LOCOMOTORY AND DEHYDROGENASE ACTIVITIES OF REDSTARTS *PHOENICURUS PHOENICURUS* L. (AVES) GIVEN PCB AND DDT

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ABSTRACT

Caged redstarts trapped during the autumn migration were given PCB and DDT injected into meal-worms. There was a tendency, although not statistically significant, of increased locomotory activity in PCB-treated birds in the early night hours, that is, at the peak of normal migratory activity. Similarly, there was a tendency in both PCB- and DDT-treated birds to altered dehydrogenase activities, suggesting metabolic changes presumably due to the contaminants accumulated during treatment. The organ residue levels found at analysis were comparable to those reported from wild birds in the field. The changes in the dehydrogenase activities were more pronounced for liver and breast muscle of the DDT-treated birds.

INTRODUCTION

Non-lethal doses of organochlorine substances have been shown to have, or have been suspected of having, effects on vertebrate organisms and populations, as well as on whole ecosystems (*e.g.* Peakall, 1967; Lincer & Peakall, 1970; Cade *et al.*, 1971; Ratcliffe, 1972). On the other hand, Persson (1971) found no effects on the reproduction in the whitethroat, *Sylvia communis*, Lath, neither did Dyck *et al.* (1972) in some other passerine species.

We have carried out a series of experiments in which caged redstarts *Phoenicurus phoenicurus* L., a turdine passerine, trapped during their autumn migration, were given PCB and DDT. Subsequently, the locomotory activity and the activities of

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four dehydrogenases in these birds were compared with those of control birds. After the termination of the experiments the amounts of test substances in various tissues of the birds were measured.

MATERIAL AND METHODS

Treatment of birds and measurement of their locomotory activity

The redstarts were trapped at Falsterbo Bird Station in Southern Sweden in September 1971. In the laboratory they were kept and fed as described by Södergren & Ulfstrand (1972). The test substances were administered after at least five days of acclimatisation, and each bird received daily one meal-worm injected with either 11·0 μg of PCB (Clophen A 50) or 10·5 μg of p,p'-DDT dissolved in ethanol. In all, the birds ingested 132 μg of PCB or 126 μg of DDT in the course of 12 days. The birds were weighed to the nearest 0·1 g every other day. The control birds were daily given one meal-worm injected with a corresponding volume of ethanol (2 μl).

The locomotory activity was measured according to Cavallin *et al.* (1972). The activity was monitored during three successive 24-h periods, starting one day after the last contaminated meal-worm had been fed to the birds. During the measurement period the birds were given food once daily, at about noon.

Enzyme assay technique

After the end of the locomotory activity measurements the birds were anaesthetised with ethyl ether. Kidney, liver, breast muscles and brain were excised and divided into two parts (except kidney). One portion was used for the determination of organochlorine residues (*see* below), the other portion, including the kidney, for enzyme assay. The latter portion was treated as follows. The tissues were weighed and then stored at −20°C for one to four weeks. Homogenates of the organs were prepared in a saline-phosphate buffer of pH 7·2 (composition: 8·5 g NaCl, 1·07 g Na_2HPO_4, 0·39 g NaH_2PO_4. $2H_2O$ per litre distilled water) in the weight/volume proportions 1:10 using a glass-homogeniser with Teflon pestle. The homogenate was centrifuged at 3000 × g for 30 min (+4°). The resulting supernatants were used for the enzyme analyses (= 'organ extracts').

The activities of lactic dehydrogenase (LDH), malic dehydrogenase (MDH), α-glycero-phosphate dehydrogenase (α-GPDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) were determined in a Unicam SP 800-A spectrophotometer. The activity was measured from the initial rate of the increase in amount of NADH and NADPH in the reactions NAD → NADH and NADP → NADPH at 340 mμ using distilled water as reference. The determinations were performed at +30°. The final volume in each cuvette was 3 ml.

Reactions were started by adding supernatant to the corresponding reaction mixtures. Sometimes the activity was too high, giving non-linear changes in extinction during the first minute of registration. In such cases the supernatant was diluted before analysis until linear curves were obtained. The changes in extinction were recorded for three minutes.

Enzyme activities are expressed as enzyme units/g wet weight. One enzyme unit is defined as the amount of enzyme which catalyses the transformation of one micromole of substrate/minute.

Amounts of supernatants and composition of reaction mixtures for assay of the four enzyme systems were as follows (cf. Bergelin & Karlsson, 1973).

