Toxicity of an Organic Arsenical, 3-Nitro-4-hydroxyphenylarsonic Acid

Residues in Chicken Tissues

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Build-up, plateau, and depletion of tissue residues of an organic arsenical (roxarsone: 4-hydroxy-3-nitrobenzene-arsonic acid) administered in the feed to growing chickens are compared to total arsenic residues in chickens receiving nonmedicated feed. Spectrophotometric determination of residues was based on the procedure given by Winkler, using silver diethylthiocarbamate. Total residues and rates of depletion were established using four tissues (muscle, skin, liver, and kidney) from each of four birds of each sex sacrificed on scheduled days. Highest residues were found in the liver, and significant sex differences were not observed. Rapid depletion of residues occurred during the first five days off medication.

Roxarsone (4-hydroxy-3-nitrobenzenearsonic acid or, more commonly, 3-nitro-4-hydroxyphenylarsonic acid) has been administered in the feed of poultry and swine for about 20 years for growth promotion and improvement of the efficiency of feed utilization. Medication with roxarsone produces residues in edible tissues which are measured as elemental arsenic as reported by Kerr *et al.* (1963). This report, which was a compilation of data from animals maintained under varying conditions, also included levels of arsenic found upon withdrawal of the drug. Hüni and Zanetti (1963) reported on a small group of growing chickens and compared the arsenic levels found during medication and postmedication to that in nonmedicated birds.

The purpose of this study was to obtain a better evaluation of the arsenic residues occurring in growing chickens during medication with roxarsone and following its withdrawal from the feed with relation to the arsenic levels found in chickens of the same hatch and fed the same but nonmedicated feed.

MATERIALS AND METHODS

Arsenic Analysis. Ten-gram samples of each tissue were wet-ashed in a nitric- and sulfuric-acid mixture (Robertson, 1921). The arsenic assay was based on the procedure given by Winkler (1962) in which arsenic is determined spectrophotometrically, using silver diethyldithiocarbamate. All samples were assayed on an individual-bird basis.

Chickens and Their Treatment. Broiler-type chickens (Pilch-Ledbrest Cross) were used in two tests. Each of the tests, CS-270-66 and CS-277-66, consisted of 300 chicks with equal numbers of males and females. The chicks used in CS-270-66 were maintained in floor pens while those in CS-277-66 were housed in wire-floored chick-growing batteries after they were four weeks of age. For each of the tests, a single batch of broiler-growing ration was prepared and divided—one portion being used for the nonmedicated controls and the other portion being mixed with roxarsone at the level of 0.005% provided by 3-Nitro-10 at 1 lb. per ton of feed. Medication was started at four weeks of age in both tests.

The chickens were killed, and samples of breast muscle, liver, kidney, and skin with adhering fat were taken, identified by bird number, and frozen according to the following plans: CS-270-66—day 1, 2, 3, 4, 5, 7, 9, 11, 14, 28, 42, 56, and 70 of medication, and daily—day 1 through 14 after withdrawal of medication, five males and five females per day, nonmedicated and medicated. CS-277-66—day 1, 2, 3, 4, 5, 7, 9, 11, and 14 of medication, and day 1, 5, and 14 without medication, 10 males and 10 females per day, nonmedicated and medicated. Except for kidney, the sample size was considerably greater than 10 grams. Entire kidneys were taken, but in younger chickens these frequently did not weigh 10 grams (weight at 8 weeks of age, 8–15 grams).

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RESULTS AND DISCUSSION

Nonmedicated Tissues. The amount of arsenic found in the nonmedicated tissues from these two tests was less than that reported by Kerr *et al.* (1963). The data in Table I show that in muscle the level obtained was 0.02 ± 0.02 p.p.m. while Kerr *et al.* showed 0.15 ± 0.19 p.p.m. Although the latter data were obtained on leg muscle and the present data from breast muscle, other experiences in our laboratory have not indicated a difference in arsenic content in the two types of muscle. Kerr *et al.* used a different procedure, that of Cassil and Wichmann (1939). Hüni and Zanetti (1963) reported 0.56 p.p.m. arsenic on a dry tissue basis, which if one assumes 70% water would be 0.17 p.p.m. on a wet tissue basis.

