Organochlorine Compounds in Neoplastic and Adjacent Apparently Normal Breast Tissue

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In previous papers (9,10) we summarized our findings regarding the concentration of organochlorine compounds in the adipose tissue of presumably healthy people who died accidentally.

In this paper we report some features of the storage of organochlorine compounds (OCC), organochlorine insecticides (OCI), and polychlorinated biphenyls (PCBs) in neoplastic breast tissue in comparison with those in adjacent, apparently normal, tissue.

Materials and Method

Malignant tissue, adjacent apparently normal glandular and adipose tissue of nine women with adenocarcinoma of the breast obtained from the A.C. Camargo Hospital, Foundation Antonio Prudente, Sao Paulo, were studied. Mammary gland and adjacent adipose tissue of five presumably healthy women who died accidentally received from the Medico-Legal Institute of Sao Paulo served as controls.

Fragments of 1-2 g. of tissue were kept in glass jars containing a 4% formalin solution until OCC assessment. The tissue lipids were extracted by the method of Folch (2) : 0.5 g. of tissue was homogenized with 19 cm. of a mixture of 2:1 chloroform - methanol v/v, filtered through a fat free paper into a centrifuge tube. 2 ml. of water were added. The whole was mixed with a stirring rod, and afterwards centrifuged (about 20') until complete separation into two phases was obtained. The upper phase was removed as completely as possible, the lower phase was

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transferred into a weight tube, evaporated by a nitrogen flow until constant weight was reached. The figure obtained was a reference point for the calculation of the amount of organochlorine compounds (organochlorine insecticides and polychlorinated biphenyls) in extracted lipids.

Organochlorine insecticides were separated from polychlorinated biphenyls using the Armour and Burke method (chromatography on a silicic acid - celite column (1). The extracted lipids were dissolved in 20 ml. petroleum ether and allowed to pass through the column. Polychlorinated biphenyls were obtained in the eluate. Organochlorine insecticides were eluted afterwards with 20 ml. of a mixture of acetonitrile 1%, hexane 19% and methylene chloride 80%. Each of the two eluates was concentrated to a volume of 0.5 ml. The organochlorine compound levels were determined by gas chromatography with electron capture detection and spiral glass column (6 feet/ 4 mm.), containing a mixture of equal parts of 15% QF and 10% DC - 200 on 80-100 mesh chromosorb WHP for polychlorinated biphenyls and 5% QF - 1 on chromosorb WHP on 80-100 mesh for organochlorine insecticides.

Arochlor 1254 and a mixture of pure organochlorine insecticides were used as standards. The values of OCI and PCBs referred to in this paper represent the concentration of the respective compounds in extracted lipids of abovementioned tissues.

Results and Comments

Radomski et al. found "a suggestive relationship between elevated pesticide concentrations in adipose tissues in cases of portal cirrhosis, carcinoma and hypertension." (3)

From previous studies of storage of organochlorine insecticides in people occupationally (7) or non occupationally exposed to organochlorine insecticides (9, 10), values ranging from ppbs to tens of ppm have been reported. The data in this study based on a few cases, are inadequate for conclusions regarding the amount of the organochlorine compounds in cancer patients. Nevertheless, comparison of the different compounds or metabolites regarding individual cases may lead to suggestive conclusions.

In the breast and adipose tissue of control and cancer patients p,p'-DDE is larger than p,p'-DDT, a feature of chronic exposure to DDT. In the neoplastic breast tissue, the value of p,p'-DDE is lower than that of p,p'-DDT (Table 1). This fact may be seen as a slowing of the process of metabolisation of p,p'-DDT to p,p'-DDE, but also as a storage of more recent date, the malignant tissue growing in a relatively short time in comparison to the normal. O,p'-DDT is much increased in the malignant tissue when compared to the normal breast and adjacent adipose tissue. The proportion between the o,p'-DDT and o,p'-DDE

Table 1. Organochlorine insecticides (ppm) in extracted lipids of malignant and normal breast and adjacent adipose tissue.