LDH (EC 1.1.1.27): 50 ml 0·5M Tris-HCl, pH 8·9; 50 ml 2M Li-lactate and 400 ml distilled water. 10 mg NAD was added to each 15 ml of this reaction mixture. The reaction was started by adding 10 μl organ extract to 3·0 ml reaction mixture.

MDH (EC 1.1.1.37): 51 ml 0·1M glycin-buffer pH 9·8; 7 ml 0·1M Na-malate. 30 mg NAD was added to each 58 ml of this solution. The reaction was started by adding 10 μl organ extract to 3·0 ml reaction mixture.

G-6-PDH (EC 1.1.1.49): 21·6 ml 0·1M Tris-HCl pH 8·0; 0·83 ml 0·03M glucose-6-phosphate; 0·83 ml 0·01M NADP; 1·65 ml 0·15M $MgCl_2$. The reaction was started by adding 50 μl organ extract to 3·0 ml reaction mixture.

α-GPDH (EC 1.1.1.8): 20·0 ml 0·1M glycin-buffer, pH 9·2; 2·5 ml 0·1M α-glycero-phosphate; 2·5 ml NAD solution (3·85 mg/ml). The reaction was started by adding 50 μl organ extract to 3·0 ml reaction mixture.

Organochlorine residue analysis

The tissue portion not used for enzyme assay was treated as described in Ulfstrand *et al.* (1971) and Södergren & Ulfstrand (1972).

The term 'carcass' denotes the whole body of the bird after brain, breast muscles, liver, kidney, tarsi, bill and feathers had been removed.

RESULTS

Levels of organochlorine residues

Administration of test substances, as described above, resulted in a thirty-fold rise of PCB and a sixfold rise in DDT total quantities in the contaminated birds in comparison with their controls (Table 1). In the contaminated birds approximately

TABLE 1

QUANTITIES AND DISTRIBUTION OF Σ DDT AND PCB IN THE EXPERIMENTAL AND CONTROL BIRDS. Σ DDT = P,P'-DDT + P,P'-DDD + P,P'-DDE. BR. = BRAIN, BRM. = BREAST MUSCLES, LIV. = LIVER, CARC. = CARCASS, N.D. = NOT DETECTED

Substance administered: p,p'-DDT $n = 10$			*Controls* $n = 10$	
	Σ DDT (μg)	Σ DDT (%)	Σ DDT (μg)	Σ DDT (%)
br.	0·1 (0·0–0·1)	0·3	n.d.	0·0
brm.	0·7 (0·2–1·3)	1·9	0·1 (0·0–0·1)	1·7
liv.	0·2 (0·1–0·2)	0·5	n.d.	0·0
carc.	36·5 (25·5–51·8)	97·3	5·6 (1·4–12·8)	98·3
tot.	37·5	100·0	5·7	100·0
Substance administered: PCB $n = 9$			*Controls* $n = 5$	
	PCB (μg)	PCB (%)	PCB (μg)	PCB (%)
br.	0·1 (0·0–0·3)	0·3	n.d.	0·0
brm.	0·5 (0·3–0·9)	1·5	n.d.	0·0
liv.	0·3 (0·1–0·4)	0·9	<0·1 (0·0–0·3)	9·1
carc.	33·0 (10·5–41·7)	97·3	1·0 (0·0–1·8)	90·9
tot.	33·9	100·0	1·1	100·0

39% of the PCB and 34% of the DDT administered was recovered. The major portion of the substances was stored in the carcass which also held the highest concentrations (Table 2). This distribution was found in both contaminated and control birds, so that obviously the substances administered dispersed according to the same pattern of distribution as that found in the control birds.

Locomotory activity and weight

Mean total locomotory activity/h of all redstarts is illustrated in Figs. 1 and 2. A comparison between contaminated and control birds revealed no significant differences, but certain interesting tendencies, in terms of activity patterns. Nor were any differences of total body weight established between contaminated and control birds, or between PCB and DDT birds, at the end of the experiment. The same also applied when each organ was examined separately.

