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# Diphenylamine-Induced Renal Lesions in the Chicken

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Summary. Chronic intoxication with diphenylamine (DPA), which causes a cystic kidney disease in the rat and the guinea-pig, caused degeneration of the renal tubular epithelium in the chicken. This was similar to but much more serious than that preceding the formation of cysts in the rodents, but did not actually result in cyst formation, probably because of the high mortality rate observed in the birds even at this early stage. In the chicken until now it had been possible to obtain a pattern of renal cysts only with polychlorinated biphenyls (PCB) which also induce the "chick oedema" syndrome. The renal lesions due to DPA in the chicken were similar to those produced by PCB, but were not accompanied by oedema, which suggests that "chick oedema" caused by PCB is not due to renal insufficiency. The differences in the renal lesions noted in the various animal species give credit to the hypothesis that DPA may have two effects on the tubular epitelium, one stimulating cell proliferation and one leading to degeneration. Cysts may be formed only in those species in which there is cell proliferation.

Key words: Cystic kidney disease - Diphenylamine - Chick oedema.

In recent years the toxic theory of the pathogenesis of the adult type of polycystic kidney disease, in genetically susceptible subjects has gained wider acceptance. Normally formed nephrons are thought to be affected by cystic degeneration due to toxic substances as yet unidentified, (9, 10, 15). This theory is based on three fundamental points:

Previous theories regarding the polycystic kidney as a congenital malformation are contradicted by morphological and functional observations.

In the experimental animal it is possible to produce a cystic kidney similar to the human polycystic kidney by administering toxic substances.

In Europe, according to the data from the European Dialysis and Transplant Association (EDTA), cases of chronic uraemia from cystic nephropathies are increasing each year (2), a fact which it is difficult to explain without considering environmental factors.

Nothing is known of these hypothetical toxic substances responsible for the human pathology. The aim of the present studies was to find a substance which is cystogenous in the greatest variety of animals, and therefore a prototype for further research, and also to attempt to explain the mechanism of toxic cyst formation. For Diphenylamine (DPA), the most well-known substance causing experimental cystic nephropathy in rats and adult guinea-pigs (4, 6, 11, 12, 16, 17, 19) there is some controversy as to whether the nephrotoxic action is limited only to these rodents or is common to many animal species. This study investigated its effects in the chicken.

## MATERIAL AND METHODS

Twelve chickens, sixty days old and able to eat and drink ad libitum, were treated with diphenylamine (D. P. A.) added to their feeds in the ratio of 2 : 100. The diphenylamine used (E. Merck Company), had been produced previously, and preserved in our laboratory for over five years. The experiment lasted 120 days; the animals that died during this period and those killed at the end of the study were

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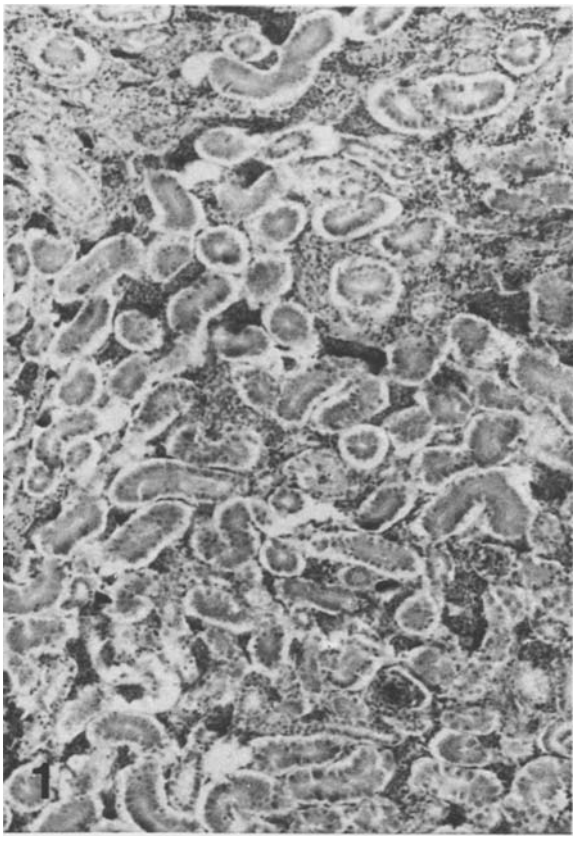

