Effects of Dietary DDT on Blood Plasma Levels of Calcium and Magnesium in Laboratory Rats

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The insecticidal action of DDT was discovered in 1939 but no one yet knows what its toxic mechanisms are. This paper reports on a study of the plasma levels of calcium and magnesium as affected by the presence of DDT in the diet of laboratory rats.

DDT alters the observed electrical activity of the nerve. GORDON and WELSH (1948) reported repetitive firing and spontaneous activity from DDT-poisoned nerve in the crayfish. This effect could be inhibited by the addition of calcium or magnesium ions.

It has been suggested (MATSUMARA, et al. 1969) that the nerve enzyme adenosine triphosphatase (ATPase) might actually be the site of DDT attack. Two kinds of ATPase are of concern and have been identified (PUSKIN, et al. 1968). Both kinds are dependent upon the presence of certain ionic species. One requires Na^+, K^+, and Mg^{++} and is referred to as the Na-K-Mg type. The other requires Ca^{++} or Mg^{++}. The Na-K-Mg type is assigned a governing role in cation transport across the cell membrane (GERMAIN and PROULX 1965, SKOW 1965). It has been suggested (GERMAIN and PROULX 1965) that the Ca-Mg type may be involved in the release of acetylcholine.

In a study of human bood plasma, WALSER (1961) reported that calcium and magnesium ions occur in several forms. The predominant species are the free ion and the protein-bound ion. Several complexed forms occur, the mono-hydrogen phosphate and citrate complexes being the only two WALSER identified. Apparently no studies have been done on the effects of DDT on magnesium levels and the only calcium studies were on birds in which no change in total calcium is detected (PUJMAN, et al. 1970, BITMAN, et al. 1969, PEAKALL 1969 and 1970, SIMPSON, et al. 1972). The studies reported herein involved total and non-protein-bound levels of calcium and magnesium as a function of dietary DDT. Feeding the insecticide was chosen as an approximation to an environmental possibility.

EXPERIMENTAL

Twenty-four of the rats used were hooded females (LONG-EVANS) and two were albinos (SPRAGUE-DAWLEY). One of the albinos was male. The rats were ten to twelve months old and averaged 259 grams. The females were paired and housed in wire cages; the male was housed singly in a metal cage. All animals were on free feed and water. The feed was a vitamin- and mineral-supplemented grain mixture.

The DDT was obtained from Montrose Chemical Corporation of

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California. The analysis showed 76.2% of the p,p'-isomer, usually considered the "active" form. There was 17.5% of the o,p'-form and lesser amounts of both isomers of DDD and DDE. The DDT was mixed with soybean oil as a solvent and peanut butter as an appetizer, then mixed progressively with the feed. A typical mix, for 0.1% DDT, consisted of 6 g DDT thoroughly mixed with 100 g soybean oil and 100 g peanut butter, then mixed completely with 200 g meal. This was mixed with 1 kg meal, then meal added to 6 kg and the mixing continued until a homogenous mix was obtained.

Blood samples were taken using heparinized syringes fitted with 23 gauge needles. Because heparin will sometimes complex calcium and magnesium, it was necessary to test the heparin to secure a batch lot that did not significantly complex either ion. That lot of heparin was then used for all further studies. All animals were etherized prior to obtaining a sample.

The blood plasma was separated by centrifugation. To determine the total ion concentration the plasma proteins were precipitated with trichloroacetic acid and the sample diluted for atomic absorption analysis. The non-protein-bound ion (hereafter referred to as ultrafiltrable ion) was determined after separation of the protein by means of membrane ultrafiltration. The filtrate was analyzed by atomic absorption at the resonance line. Details of the atomic absorption method may be found in SUNDERMANN and CARROLL (1965). Further information on membrane ultrafiltration occurs in FARESE and MAGER (1970).

The rationale behind analyzing both total and ultrafiltrable ions was to establish at what level any observed change occurred. It was anticipated from the work of GORDON and WELSH (1948) that a change might occur in the free ion form and none, based on the bird studies, was expected in the total concentration.