Dehydrogenase activities

No statistically significant differences were found in terms of dehydrogenase activity between PCB- and DDT-treated birds, nor between contaminated birds and their corresponding controls (Tables 3 and 4). This holds for all organs examined. Throughout, however, there was a tendency of increased activity of α-GPDH and MDH in both PCB- and DDT-treated birds compared with their controls. This tendency was most pronounced for the activities of MDH in liver

TABLE 2

RESIDUE LEVELS IN EXPERIMENTAL AND CONTROL BIRDS CALCULATED AS PPM FAT WEIGHT. BR. = BRAIN, BRM. = BREAST MUSCLES, LIV. = LIVER, CARC. = CARCASS, N.D. = NOT DETECTED

		% Extractable fat	*p,p'DDE*	*p,p'-DDD*	*p,p'-DDT*	*PCB*
Compound administered: p,p'-DDT						
	br.	7·3 (4·9–12·0)	0·8 (0·4–2·7)	n.d.	1·4 (0·0–2·5)	0·2 (0·0–1·9)
$n = 10$	brm.	5·0 (1·8–7·3)	2·6 (0·4–6·6)	0·6 (0·2–2·4)	12·8 (2·1–33·1)	<0·1 (0·0–0·3)
	liv.	3·4 (0·6–8·4)	4·1 (0·6–12·7)	3·7 (0·5–9·8)	13·4 (2·0–45·3)	n.d.
	carc.	11·1 (6·1–17·9)	12·2 (0·3–58·9)	4·0 (0·7–16·6)	28·8 (10·1–65·8)	n.d.
control						
	br.	5·7 (4·7–6·3)	0·2 (0·1–0·5)	n.d.	0·1 (0·0–0·2)	n.d.
$n = 10$	brm.	6·0 (2·0–9·4)	0·9 (0·4–2·0)	0·1 (0·0–1·0)	0·5 (0·0–1·9)	0·3 (0·0–0·5)
	liv.	1·9 (0·5–3·4)	1·4 (0·3–2·9)	0·2 (0·0–0·1)	0·5 (0·0–1·5)	n.d.
	carc.	13·3 (6·5–20·2)	3·2 (0·6–6·1)	0·6 (0·0–3·4)	1·4 (0·0–3·4)	0·4 (0·0–2·4)
Compound administered: PCB						
	br.	5·2 (2·3–7·7)	0·1 (0·0–1·0)	n.d.	n.d.	2·5 (0·9–3·7)
$n = 9$	brm.	3·6 (2·9–4·4)	0·4 (0·1–0·8)	n.d.	0·1 (0·0–0·3)	7·6 (1·1–13·2)
	liv.	4·4 (3·7–6·0)	0·5 (0·2–1·1)	n.d.	0·1 (0·0–0·8)	10·0 (3·2–20·2)
	carc.	15·5 (7·3–24·3)	1·2 (0·4–2·2)	0·1 (0·0–0·1)	0·2 (0·0–1·3)	20·6 (4·5–39·6)
control						
	br.	4·7 (3·4–5·8)	0·1 (0·0–0·3)	n.d.	n.d.	n.d.
$n = 5$	brm.	3·8 (3·0–5·1)	0·5 (0·4–0·7)	n.d.	0·2 (0·1–0·5)	1·0 (0·0–1·9)
	liv.	4·6 (2·7–6·1)	0·5 (0·4–0·7)	n.d.	0·2 (0·2–0·3)	0·5 (0·0–0·9)
	carc.	11·8 (5·5–23·9)	2·3 (1·7–2·9)	0·1 (0·0–0·1)	2·2 (0·7–3·8)	1·4 (0·0–3·1)