Fig. 1. Kidney of a chicken treated with diphenylamine. There are clear signs of the degeneration and epithelial scaling affecting all the tubules and the interstitial infiltration. x 140

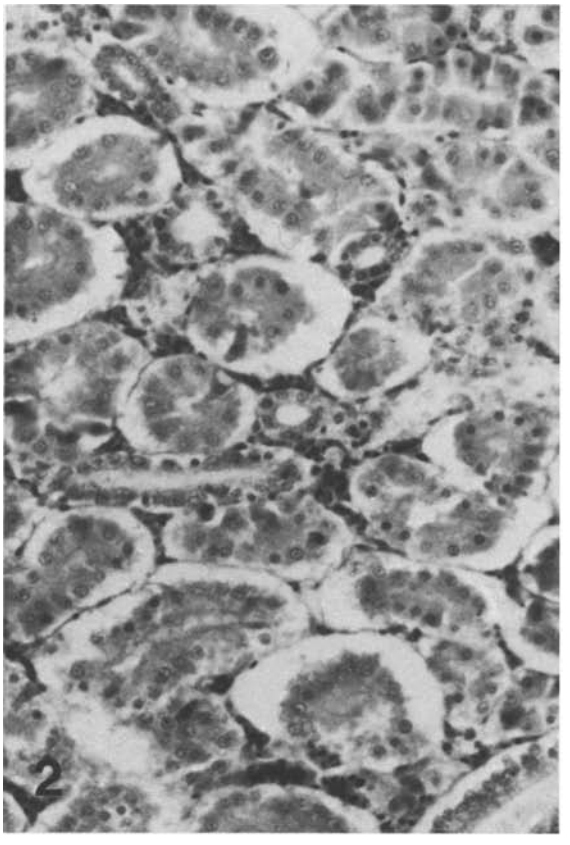

Fig. 2. Degeneration and detachment of the basal membrane from all the epithelium with occlusion of the tubules. x 350

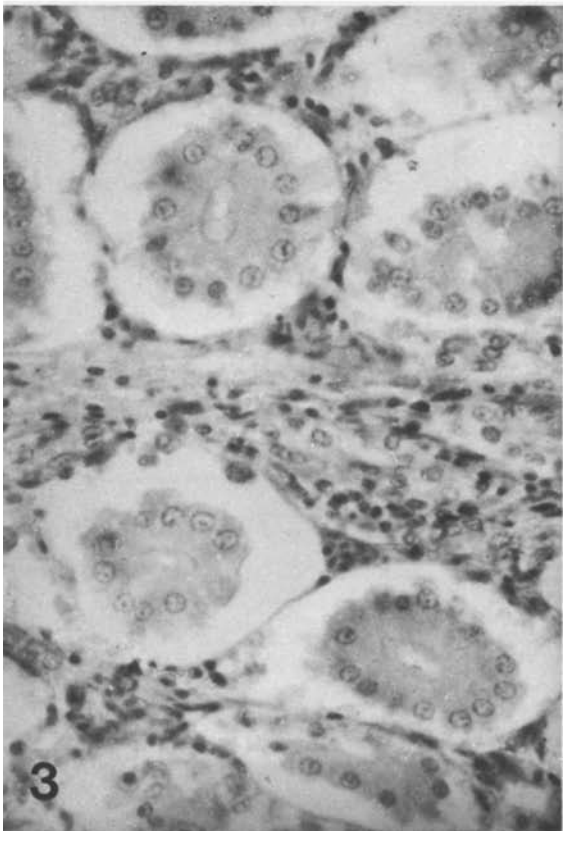

Fig. 3. Degeneration and detachment of all the epithelium from the basal membrane with signs of regeneration of the fibroblasts. x 400

autopsied and the principal organs were examined histologically, with special attention to the kidneys.

