

LIVER AND KIDNEY DAMAGE FOLLOWING ACETAMINOPHEN INJECTION IN THE CHICKEN EMBRYO

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ABSTRACT

Chicken embryos were injected after four days of incubation with 60 mM or 80 mM acetaminophen via yolk sac. Livers and kidneys were reduced in size and some kidneys exhibited large clear blisters. Histological analysis demonstrates significant damage to both organs. Brush borders were absent from most proximal kidney tubules. Hepatic cells were generally smaller and hepatic cell rows were distorted.

INTRODUCTION

Reports of liver damage in humans, as a result of acetaminophen overdosages have been well documented (Lesna et al., 1976; McJunkin et al., 1976; Baker et al., 1977; Johnson and Tolman, 1977; Nogen and Bremmer, 1978; Gerber et al., 1980 and Black et al., 1982). Liver biopsies generally exhibit centrilobular degeneration and necrosis. Bile ductule proliferation and fibrosis of the portal area has been reported in some cases (Gerber et al., 1980; Black et al., 1982).

Renal damage has been observed in some severe cases (Stokes, 1976; Wilkinson et al., 1977; Kleinman et al., 1980; and Nogen and Bremner, 1978). Damage has also been observed in rats, following a subcutaneous injection of acetaminophen (McMurtry et al., 1978 and Kleinman et al., 1980).

The gross toxic effects of acetaminophen upon the four-day chicken embryo have been previously reported (Walton et al., 1986). Severe leg anomalies resulted. Of course, the severe skeletal defects reported for the chicken did not indicate that the human embryo and fetus would respond in a similar manner, but that caution should be used in the quantity of acetaminophen recommended for use by pregnant human individuals.

This paper reports the effects of acetaminophen on the liver and kidney of the four-day chicken embryo.

MATERIALS AND METHODS

Chicken embryos (Cornish versus White Rock; Keith Smith Farms, Hot Springs Arkansas) were incu-

bated in a forced draft incubator at 37 °C and 55-22% relative humidity. An injection stage of 22+ (Hamburger and Hamilton, 1951) was determined by sacrificing and staging four-day old embryos (15 embryos).

The procedure for preparation of acetaminophen concentrations of 60 mM (225 mg/Kg yolk weight) and 80 mM (300 mg/mg yolk weight) was the same as previously reported (Walton et al., 1986). The injection procedure was also the same.

TREATMENT GROUPS. Two control groups were made. Sham controls received a needle probe and the other controls received 4 ml of distilled water. Experimental group one received 60 mM acetaminophen in 4 ml distilled water and experimental group two received 80 mM acetaminophen in 4 ml distilled water.

ANALYSIS OF EMBRYOS. Eggs were rotated three times a day before and after treatment. Embryos were sacrificed after a total of 12 days of incubation. As embryos were sacrificed, they were examined for gross defects. Control embryos were staged according to Hamburger and Hamilton (1951). Experimental embryos were approximately staged.

HISTOLOGICAL ANALYSIS. Livers and kidneys were removed from three embryos of each control group and from five embryos of each experimental group. Organs were removed from experimental embryos that represented the varying forms of limb anomalies described previously (Walton et al., 1986). A stereoscopic dissecting microscope was used to examine the organs. Organ weights and widths were determined.

Organs were fixed in Bouin's solution and then embedded in paraffin (Peel-A-Way, Lipsaw Manufacturing Co.). Specimens were serially sectioned at 10 μ m and stained in Harris' Hematoxylin and in Eosin in 95% ethanol.

RESULTS

Kidney: Gross examination. Experimental kidneys exhibited large clear blisters over their surfaces (Figure 1). The blisters were filled with a clear fluid that contained scattered blood cells, in some cases. Control kidney weights averaged 21.9 mg while experimental kidney weights averaged 13.6 mg when treated with 60 mM acetaminophen and 6.8 mg

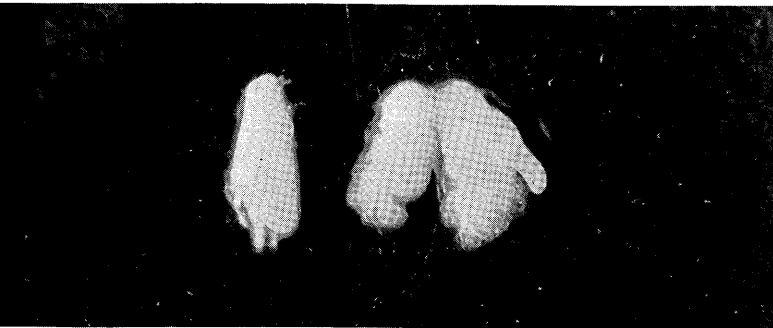


Figure 1 Single control kidney (left) and experimental (0.06 M) kidneys (right). Large clear blisters extend from the surfaces of both experimental kidneys.