Similar differences were obtained for liver tissue. The data in Table I show 0.09 ± 0.10 and 0.03 ± 0.02 p.p.m. arsenic for males and females, respectively, while Kerr *et al.* found 0.18 ± 0.14 p.p.m. in 59 samples. The 0.09 figure for the male livers is influenced by one high result, 1.13 p.p.m., which is so out-of-line with all the other results that it is probably of spurious value. It is believed that the 0.06 p.p.m., found for female chickens is probably a truer value for all liver tissues. Hüni and Zanetti reported a lower average for liver than for muscle which does not agree with our findings.

For kidney and skin, no valid comparisons are possible because Kerr *et al.* gave data from four kidney samples and no data from skin samples. Hüni and Zanetti did not examine these tissues.

The only explanation for these differences which can be offered is a difference in the feed used. Our present feeds do not contain any fish meal, which is frequently high in arsenic (3.00–4.00 p.p.m.), while that used by Kerr *et al.* contained this ingredient. Huni and Zanetti did not report their feed formulation.

Liver. Of the four tissues examined, the greatest amount of arsenic is found in the liver. Figure 1 shows that at one day of medication there has been a marked increase in arsenic

Table I. Mean P.P.M. Arsenic and Range of Values by Sex in Tissues from Nonmedicated Chickens

Test	Tissue	Arsenic Found: Males No. of samples	Males P.p.m. + σ	Females No. of samples	Females P.p.m. + σ	Range: Males	Range: Females
CS-270	Liver	135	0.09 ± 0.10	134	0.06 ± 0.03	0.00–1.13	0.01–0.18
CS-277	Liver	29	0.03 ± 0.02	30	0.03 ± 0.01	0.00–0.11	0.00–0.09
CS-270	Kidney	134	0.05 ± 0.03	134	0.05 ± 0.03	0.00–0.13	0.01–0.19
CS-277	Kidney	30	0.04 ± 0.02	29	0.04 ± 0.02	0.00–0.14	0.00–0.14
CS-270	Muscle	135	0.02 ± 0.02	133	0.02 ± 0.02	0.00–0.12	0.00–0.23
CS-277	Muscle	30	0.02 ± 0.02	30	0.02 ± 0.01	0.00–0.08	0.00–0.07
CS-270	Skin	135	0.02 ± 0.02	134	0.02 ± 0.02	0.00–0.22	0.00–0.10
CS-277	Skin	30	0.03 ± 0.02	30	0.03 ± 0.01	0.01–0.13	0.02–0.05

