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THE TRANSPORT AND MOBILIZATION OF FAT SOLUBLE POLLUTANTS IN THE RAINBOW TROUT: THE EFFECT OF CHANGES IN PHYSIOLOGICAL STATUS

by

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Summary

The lipophilic pollutants DDT, hexadecane and 3, 4-benzpyrene are transported in the blood plasma of rainbow trout mainly in association with plasma lipoproteins. The concentration of DDT in plasma is raised by the stress hormones adrenalin and cortisol. The latter compound causes, additionally, a transport of pollutant from depot lipids to the plasma. It is shown that the changes in lipid dynamics which precede gonadal development induce an increased translocation of DDT to brain tissues and an increased rate of excretion.

Introduction

It is known that DDT contaminated animals are acutely sensitive to environmental stress, (Ecobichon and Saschenbrecker, 1969; Youngson, unpublished observations) and that the neurotoxic symptons are associated with elevated levels of pollutant in the blood plasma (Mount, Vigor and Schafer, 1966; Hogan and Roelafs, 1971). It seems likely that the sequence of events is that stress elicits a hormone mediated mobilisation of stored pollutant, which is then translocated via the blood plasma to sensitive brain sites.

The low polarity of organochlorine pesticides suggests that they are transported in association with plasma lipoproteins; such associations in mammals have been described for other lipophilic materials (Chalmers, 1955; Avigan, 1959; Moss and Hathaway, 1964; Wahlquist, Nilsson, Sandberg, Agurell and Granstand, 1970).

In the present study of the transport of low polarity pollutants in the rainbow trout the substrates used were, DDT, representing the broad class of organochlorine pollutants and n-hexadecane and 3,4-benzpyrene representing respectively, the paraffinic and more objectionable aromatic constituents of fossil fuels.

Materials and Methods

DDT - C^{14}, 3, 4 - benzpyrene - H^3 and n - hexadecane - H^3 (Radiochemical Centre Ltd) were administered intravenously, in a 60:40 v/v isopropanol isotonic saline vehicle, to rainbow trout at 250 ug/kg body weight. Pooled plasma samples obtained after 24 hours were subjected to preparative ultracentrifugation by the method of Barclay, Barclay, Terebus-Kekish, Shah and Skipski, (1963). Radioactivity was determined in aliquots of the lipoprotein fractions obtained, by liquid scintillation spectrometry. Their lipid class composition was determined; phopholipid phosphorus was determined by the method of Shin (1962), neutral lipids were fractionated by the thin layer chromatographic method of Skipski (1965) and identified and quantitated, after charring, by photodensitometric comparison with known standards.

Aliquots of the ultracentrifugal fractions were subjected to electrophoresis on polyacrylamide gel and portions of these gels were counted for radioactivity. Samples prestained for lipoprotein by the method of Narayan, Creinin and Kummerow,(1966) were also examined.

Fish injected previously with tritiated DDT (New England Nuclear) at about 320 ug/kg body weight were maintained in the neutralised anaesthetic M.S. 222 (Sandoz) at an appromimate final concentration of 1:15000. Plasma samples were prepared from blood samples withdrawn by caudal puncture before, and at intervals after the intravenous administration of adrenalin and cortisol at 40 ug/kg and 10 mg/kg body weight respectively. Plasma samples were taken and microhaematocrit and radioactivity determinations made.

Tritiated DDT was administered to two groups of rainbow trout in association with $^{47}Ca^{++}$ and determined accurately as previously described (Simpson, Johnstone and Youngson, 1974). One group of fish received pollutant in autumn, in the pre-spawning season, while the other was treated in spring. The retention of pollutant and its representation in the brains of these fish was determined after 10 weeks.

Results and Discussion

DDT was present in the plasma lipoprotein fractions in amounts approximately proportional to their total lipid content, (Table 1). DDT and lipid were present only in small amounts in the material remaining after lipoprotein flotation (density >1.21 g/ml).

After electrophoresis on 7.5% polyacrylamide gel, DDT in unfractionated plasma was associated with an immobile and a diffuse mobile band of sudanophilic protein (Fig. 1). The occurrence of these components in the lipoprotein fractions was examined. On 7.5% polyacrylamide, DDT was shown to be associated in Fractions 1-4 with an immobile lipoprotein (Table 1 and Fig. 1). Each of the two most dense lipoprotein fractions (Fractions 3 and 4) also bore DDT in association with a mobile component. All the immobile and both the mobile components were shown to be electrophoretically heterogeneous on 5% polyacrylamide gel (Fig. 2).