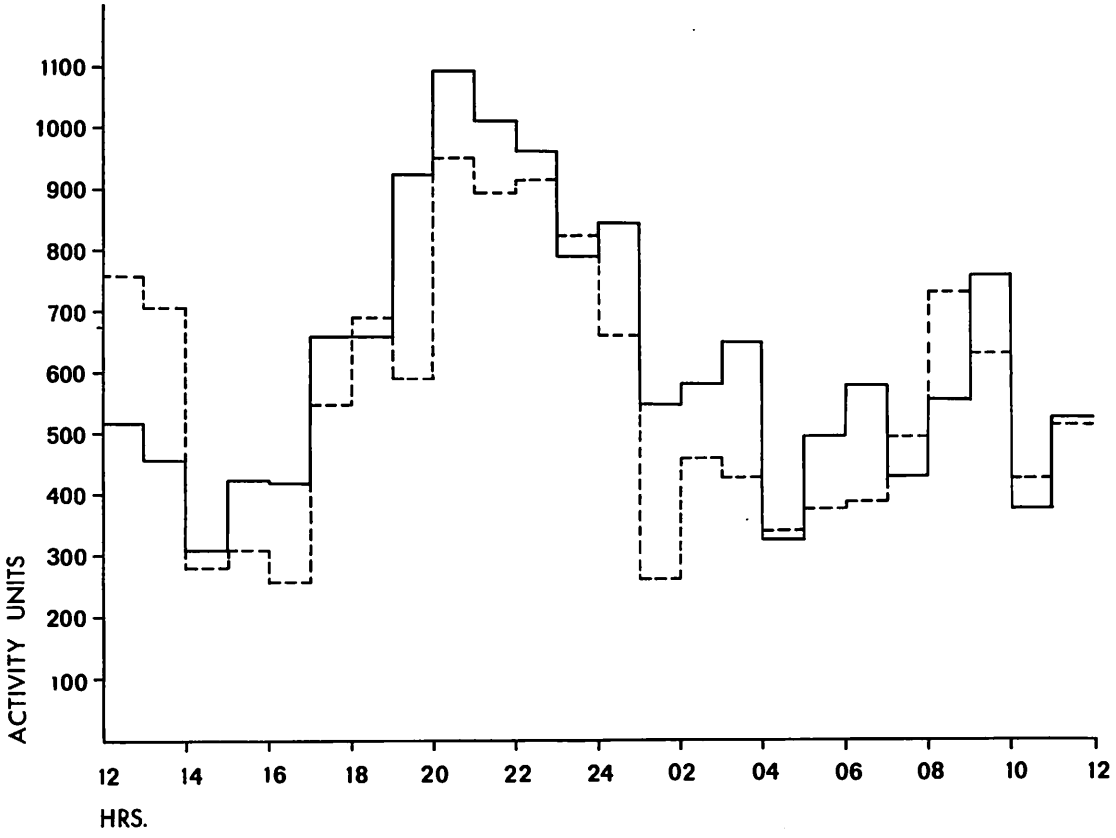

Fig. 1. Mean hourly activity of ten DDT-treated and ten control redstarts (*Phoenicurus phoenicurus*) during the 24-h study period. — — — — = DDT-treated birds, ———— = control birds.

