THE EFFECT OF STARVATION ON THE TISSUE DISTRIBUTION METABOLISM AND EXCRETION OF DDT IN THE RAINBOW TROUT

by

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Summary

It has been shown that food deprivation in the rainbow trout causes an increase in the rates of metabolism and excretion of DDT. Food deprivation further induces a mass transport of pesticide, first from visceral and later from carcass lipid depots to the liver and brain. The rapid transport which occurs at high levels of lipid depletion far outweighs any protective effect of increased excretion. A conceptual model of pesticide dynamics is advanced and current methods of assessing the toxicological threat of pesticides are criticised.

Introduction

In consequence of their low polarity and high resistance to metabolism, chlorinated hydrocarbons become concentrated in the body lipids of animals exposed to contamination. While they remain in these lipid depots they must be regarded as being relatively innocuous, becoming toxic only when they are released to sensitive target areas. Although it has been shown that, in homeotherms, food deprivation results in increases in the concentrations of organochlorine pesticides in the tissues and organs of contaminated animals, most studies have failed to demonstrate a transport of the pesticide from the depot lipids or to describe the fate of the mobilised compounds (Brown, 1970, Dale, Gaines and Hayes, 1962, Donaldson, Sheets and Jackson, 1968, Ecobichon and Saschenboecker, 1969, Hukuhara, 1962).

The sole, unequivocal demonstration of pesticide translocation is due to Finley & de Freitas (1971) who showed that, in the pigeon, a 48 hour period of starvation, during which body lipid levels were reduced by 50%, resulted in the transport of DDT from adipocytes to muscle cells; no change in the concentration in brain tissues was observed.

In contrast to the homeotherms investigated above, many fish species undergo chronic and extreme variations in lipid content (Lovern, 1934; 1938). It was therefore considered important to investigate the effect of starvation on the distribution of organochlorine compounds in fish. The present communication describes the effect of food withdrawal on the mobilization and metabolism of DDT in the rainbow trout, Salmo gairdnerii (Rich).

Methods

$^3$H labelled DDT (New England Nuclear) was injected intravenously into rainbow trout (100-200 g) in a 60/40 v/v isopropanol/isotonic saline vehicle to give a dose concentration of approximately 160 µg/kg body weight. The size of the injected dose was determined to an accuracy of ± 1% by
incorporating \(^{47}\)Ca into the vehicle at a known DDT/Ca ratio and by
determining the amount of \(^{47}\)Ca administered with a whole body gamma
scanner (Simpson, Johnstone & Youngson, in press). Twenty fish treated
in this way were divided randomly into two equal groups, one of which was
starved and the other fed ad libitum with a proprietary trout food for the
70 day period of the experiment. Water temperature was 8-10°C. At
sacrifice plasma was prepared from blood removed by caudal puncture and
brain, liver, gall bladder, viscera and a dorsal muscle sample were
excised. The remainder ("carcass") was also retained for analysis. Lipid
and DDT contents of these samples were determined gravimetrically and by
liquid scintillation spectrometry respectively. Qualitative analyses of the
tritium labelled compounds recovered from liver and carcass were
accomplished by a combination of thin layer chromatography, liquid
scintillation counting and radio gas-liquid chromatography.

Results and Discussion

At the completion of the experiment, the fed fish had increased their body
weight by 20-60% and had fat contents of between 6 and 12%; the starved fish
had lost 10-35% in body weight and had lipid contents of 0.5-3%.

Figure 1 shows plots of the total amount of DDT and its metabolites
retained in the fish against final lipid concentration. The mean retention
of pesticide in the fed fish was 88%, greater elimination being associated
with low lipid content. Six of the starved fish retained a mean 99% of
the injected dose, statistically higher \((p > 0.05)\) than the fed fish; the
remaining four starved fish, of lipid concentration < 1.3% had suffered
massive elimination of pesticide.

Pesticide elimination is further shown in Figure 2 in which the change in
body weight is plotted against the change in the whole body concentration
of pesticide. The extent of elimination is indicated by the interval,
on the vertical axis, between each of the points and the calculated
no elimination curve.

The distribution of lipids in the various body compartments of rainbow
tROUT is indicated in Figure 3. This shows that visceral lipids are
depleted rapidly during starvation, "carcass", liver and brain lipid
concentrations tending to remain relatively constant until the body lipid
concentration falls below 2%. The corresponding dynamics of pesticide
residues in the tissues are indicated in Figure 4. This shows that visceral
residues are depleted during food deprivation and undergo a translocation
to carcass, liver and brain. When body lipids were depleted below a level
of 2%, a rapid reduction in carcass lipids occurred with a consequent mass
transport of carcass pesticide to viscera, liver and brain. The rise in the
quantities of pesticide associated with the last two tissues was particularly
rapid, the levels increasing five-fold and ten-fold respectively.