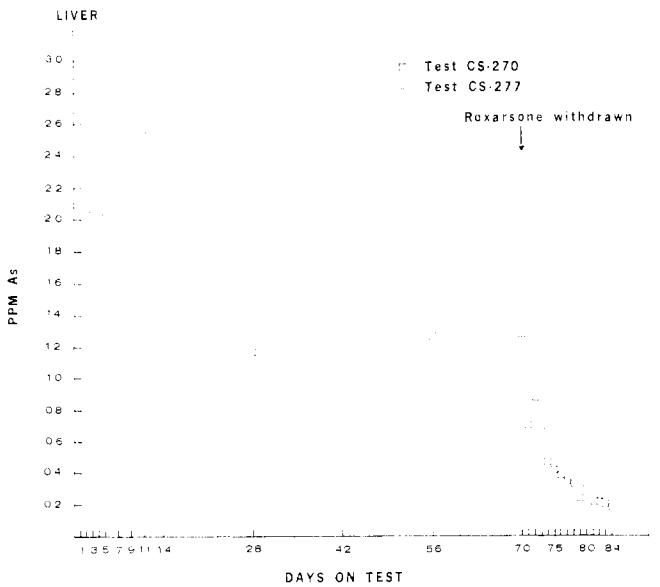

Figure 1. Levels of arsenic in p.p.m. found in liver

present in the liver. The figure indicates an increase in arsenic content through the eleventh day, then a falling-off to a plateau. This occurs in spite of the fact that the feed consumption, and hence the drug consumption, is increasing as the chickens grow older. The same general pattern was found in both tests. After eight weeks of medication, a time when many broilers are marketed, the arsenic found was 1.25 ± 0.66 p.p.m. The range in the 10 samples was 0.71 to 2.60 p.p.m. Upon withdrawal of the roxarsone, the level of arsenic immediately falls, and by the fourth postmedication day, the levels of arsenic are well below the tolerance of 1.0 p.p.m. established by the Food and Drug Administration as published in the Federal Register (paragraph 121. 1138, Arsenic). From that time on, the decrease is slow, and even at 14 days postmedication, the mean is about 0.13 p.p.m. higher than that found in livers of nonmedicated chickens.

Hüni and Zanetti reported 4.13 p.p.m. arsenic in dry liver which on a wet basis (70% water) would be equivalent to 1.24 p.p.m., which is in agreement with our on-medication result. However, at 14 days postmedication these authors found 1.45 p.p.m. on a dry basis equivalent to 0.44 p.p.m. on a wet basis (70% water) which is two fold higher than the 0.2 p.p.m. found in the present study (Figure 1) but comparable to that found by Kerr *et al.* (1963).

Kidney. The results obtained from kidney, as given in Figure 2, show that after one day of medication the level

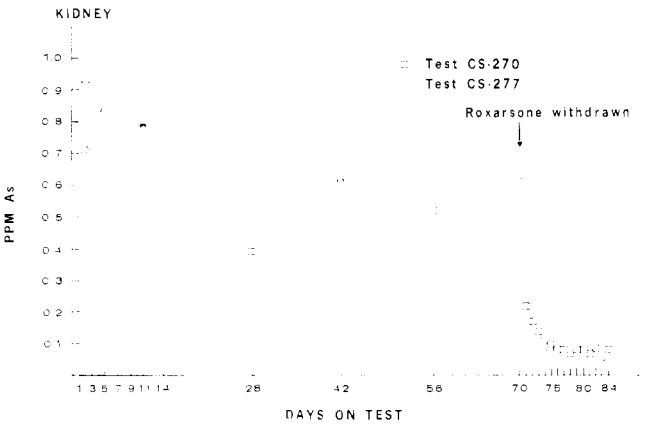

Figure 2. Levels of arsenic in p.p.m. found in kidney

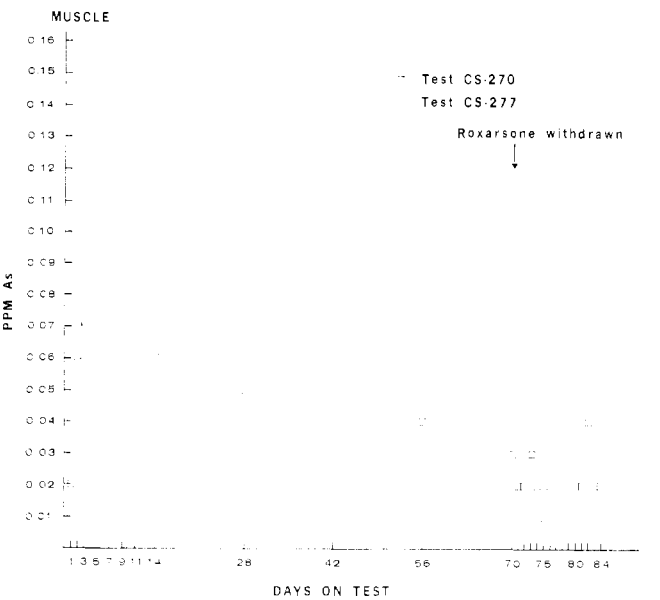

Figure 3. Levels of arsenic in p.p.m. found in muscle

of arsenic is as high as it is at seven days, *i.e.*, above 0.7 p.p.m. Following this, it decreases slightly but upon withdrawal of the drug, it immediately falls, and at five days off-medication, it is about 0.1 p.p.m. or about twice that found in nonmedicated chicken kidney.