Twelve more chickens maintained under exactly the same conditions, but not treated with DPA, were used as controls.

## RESULTS

### Chickens Treated with DPA

Nine of the twelve chickens died between the 90th and 120th day; the three survivors were

killed at 120 days. All the chickens weighed 20 % less than the controls. The results of autopsy were the same for all the birds: there was no ascites, pericardial effusion or oedema. No particular signs were noted in any organ except the kidneys, which appeared swollen and oedematous.

Microscopic examination revealed that in the outer part of the kidney (the part corresponding to the medulla of the kidney of a mammal) there were areas in which the epithelium of all the tubules had degenerated and become detached from the basal membrane (Fig. 1). The tubular lumina appeared full of either recognisable cellular masses or amorphous PAS-positive material (Fig. 2). Only a few of the tubules showed evidence of regeneration of the epithelial cells and fibroblasts (Fig. 3). In some cases the basal membrane of two adjacent tubules appeared to be disrupted so that they constituted one cavity. In no case however was the formation of actual cysts observed. Interstitial lymphocytic infiltration was noted. Adjacent glomeruli and tubules were generally unaltered.

In the birds that died spontaneously the areas containing degenerated tubules appeared more extensive than those in the kidneys of the chickens killed at the end of the four-month period. Otherwise, the lesions observed were similar in all the animals.

### Control Chickens

None of the control chickens died spontaneously in the course of the 120 days; they were all killed between the 90th and the 120th day. Nothing abnormal was noted in the kidneys or the other organs.

## DISCUSSION

In order to explain how genetically determined human diseases can be induced by toxic substances, two main subhypotheses have been formed:

1. The toxin is environmentally endogenous but only affects genetically susceptible cases.
2. The toxin is endogenous, produced by a congenital error of the metabolism.

This second hypothesis is held almost exclusively by Darmady and colleagues (4, 8). Without excluding it entirely, since it may well be valid for some of the rare cystic nephropathies, we feel that it must be discarded for the polycystic kidney. The increased incidence of this disease as a cause of uraemia in Europe is an argument in favour of the influence of environmental factors.

It has been proved that the administration of DPA produced some time previously causes more severe cystic changes in the rat kidney than those caused by the administration of recently-produced DPA (3). This is probably due to the presence of degeneration products of out-dated DPA which may be more toxic than the DPA itself. This is why we preferred to use DPA which had been in our laboratories for over five years rather than recently produced DPA, even if this imposed a limit on the number of animals treated.

There is little agreement regarding the pathogenesis of renal cysts produced by toxins because the results have been observed in different species, at ages varying from embryonic life to adulthood, and using a variety of chemical substances.

Sorrentino, 1967 (12) observed in the adult guinea-pig treated with DPA an initial hyperplasia of tubular epithelium which gradually increased in severity, leading to an increase in volume of the tubules and formation of pseudo-adenomatous areas. This was followed by a phase of cell vacuolisation with consequent intraluminal scaling of the epithelium, and the tubules which remained empty assumed a cystic appearance. The third phase was epithelial regeneration. There was no clear distinction between the various phases and at the same time in the same microscopic field or the same tubule both scaling and regeneration could be noted.

Darmady et al. in 1970 (4) observed that in the adult rat treated with DPA, the first sign was degeneration of tubular cells with intraluminal breakdown followed by regeneration of new epithelium or even of fibroblasts. This cycle was repeated several times and each time the tubule was distended a little further until it assumed a cystic appearance.

Carone and colleagues (1) who used aminodiphenyltiazol in the rat, also described degeneration and scaling of the epithelium, but maintain that the toxin acts directly on the basal membrane of the tubules, causing them to lose their elasticity. The normal intratubular pressure, no longer balanced by the elasticity of the wall may then cause progressive dilation of the tubule. This interpretation, which had been favourably accepted by several authors, was not confirmed by Gardner et al. (5). According to these workers the intraluminal pressure is not normal but increased as the result of partial obstruction due to collapsed epithelium.