Compound \ Tissue		Control (n: 5)		Cancer of breast (n: 9)		
		Breast tissue	Adjacent adipose tissue	A. Malignant tissue	B. Adjacent breast tissue	C. Adjacent adipose tissue
p,p'-DDT	Range	1.58-8.24	1.24-5.78	1.35-6.40	0.39-2.81	0.15-1.46
	Mean	3.5516	3.4004	4.3978*	1.4266	0.7582
p,p'-DDD	Range	0.33-0.86	0.0 -1.58	-	0.0 -0.84	0.0 -0.83
	Mean	0.5493	0.5378	-	0.2426	0.1178
p,p'-DDE	Range	4.81-8.75	2.63-6.50	0.44-6.61	0.22-5.35	0.33-4.46
	Mean	6.6697	4.3215	2.7149	2.0276	1.5291
o,p'-DDT	Range	0.21-1.17	0.0 -0.12	0.0 -12.19	0.0 -2.28	0.0 -5.80
	Mean	0.7775	0.0646	2.8487	1.0044	1.1417
o,p'-DDD	Range	-	-	0.0 -4.08	0.0 -4.28	0.0 -3.72
	Mean	-	-	1.4115**	0.7292	0.6000
o,p'-DDE	Range	0.06-0.32	0.04-0.19	0.34-4.70	0.07-1.47	0.07-1.20
	Mean	0.1639	0.0890	1.8589	0.5689	0.3043
Total p,p'-DDT	Range	8.01-16.78	5.36-13.09	1.84-12.38	0.82-7.50	0.52-5.37
	Mean	11.5309	8.7597	7.4237	3.9327	2.5630
Total o,p'-DDT	Range	0.28-1.52	0.04-0.33	2.23-17.01	0.40-8.45	0.18-5.88
	Mean	0.9601	0.1619	6.0854	2.3674	2.0796
T. DDT	Range	8.29-18.30	5.44-13.13	4.07-29.39	1.99-13.56	0.95-11.26
	Mean	12.4910	8.9216	13.5091	6.3001	4.6426
Y - BHC	Range	0.19-0.81	0.14-0.34	0.21-1.28	0.10-2.57	1.02-0.36
	Mean	0.3905	0.2586	0.6100	0.5698	0.1403
Dieldrin	Range	0.16-0.47	0.05-0.33	0.0 -2.67	0.0 -1.25	0.26-1.44
	Mean	0.2891	0.1699	0.9056	0.4590	0.7960
Hexachl. epoxide	Range	0.0 -0.59	0.0 -0.18	0.0 -1.61	0.0 -0.51	0.0 -1.20
	Mean	0.2336	0.0444	0.3506	0.1282	0.2740

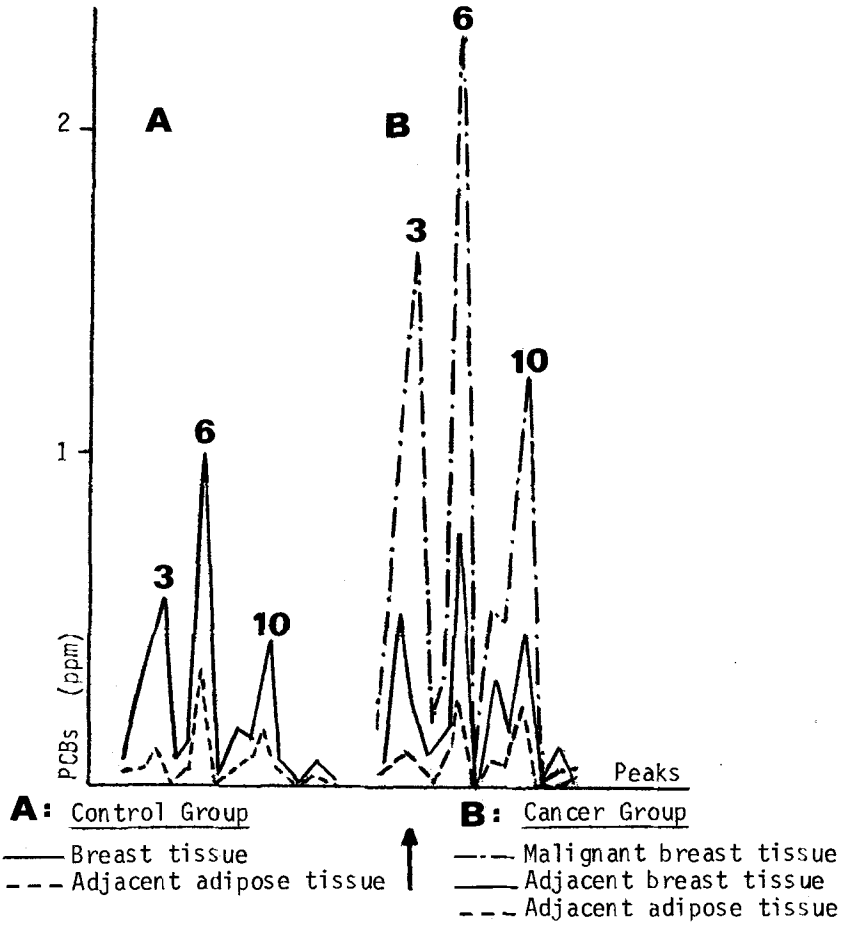
A versus B $^{*}p < 0.01$, $^{**}p < 0.10$

Table 2. Polychlorinated biphenyls (ppm) in extracted lipids of malignant and normal breast and adjacent adipose tissue.