The ultracentrifugal distribution of tritiated hexadecane and benzpyrene in plasma obtained from fish injected simultaneously with one of these compounds and DDT - C14 was examined, (Table 2). The profiles of their representation in the lipoprotein fractions were similar to that of DDT, except in the greater occurrence of pollutant in the ultracentrifugal residue. A mean 6% of DDT was accommodated in this fraction while benzpyrene and hexadecane were present at 20% and 22% respectively. These latter data are broadly in agreement with those of Moss and Hathaway (1964) who found 26% representation of Telodrin in the same fraction of rabbit serum. Avigan (1959) found dibenzanthracene and methylcholanthrene were similarly represented at 5% and 17% respectively, in rat serum. These differences between the binding affinities of similarly highly lipophilic substances for the non-lipoprotein constituents of the circulatory fluids are likely to be the result of stereochemical differences.

The dominant role of plasma lipoids in the transport of non-polar pollutants suggests that changes in physiological status are likely, by affecting lipid mobilisation and transport, to effect translocations of such pollutants. The effect of food deprivation on the mobilisation of DDT has been discussed by Simpson, Youngson and Johnstone (1974). Transport of DDT must also be expected to be effected by the hormones of the adrenal gland and the gonads. Although the effect of adrenalin in mammals is invariably one of increasing the concentration of non-esterified fatty acids (NEFAs), its effect in fishes has not yet been resolved, reportedly increasing

(Leibson, Plisetskaya and Mazina, 1968; Mazeaud, 1973) or decreasing the concentration of NEFAs (Farkas, 1967; Minick, 1970). Cortisol has been shown to elevate plasma NEFA concentration in fishes, (Minick, 1970). The following studies were performed to determine the effects of these hormones on the representation of DDT in the blood plasma of rainbow trout.

Plasma DDT titres were determined after the administration of hormone and microhaematocrit determinations were used to define changes in plasma volume (Van Beaumont, 1972). The control procedure itself caused residue concentrations to fall. This was shown to result from increased plasma volume consequent on anaesthesia. No net mass transport of DDT to or from the plasma volume occurred.

Intravenously administered adrenalin caused plasma residue levels to be elevated significantly ($p > 0.05$) above control values by a mean 19%, with a mean latency of 10 minutes. Peak response occurred 30 minutes after injection. Consideration of the haematocrit data led to the conclusion that this elevation of pollutant levels was entirely attributable to a reduction of plasma volume to a mean 70% of the pre-response level, the consequence of the adrenalin induced increase in vascular resistance and transcapillary pressure. There had, indeed, occurred a slight net outflow of DDT from the plasma to the extra-vascular compartments. Cortisol increased pollutant concentrations significantly ($p > 0.05$) by a mean 14%. It acted with a latency of 30 minutes to achieve peak response 15 minutes later. The haematocrit data showed that no reduction in plasma volume had occurred and that cortisol had induced a net mass transport of DDT.

The elevation in the concentration of DDT in blood plasma which occurs as a result of the secretion of adrenalin and cortisol must be expected, in the dynamic situation, to cause a flux of pollutant to all tissues including the brain.

It is known that there is an increase in plasma lipoproteins during sexual development as nutrient is mobilized to the developing gonad. Such an increase must be expected to be mirrored by increases in the concentration of lipophilic pollutants in blood plasma and in corresponding increases in the transport of pollutant from the depot lipids to tissues. In agreement with this, it has been observed that the mean quantity of DDT in the brain of pre-spawning rainbow trout was five times higher (significantly higher at $P < 0.001$) than that in trout examined in spring. Similarly, the retention of pollutant in trout during the spring was significantly higher at 88% than in the pre-spawning fish (24%)

It has been reported that sexual maturation and spawning, in the barracuda (Deichmann, Cubit, MacDonald and Beasley, 1972) and sea-trout (Butler, Childress and Wilson, 1970) results in a reduction in the body load of pesticide. The present observations, show that though an increase in excretion does occur during sexual development, any protective effect which this may have is far outweighed by the increase in pollutant transport to the brain.

It is believed that these observations provide a rationale for field observations that the toxic hazard of pollutants to fish is greatly increased when they are subjected to stress or are in the course of sexual maturation.