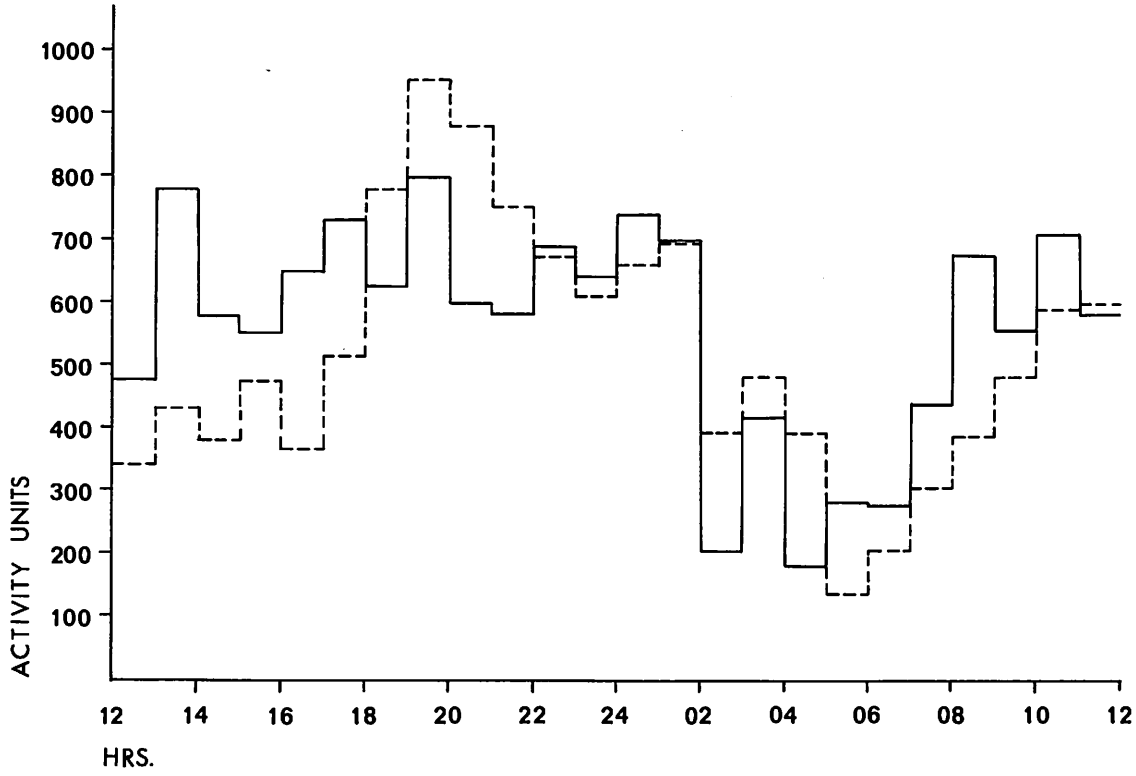

Fig. 2. Mean hourly activity of nine PCB-treated and five control redstarts (*Phoenicurus phoenicurus*) during the 24-h study period. — — — — = PCB-treated birds, ———— = control birds.

TABLE 3

DEHYDROGENASE ACTIVITIES (ENZYME UNITS/WET WEIGHT) OF LIVER, BRAIN, BREAST MUSCLE AND KIDNEY OF REDSTARTS CONTAMINATED WITH PCB, AND CONTROL BIRDS. THE VALUES ARE MEANS ±SD (*n*)

		Liver	*Brain*	*Breast muscle*	*Kidney*
LDH	PCB	3·5 ± 0·8 (9)	4·5 ± 0·6 (9)	5·7 ± 1·1 (9)	3·4 ± 0·6 (9)
	controls	3·8 ± 0·9 (5)	4·2 ± 1·1 (5)	5·5 ± 1·2 (5)	3·5 ± 0·8 (5)
MDH	PCB	14·9 ± 2·4 (9)	5·5 ± 0·8 (9)	27·5 ± 6·3 (9)	10·3 ± 1·5 (9)
	controls	14·1 ± 2·6 (5)	5·1 ± 1·3 (5)	25·0 ± 5·0 (5)	9·3 ± 1·4 (5)
α-GPDH	PCB	1·6 ± 0·6 (9)	0·07 ± 0·01 (9)	0·41 ± 0·10 (9)	0·12 ± 0·05 (9)
	controls	1·2 ± 0·4 (5)	0·06 ± 0·01 (5)	0·35 ± 0·07 (5)	0·11 ± 0·06 (5)
G-6-PDH	PCB	0·03 ± 0·00 (9)	0·03 ± 0·00 (9)	0·01 ± 0·00 (9)	0·04 ± 0·00 (9)
	controls	0·03 ± 0·00 (5)	0·03 ± 0·01 (5)	0·01 ± 0·00 (5)	0·03 ± 0·00 (5)
LDH/MDH	PCB	0·237 ± 0·037	0·823 ± 0·064	0·216 ± 0·055	0·330 ± 0·028
	controls	0·266 ± 0·033	0·815 ± 0·062	0·222 ± 0·024	0·372 ± 0·045

TABLE 4

DEHYDROGENASE ACTIVITIES (ENZYME UNITS/WET WEIGHT) OF LIVER, BRAIN AND BREAST MUSCLE OF REDSTARTS CONTAMINATED WITH DDT, AND CONTROL BIRDS. THE VALUES ARE MEANS ± SD (*n*)

		Liver	*Brain*	*Breast muscle*
LDH	DDT	5·1 ± 1·4 (10)	3·8 ± 1·0 (10)	5·6 ± 1·0 (10)
	controls	4·6 ± 0·8 (10)	3·9 ± 0·5 (10)	5·5 ± 0·9 (10)
MDH	DDT	19·4 ± 4·9 (10)	5·5 ± 1·2 (10)	33·0 ± 6·0 (10)
	controls	16·7 ± 3·3 (10)	5·2 ± 0·9 (10)	27·2 ± 6·1 (10)
α-GPDH	DDT	2·4 ± 0·8 (10)	0·07 ± 0·03 (10)	0·34 ± 0·10 (10)
	controls	2·1 ± 0·4 (10)	0·06 ± 0·01 (10)	0·35 ± 0·12 (10)
G-6-PDH	DDT	0·03 ± 0·00 (10)	0·04 ± 0·01 (10)	0·01 ± 0·00 (10)
	controls	0·03 ± 0·01 (10)	0·04 ± 0·00 (10)	0·01 ± 0·00 (10)
LDH/MDH	DDT	0·264 ± 0·039	0·682 ± 0·088	0·172 ± 0·026
	controls	0·289 ± 0·110	0·761 ± 0·077	0·215 ± 0·074

and breast muscle of DDT-contaminated birds as compared with their controls. Higher MDH activity was found in liver and breast muscles of the DDT-treated birds in comparison with those given PCB. Because of these differences in the MDH activities and the lack of differences in the activities of LDH between DDT- and PCB-treated birds and their controls, it follows that the LDH/MDH quotients become rather low for the liver and the breast muscle of the DDT-treated redstarts (Tables 3 and 4).