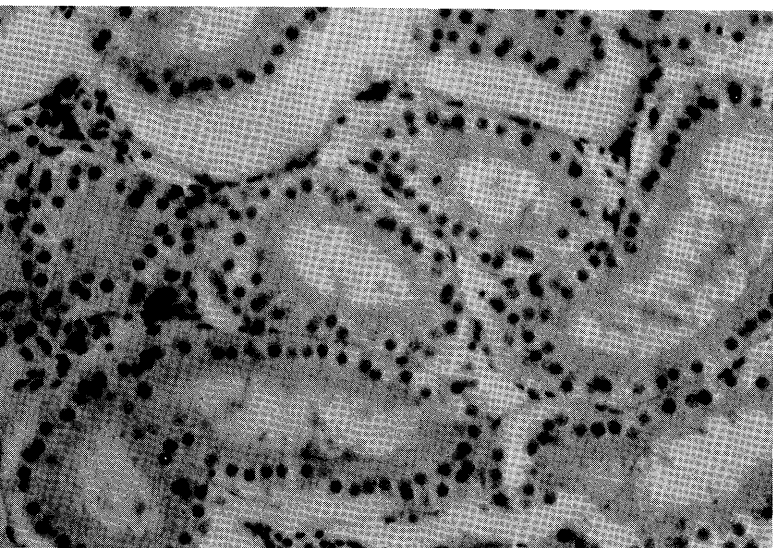


Figure 2 Control kidney section (200 \times). Proximal tubule cells appear normal, with prominent brush borders on their apical surfaces.



Figure 3 Experimental (0.06 M) kidney section (200 \times). Proximal tubules are dilated and brush borders are almost totally lacking.

when treated with 80 mM acetaminophen.

Kidney: Histological examination. Control kidneys appeared normal (Figure 2), but large dilated tubules were present in experimental kidneys, (Figure 3). Cells in the tubule walls were sometimes swollen and resembled columnar cells, rather than the typical cuboidal cells of control kidneys. However, the majority of cells lining swollen tubules appeared compressed or flattened. Necrotic cells and an occasional macrophage were found in some tubule walls. Brush borders were absent from most tubules, most cells of which had pale cytoplasm and a frothy appearance. In some cases, the apical cytoplasm of cells lining tubules appeared to be sloughing.

Mitotic figures were present in 60-70% of control tubule walls. In acetaminophen treated embryos mitotic activity was evident in only 10-30% of the tubule walls. Erythrocytes were often found in the lumen of some treated tubules. Necrotic RBCs and some RBCs with pyknotic nuclei were present within some treated tubules. Eosinophilic casts occurred in some tubules. Scattered necrosis was observed in the stroma between tubules in treated kidneys.

Liver: Gross examination. Other than size reduction, the external morphology of experimental livers did not appear to be altered (Figure 4). Control liver weights averaged 176.1 mg, while experimental liver weights averaged 98.8 mg when treated with 60 mM acetaminophen and 61 mg when treated with 80 mM acetaminophen.

Liver: Histological examination. Control tissue appeared normal (Figure 5). Experimental liver tissue was generally more compressed (Figure 6) and sometimes hepatic cell rows were distorted. Hepatic cells lining central veins were often pale and vacuolated in appearance. Liver cells, in general, were smaller than in control tissue (Figures 5, 6). An occasional macrophage and a few necrotic cells occurred within the hepatic cells of the tubules. More frequently, necrosis was found in the sinusoid regions. Mitotic activity was generally about 30-50% of that found in control organs.

DISCUSSION

The death of a 13 year old adolescent occurred as a result of an overdose of acetaminophen (Tylenol) and phenobarbital (Wilson et al., 1978). Examination of tissues following autopsy revealed damage in both the liver and kidneys. Tubular epithelium in the kidneys was swollen and cloudy. Casts and bile pigments were present in distal tubules and collecting ducts. Centrilobular necrosis was extensive in liver samples. Strands of fibrous connective tissue invaded lobule regions. Surviving hepatic cells exhibited basophilia and cytoplasmic vacuolization.