McGeoch and Darmady (8), who treated pregnant rats with 5, 6, 7, 8-tetrahydrocarbazole-3-acetic acid, and suggest that the mechanism with DPA may be the same,

maintain that the point of attack of the toxin is the $(Na^+ + K^+)$-ATPase of the cell membrane of the tubular epithelium. The effect is biphasal: in small doses the enzyme activity is stimulated while in larger doses it is inhibited. Since the enzyme controls the passage of ions through the membrane, variations in its activity lead to variations in the intracellular ion concentration and therefore in the membrane potential. It is known that such variations stimulate mitosis. In greater doses the toxin inhibits the enzyme and damages the cell membrane.

The alterations noted by us in the chicken kidney - dilation of the tubules and peeling back of the cells, breakdown of the basal membranes and fusion of two tubules - are fairly similar to those described by Darmady et al. (4), but differ from those observed by Sorrentino (12) particularly with regard to the absence of an initial phase of epithelial proliferation.

One explanation of the differences in the lesions observed in different animal species could be that DPA carries out two partially interdependant actions on the tubular epithelium, one toxic and degenerative, the other stimulating growth. This might be due to the biphasic action of the toxin on the enzymes of tubular cells, as demonstrated by McGeoch and Darmady (8). In different animal species, the difference between the minimum stimulating dose and the minimum inhibiting dose may vary. In those animals in which the two effects occur at different dosages, there is a stimulation phase followed by a degeneration phase in which renal cysts appear, while in other animals degeneration is immediate. This is the effect observed in the chicken and has two consequences: a greater mortality rate in the animals from kidney insufficiency at an early stage before the cysts appear, and therefore an apparently smaller tendency of the chicken to contract renal cysts. This lesser tendency in the chicken is supported by the fact that spontaneous or experimental cystic kidney disease has so far not been observed in birds.

Recently Resnick et al. (10), suggested that the tubular dilations observed by McCune et al. (7) and by Vos and Koeman (18) might in fact be cysts. In fact the microphotograph published in the article by McCune et al. recalls the initial phase of the polycystic kidney and also resembles our pictures. In both of these studies the chickens had been treated with polychlorinated biphenyls (PCB), substances which on the one hand are similar to biphenyl, but on the other hand often contain abundant traces of dioxine. PCB causes the death of chickens with a characteristic condition known as "chick oedema" consisting of general oedema, ascites, hydropericardium and hepatic necrosis as well as renal cysts. McCune et al. (7) maintain that the renal lesions are the first stage and that the ascites, hydropericardium and oedema are the result of the renal insufficiency and salt and water retention. We do not agree with this interpretation since while the chickens treated with DPA had renal lesions similar to those induced by PCB and serious enough to cause the death of most of the birds, no symptoms of "chick oedema" were observed.

In relation to human pathology, partially negative results similar to those observed in the chicken are of great interest and lead to the development of new working hypotheses. Until now the fact that the hypothetical environmental toxin required the presence of a particular gene in order to cause polycystic kidney formation had led to the supposition that the gene regulated detoxicating mechanisms. It was believed that once the toxin, chemically similar to DPA, was introduced into the organism, it was counteracted by conjugation with glucuronic acid. In the subjects who were carriers of the polycystic kidney gene this process of detoxication might be deficient either as the result of a conjugation defect, or, more probably, of an increase in the release of the active substance at the level of the kidney tubules due to beta-glucuronidase. However, experiments to show an increased enzyme activity in polycystic kidney carriere have had negative results (13).

The observation that DPA causes cysts only in certain animal species, and tubular degeneration in others, which could be due to variations in the difference between the minimum stimulating dose and the minimum inhibiting dose suggests that in the human the gene determining the appearance of polycystic kidneys may act by interfering with this process. The toxic factor therefore which in the rest of the population leads to degenerative changes at a tubular level, which either diminish or lead to chronic interstitial kidney lesions over a period of several decades, in the carrier of the gene leads to a hyperplasia of the cells of the tubular epitelium and therefore to the formation of cysts.

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