The effect of food deprivation on pesticide metabolism was examined (Figs
5 and 6). At moderate levels of lipid depletion, the proportion of
pesticide metabolised to DDD and DDE was relatively independent of lipid
content. At higher levels of lipid depletion, there was a rapid metabolism
of DDT and of the intermediate metabolite DDD, the former leading to the
stable derivative DDE and the latter to DDMU and DDMS. The polarities of
the last named compounds would preclude their appearance in lipoferic
tissues; traces of DDMS were however detected in bile by a combination of
thin layer and radio gas-liquid chromatography.

It is possible to construct a conceptual model consistent with these
observations and with general biochemical considerations. DDT, which may
be regarded as an exemplar of non-polar lipophilic pollutants, is
distributed among the body constituents according to partition law
considerations. The lipid depots associated with the "carcass", liver and viscera of rainbow trout are composed largely of low polarity neutral lipids and the concentrations of DDT in these are, as expected, similar (Figure 7). Brain lipids, being largely composed of polar, structural phospholipids whose binding affinities for DDT are relatively low, contain much smaller concentrations of pesticide. During food deprivation, the lipids of the highly labile visceral depot are rapidly metabolised, with a consequent rise in their pesticide concentration and a corresponding rise in the concentration of pesticide in the plasma with which visceral tissues are in equilibrium. A mass transfer of pesticide from the viscera to the carcass and liver then ensues. The slower transfer to the brain is a consequence of its phospholipid composition. The later stages in the depletion of neutral lipids from the viscera is accompanied by a rise in the rate of utilisation of the large, though less labile fat depots of the skin and carcass. As a result, the residual lipid becomes increasingly composed of polar, structural lipids whose affinity for DDT is relatively low. The depletion of neutral lipids in the visceral and carcass depots is reflected in reductions in the concentrations of plasma free fatty acids (Bilinski, 1969, Timoshina & Shabalina, 1972) and in the observed fall in the concentration of plasma DDT (Fig. 7). Despite the lowering in the latter concentration, the reduction in the binding affinity is sufficient to effect a net increase in the rate of pesticide translocation, with the following consequences. Transport to the brain causes an accumulation of pesticide in that tissue while transport to the kidney, gills and liver causes an increase in the rate of pesticide excretion which is not effectively opposed, as in the fed fish by the sequestering activity of intracellular, neutral lipids. Transport to the lipid depleted liver will similarly result in a sharp increase in the rate of pesticide metabolism.

It is believed that these data disclose shortcomings in the usual methods of assessing the toxic threat offered by pesticides to vertebrate organisms. This is commonly assessed by determinations of quantities of pesticide per unit weight of homogenised tissues, or, less usually, by determinations of pesticide concentrations in the body lipid. By both these criteria the later stages of food deprivation would be regarded as serving a protective function by inducing excretion (Figure 8) and metabolism. Symptoms of acute organochlorine toxicity are attributable to dysfunction of the central nervous system (Dale, et al., 1963), whilst sub-acute effects are due to interference with liver enzymes (McLean and McLean, 1966), gonadal function (Burdick, 1964) and C.N.S. directed behaviour (Anderson and Prins, 1969, Davy, Kleerkoper & Gensler, 1972). The present observations indicate that far from serving to protect the organism, food deprivation results in massive shifts of pesticide to those centres where toxic effects may be mediated.

References


Bilinski, E. 1969

Brown, J.R. 1970


The effect of environmental and dietary stress on the concentration of 1,1-bis (4-chlorophenyl) -2,2,2-trichloroethane in rats. Toxic. appl. Pharmac., 12: 504-510.


The redistribution of stored DDT in cockerels under the influence of food deprivation. Toxic. appl. Pharmac., 15: 420-432.


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Figure 1. The effect of food deprivation on the retention of pesticides.
Figure 2. Relationship between changes in body weight and changes in pesticide concentration (ng/g wet weight).

- \( x \) = starved
- \( o \) = fed

% Change in Pesticide Conc. (ng/g) vs. % Change in Body Weight
Figure 3. The effect of food deprivation on tissue lipid mass.
Figure 4. The effect of food deprivation on pesticide mass in tissues.
Figure 5. Distribution of pesticide metabolites in liver.

- DDT
- DDD
- DDE

Metabolites [%]

Body Lipid [%]
Figure 6. Distribution of pesticide metabolites in "carcass".
Figure 7. The effect of food deprivation on pesticide concentrations in organs and tissues.
Figure 8. The effect of food deprivation on pesticide concentrations in whole body lipid and wet weight mass.