Necrosis of proximal tubules with loss of brush borders has been reported in overdose cases (Wilkinson et al.,

1977; Kleinman et al., 1980) and in animal experimentation (McMurtry et al., 1978). Tubular cells were swollen and necrotic debris was found in some tubule lumens (Nogen and Bremner, 1978 and Kleinman et al., 1980).

Centrilobular necrosis and invasion of lobules by fibrous connective tissue has been observed in numerous overdose cases in humans (Clark et al, 1973; Lesna et al, 1976; Barker et al., 1977; Johnson and Tolman, 1977; Wilson et al., 1978; Gerber et al., 1980; Black et al., 1982;) and as a result of experimentation with animals (Davis et al., 1974; and McMurtry et al., 1978). Vacuolization of hepatic cells was observed in a number of cases (Lesna et al., 1976; Wilson et al., 1978; Black et al., 1982).

While hepatic and kidney necrosis was not extensive in our results, an important point is that the histological analysis was made on organs eight days after injection of acetaminophen. Judging by the reduced embryonic sizes, it is felt that a more massive necrosis may have occurred several days prior to the time of sacrifice.

Liver biopsies carried out on a series of human patients four days after acetaminophen ingestion showed that most necrotic cells had been removed by macrophage activity (Lesna et al., 1976). Analysis of rat kidneys following subcutaneous acetaminophen injection demonstrated that necrosis was greatest at 24 to 48 hr post injection and that regeneration was evident by 72 to 96 hr post injection (McMurtry et al., 1978).

With the extreme distortion of tubule walls and linings reported in our results, it is questionable whether such severely injured kidney tissues could recover enough to function normally. While necrosis was sparse and mitotic activity was evident, brush borders were still absent.

Liver tissues, however, appeared to be recovering. Even though mitotic activity is reduced general liver architecture resembles that of control livers.

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LITERATURE CITED

- Black, M., J.F. Cornell, L. Rabin and N. Shachter. 1982. Late presentation of acetaminophen hepatotoxicity. *Dig. Dis. and Sci.* 27: 370-374.
- Barker, J.D., D.J. de Carle and S. Anuras. 1977. Chronic excessive acetaminophen use and liver damage. *Ann. Intern. Med.* 87: 299-301
- Clark, R., R.P.H. Thompson, V. Borirakchanyant, B. Widdop, A.R. Davidson, R. Goulding and R. Williams. 1973. *The Lancet* 1: 66-69.
- Davis, D.C., W.Z. Potter, D.J. Jollow and J.R. Mitchell.

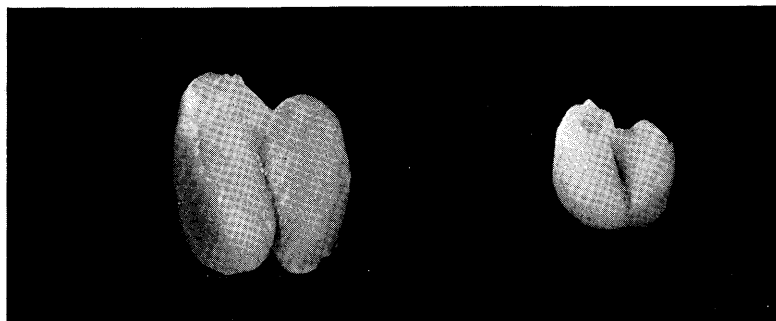


Figure 4 Control liver (left) and experimental liver (right). Experimental contours appear normal, but size reduction is considerable.

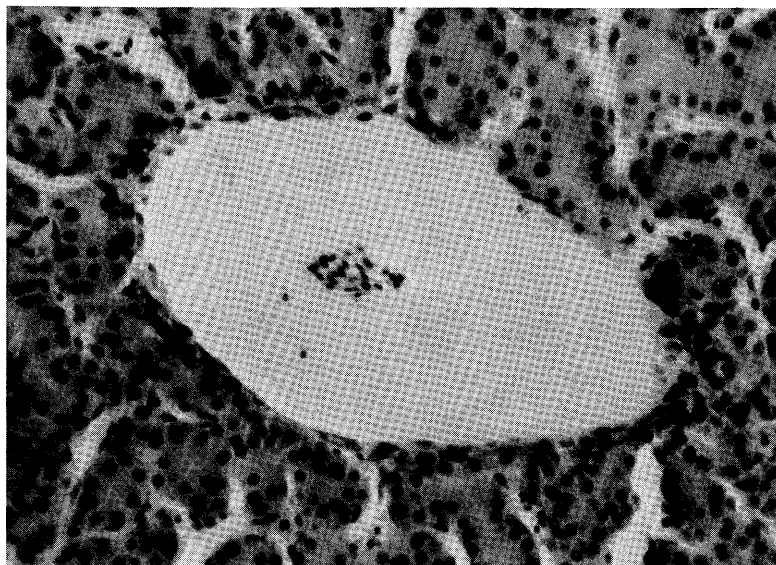


Figure 5 Control liver section (200x). Liver cells surrounding a central vein appear normal.