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Fraction	Density	Lipid Content (mg/100 g plasma)					Lipid % of total	DDT % of total	Electrophoretic Behaviour	
		Sterol ester	Sterol	Tri-glyceride	Phospho-lipid	Total			Origin DDT %	Mobile DDT %
1	<1.0086	16	4	23	6	49	12	22	18	4
2	<1.0635	32	22	38	18	110	27	28	24	4
3	<1.125	55	13	46	70	184	44	36	7	29
4	<1.21	12	6	8	20	46	11	12	2	10
5	>1.21	12	2	3	8	25	6	3	1	2

Table 2

Fraction	Density
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32	47
31	27
20	7

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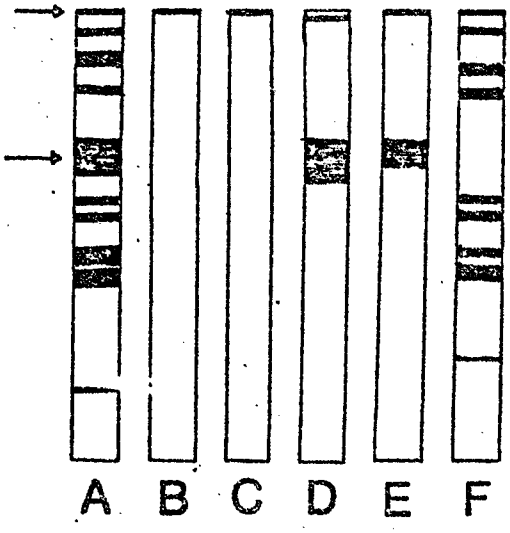
4	3
18	22
37	49
20	22
22	5

FIG 1

Electropherograms on 7.5% polyacrylamide gel in Tris buffer, pH 9.2. Stained with Naphthalene Black.

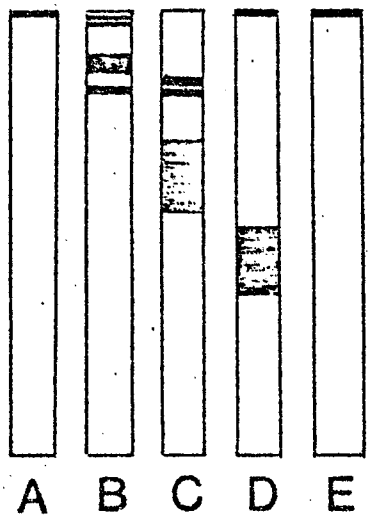


A. Unfractionated plasma. Arrows indicate those protein bands with which DDT is associated.

B-F. Ultracentrifugal fractions 1-5 respectively.

FIG 2

Electropherograms on 5% polyacrylamide gel in Tris buffer, pH 9.2. Prestained with Sudan Black.



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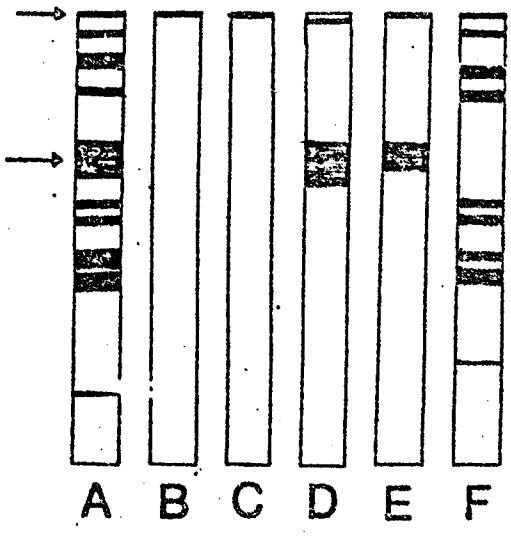
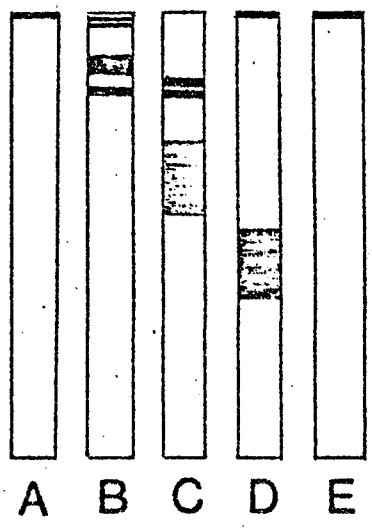


A. Unfractionated plasma. Arrows indicate those protein bands with which DDT is associated.

B-F. Ultracentrifugal fractions 1-5 respectively.

FIG 2

Electropherograms on 5% polyacrylamide gel in Tris buffer, pH 9.2. Prestained with Sudan Black.



A-E. Ultracentrifugal fractions 1-5 respectively.