DISCUSSION

Dosages of test substances were deliberately held low in an attempt not to deviate from conditions in wild birds. We regard this precaution as essential for acquiring ecologically-useful information. In comparison with available data, the levels attained in our experimental birds corresponded to those found in other Swedish wild birds (Odsjö, 1973). In every respect our caged birds appeared healthy, and during the study period (3 to 4 weeks) only one of them died.

The retention of PCB and DDT in the birds was close to the values found in a previous study of robins *Erithacus rubecula* L., another turdine passerine of similar size (Södergren & Ulfstrand, 1972).

The four dehydrogenases studied were selected because they have more or less key positions in the carbohydrate metabolism. LDH catalyses the final step in anaerobic metabolism of glucose. MDH is a participant in the citric acid cycle which is of importance for the aerobic metabolism in the cell. MDH also has a function in gluconeogenesis. G-6-PDH catalyses the first step in the pentose shunt which is of importance for the production of $NADPH_2$ and ribose. α-GPDH serves as a link between glycerol catabolism and glycolysis. The ratio of the enzyme activity quotient LDH/MDH is considered to give some information about the intensity of the anaerobic metabolism as compared with that of aerobic metabolism (Bücher & Klingenberg, 1958).

The slight increase in the activity of α-GPDH can be interpreted as an increased turnover of glycerol and thus the rate of lipid metabolism. The higher increase in the α-GPDH activity of the PCB-treated birds should indicate that PCB affects the metabolism of fat slightly more than DDT. However, no differences in terms of body weight nor of liver weight were found, neither between contaminated and control birds, nor between birds given DDT and PCB, respectively. By contrast, in ducks given PCB, Friend & Trainer (1970) established a significant increase in body weight.

The increased activity of MDH in liver and breast muscle, which was more pronounced for DDT- than for PCB-contaminated birds, can be an expression of increased aerobiosis or increased capacity of gluconeogenesis. This increase in the activity of MDH corresponds to the fact that high amounts of DDT and PCB were found in liver and breast muscle but not in the brain of the DDT- and PCB-treated birds.

No changes in the activity of LDH was noted in PCB- and DDT-treated birds in these *in vivo* experiments. However, Sova (1966) has shown that DDT may inhibit LDH *in vitro*.

When the quotients LDH/MDH for different organs were calculated, they were invariably found to be lower in DDT-treated birds than in the corresponding control group. This may indicate that metabolism is in a more aerobic state in birds containing high concentrations of DDT. These findings are in line with results obtained by other investigators, who have reported *in vitro* effects of DDT on the metabolism (Wadill & Keeley, 1970; Kimbrough *et al.*, 1971).

The redstart migrates in autumn from Sweden to tropical Africa. Its migratory activity is restricted to the hours of darkness. In southern Sweden, Alerstam (1972) has found that the main nocturnal migratory activity of birds culminates in the early night hours during the three to four hours after sunset; exceptions may be found, for example, in connection with weather front passages. We therefore find it particularly noteworthy that the tendency of elevated locomotory activity in PCB

birds was restricted to the hours during which, under natural conditions, the migratory activity would culminate. PCB has previously been shown to affect the migratory restlessness in caged robins (Ulfstrand *et al.*, 1971). On the other hand, during the daylight hours (0500 to 1800 h), control birds displayed a tendency towards higher locomotory activity than the PCB-dosed birds. Thus, the total activity for the period studied reached similar magnitudes in the two groups.

The present results demonstrate certain tendencies but fail to reach statistical significance. It is an interesting fact that the effect of PCB on the locomotory activity of the redstarts corresponds to small changes in the activities of the dehydrogenases in various organs. For DDT the opposite is true: the changes in enzyme activities are more pronounced, while no consistent changes are visible in the locomotory activity. These findings indicate that PCB and DDT may affect the complex processes of the cellular metabolism and the behaviour of organisms by different modes of action.

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