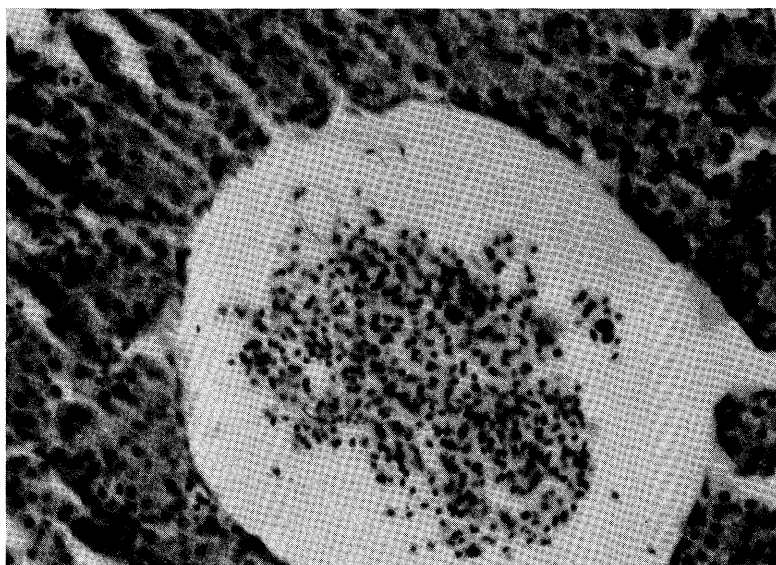


Figure 6 Experimental (0.08M) liver section (200x). Cells surrounding a central vein appear smaller than in control tissue. Nuclei are very close, indicating the smaller cells.

1974. Species differences in hepatic glutathione depletion, covalent binding and hepatic necrosis after acetaminophen. *Life Sciences* 14: 2099-2109.
- Gerber, M.A., H. Kaufman, F. Klion and L.I. Alpert. 1980. Acetaminophen associated hepatic injury. *Human Pathology*. 11: 37-42.
- Hamburger, V. and H.L. Hamilton. 1951. A series of normal stages in the development of chick embryo. *J. Morph.* 88: 49-92.
- Johnson, G.K. and K.G. Tolman. 1977. Chronic liver disease and acetaminophen. *Annals of Internal Medicine* 87: 302-304.
- Kleinman, J.G., R.V. Breitenfeld and D.A. Roth. 1980. Acute renal failure associated with acetaminophen injection-report of a case and review of the literature. *Clinical Nephrology* 14: 201-205.
- Lensa, M., A.J. Watson, A.P. Douglas, A.N. Hamlyn and O.F.W. James. 1976. Evaluation of Paracetamol-induced damage in liver biopsies. *Virchows Arch. Path and Histol.* 370: 333-334.
- McJunkin, B., K.W. Barwick, W.C. Little and J.B. Winfield. 1976. Fatal massive hepatic necrosis following acetaminophen overdose. *JAMA*. 236: 1874-1875.
- McMurtry, R.J., W.R. Snodgrass, and J.R. Mitchell. 1978. Renal necrosis, glutathione depletion and covalent binding after acetaminophen. *Toxicol and App. Pharm.* 46: 87-100.
- Nogen, A.G. and J.E. Bremner. 1978. Fatal acetaminophen overdosage in a young child. *J. Pediat.* 92: 832-833.
- Stokes, J.F. 1976. Discussion of papers presented in session III. *J. Int. Med. Res.* 4: 93-96.
- Walton, B., B. Delay, K. Mullins, P. Grimes and J. Osborne. 1986. Acetaminophen toxicity in the developing chicken embryo. *J. Tenn. Acad. Science* 61: 11-14.
- Wilkinson, S.P., H. Moodie, V.A. Arnayo and R. Williams. 1977. Frequency of renal impairment in paracetamol overdose compared with other causes of acute liver failure. *J. Clin. Pathol.* 30: 141-143.
- Wilson, J.T., V. Kasantikul, R. Harbison and D. Martin. 1978. Death in an adolescent following an overdose of acetaminophen and phenobarbital. *Amer. J. Dis. Children* 132: 466-473.