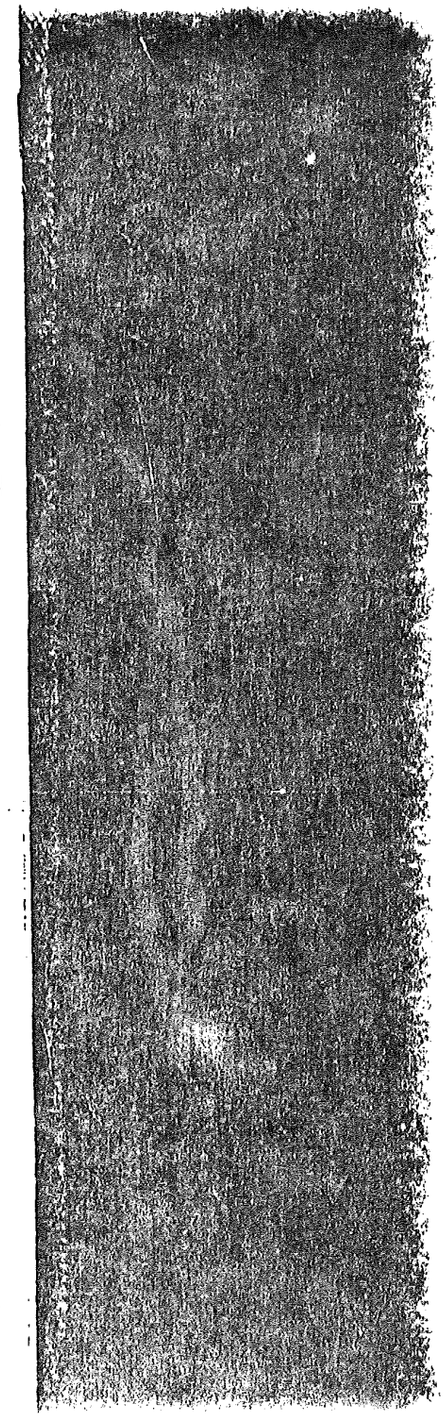

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