BEHAVIOURAL EFFECTS OF DDT

Two levels of DDT were chosen for the blood sampling experiments, 0.075% and 0.125% DDT in feed on a weight-weight basis. At the 0.075% level, the rats consumed about 25 g/day/rat (18 mg DDT) and showed few external symptoms. No deaths occurred even when fed 0.075% for as long as 56 days. The 0.125% level was lethal to two of twelve rats at five and six days but several lived for two weeks when they were sacrified. At the 0.125% level the rats consumed about 30 g/rat/day or about 37.5 mg DDT/rat/day.

Mild tremors were observed in all rats at the 0.125% level by three days. Severe tremors and tetany preceded all deaths from DDT. It was possible to "adapt" animals to the 0.125% level by first feeding at the 0.075% level for several days.

MAGNESIUM

The ultrafiltrable magnesium was found to be 11 ppm for the control animals and 12 ppm for all animals on DDT. This difference was not statistically significant.

The total magnesium results are given in Table 1. From a control value of 19 ppm, the magnesium concentration rose with feeding DDT. The magnesium concentration in the plasma increased in proportion to the level of DDT fed. Time after onset

TABLE 1
Total Magnesium

Group	Control	Acute[1]	0.075%	0.125%
Group Means	19[2]	22	20	23
Hooded Pair C	-	-	19	19
Hooded Pair K	18	-	-	26
Hooded Pair D	18	-	-	22
Hooded Pair F	20	22	-	-
Hooded Pair A	22	18	-	24

Notes:
[1]"Acute refers to animals showing tremors less than 24 hrs after onset of 0.125% DDT treatment.
[2]All magnesium concentrations are in ppm.

of feeding appears not to affect the results as there was no difference between the acute group (0.125% for less than 24 hrs) and longer feeding at the same level.

Some variability was noted between pairs which were tested in more than one group as compared with the group means. This is probably related to the fairly high standard deviations shown within each group.

The significance of the magnesium findings is not clear even though there is a statistically significant ($p = < 0.005$) difference between the controls and the 0.125% level, both the acute and long term. Depressed magnesium ion levels would be anticipated due to the nature of the clinical signs, particularly the uncontrolled tremors, but instead, we found a rather consistent increase in magnesium levels after tremors occurred.

CALCIUM

No significant difference was found in the ultrafiltrable calcium: 60 ppm for controls, 59 ppm for all animals on DDT.

Both duration and level of feeding affected the total calcium results (Table 2). Animals fed at the 0.125% first showed

TABLE 2
Total Calcium

Animal	Control	Acute[1]	0.075%	0.125%
Group Means	113[2]	107	123	119
Hooded Pair C	-	-	122	118
Hooded Pair K	114	-	-	116
Hooded Pair D	108	-	-	115
Hooded Pair F	118	104	-	-
Hooded Pair A	111	102	-	115

Notes:
[1]"Acute" refers to animals showing tremors less than 24 hrs after onset of 0.125% DDT treatment.
[2]All calcium concentrations are in ppm.

a drop in calcium and then rose to a higher level than the controls after long-term feeding. Feeding at the lower level brought even higher Ca^{+2} concentrations, although the animals' tolerance to this level also led to prolonged feeding so that some were sampled after nearly two months. All individual pairs followed the overall trends of calcium increase with continued tremors.

In the case of calcium, some sort of adaptative mechanism appears operative. The initial drop, associated with distinct tremors, is consistent with the hypothesized interference with nerve functioning (GORDON and WELSH 1948). The raised levels are associated with a relative absence of symptoms, particularly at the 0.075% level. This is consistent with the reported observation (GORDON and WELSH 1948) that spontaneous, repetitive nerve activity due to DDT poisoning could be overcome with the addition of calcium.

DISCUSSION

One possible explanation for these findings is an adaptive mechanism. While DDT initially depresses the bound calcium (possibly also magnesium) levels and thereby disrupts nerve functioning, eventually adaptation occurs resulting in levels higher than those seen in control animals. Birds may not have such an adaptive mechanism which would account for the calcium results and their greater susceptibility to DDT poisoning.

Another possibility is that the results are an artifact of experimental design. The original focus was on measuring ultrafiltrable ions since that was where change was anticipated. Furthermore, no suspicion was had of the role time would play in the observed results. Obviously further study is needed.

Because of prior commitments, none of the present authors is able to continue with the study but the findings do appear significant enough to warrant further investigation by other researchers.

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