Muscle. Very little arsenic accumulated in muscle. After the first day and up to the fourteenth day of medication, the means were between 0.06 and 0.16 p.p.m. (Figure 3); at the

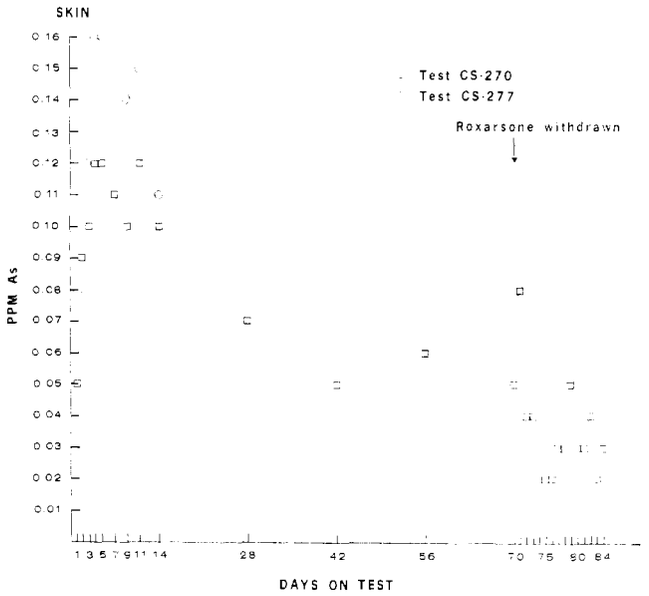

Figure 4. Levels of arsenic in p.p.m. found in skin

28th, 42nd, 56th, and 70th day, the means were between 0.03 and 0.05 p.p.m. Following the withdrawal of medication, no mean higher than 0.04 p.p.m. was found, a level which is essentially the same as that found for nonmedicated chicken muscle (Table I).

Kerr *et al.* (1963) found an on-medication level of 0.15 p.p.m. in 30 samples which was not an increase over the nonmedicated muscle finding. Hüni and Zanetti found 0.91 p.p.m. on a dry basis which is equivalent to 0.27 p.p.m. on a wet basis. At 14 days off-medication they found 0.82 p.p.m. which is equivalent to 0.25 p.p.m. on a wet basis. Our present study and the data of Kerr *et al.* are in agreement in the sense that there is no material change in arsenic level in the on- and off-medication tissues while the data of Hüni and Zanetti (1963) show a considerable difference.

Skin. The amount of arsenic found in skin is somewhat higher than that found in muscle. Figure 4 shows mean levels after the first day and prior to the 28th day between 0.08 and 0.16 p.p.m. From the 28th day on, the mean levels are less: 0.05 and 0.07 p.p.m. Upon withdrawal of the medication, the mean levels fall to about 0.03 p.p.m., essentially equivalent to the mean levels found for nonmedicated chickens.

Sex Differences. Although numerically, particularly in the liver data, there appears to be a difference between the sexes—with the females showing greater quantities of arsenic —a statistical analysis of the data for all tissues showed that the differences are due to chance.

CONCLUSIONS

These data clearly show that only in 2 of the 4 tissues examined could the arsenic level found represent a conceivable hazard for man if the medication was not withdrawn. These are the liver and the kidney. The quantity of the latter in a broiler chicken is so small that it is of little significance. In none of the tissues is there a continued accumulation of arsenic residue, in spite of the fact that as the chickens grow older, the feed intake, and hence the drug intake, increases. Withdrawal of the drug 5 days before slaughter results in arsenic levels well below the tolerance established by the Food and Drug Administration.

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