Peaks \ Tissue		Controls (n:5)		Cancer of breast (n:9)		
		Breast tissue	Adjacent adipose tissue	A.Malig-nant tissue	B.Adjacent breast tissue	C.Adjacent adipose tissue
1	Range	0.04-0.13	0.0 -0.02	0.08-0.40	0.04-0.13	0.0 -0.05
	Mean	0.0787	0.0104	0.1988	0.0827	0.0220
2	Range	0.10-0.40	0.02-0.05	0.30-0.94	0.12-0.48	0.01-0.12
	Mean	0.2840	0.0419	0.6060	0.5732	0.0625
3	Range	0.35-0.73	0.01-0.21	0.55-4.06	0.19-0.35	0.06-0.13
	Mean	0.5530	0.1068	1.6318	0.2992	0.0851
4	Range	0.0 -0.10	0.0 -0.00	0.05-0.31	0.0 -0.29	0.0 -0.07
	Mean	0.0513	0.0023	0.1868	0.0920	0.0350
5	Range	0.09-0.22	0.0 -0.04	0.09-0.57	0.04-0.48	0.0 -0.12
	Mean	0.1324	0.0114	0.3324	0.1674	0.0388
6	Range	0.67-1.41	0.30-0.43	1.47-5.40	0.37-1.15	0.17-0.45
	Mean	1.0220	0.3477	2.2998	0.7937	0.2644
7	Range	-	-	-	-	-
	Mean	0.0330	-	-	-	-
8	Range	0.10-0.23	0.0 -0.04	0.32-0.86	0.07-0.73	0.01-0.20
	Mean	0.1637	0.0260	0.5526	0.3197	0.0980
9	Range	0.01-0.19	0.0 -0.10	0.0 -1.13	0.0 -0.42	0.0 -0.20
	Mean	0.1113	0.0356	0.5059	0.1903	0.0734
10	Range	0.30-0.71	0.0 -0.28	0.51-2.33	0.0 -1.01	0.0 -0.41
	Mean	0.4418	0.1347	1.2676	0.4789	0.2551
11	Range	0.0 -0.06	0.0 -0.09	-	-	0.0 -0.25
	Mean	0.0246	0.0019	-	0.0102	0.0484
12	Range	-	-	-	-	-
	Mean	-	-	-	-	-
13	Range	0.0 -0.13	0.0 -0.04	0.0 -0.31	0.0 -0.66	-
	Mean	0.0543	0.0159	0.0440	0.1381	-
14	Range	0.0 -0.10	0.0 -0.02	-	-	-
	Mean	0.0269	0.0085	0.0656	0.0056	0.0019
Total	Range	2.38-3.92	0.38-1.02	4.34-14.25	1.33-4.24	0.28-1.44
	Mean	2.9849	0.7658	9.1430*	2.7680	0.9720

A versus B $p < 0.01$ *

Graph 1.



is the same as that described for the p,p' isomers i.e. predominance of o,p'-DDT. O,p'-DDD is also fairly high when compared to controls.

Storage levels of DDT and metabolites in the liver and in the induced hepatomas of CF-1 mice exposed to DDT, measured on wet tissues, show the same higher proportion of DDE over DDT in normal liver than in the tumors (4).

The largest concentration of total DDT in cancer patients is found in the malignant tissue, when compared to the adjacent mammary and adipose tissues (Table 1). The same may be said

about the concentration of PCBs (Table 2) that is, the concentration of PCBs in extracted lipids of malignant breast tissue is considerably higher than in the normal breast and adipose tissue. The values obtained for the last two tissues is comparable to those found in the control subjects. The concentration of individual chlorinated biphenyls (Table 2 and Graph 1) is largest in the extracted lipids of malignant tissue. There is a correlation between these and the concentration of respective compounds in the adjacent mammary tissue with the exception of peak 3. The values for this compound are more divergent than for the others. (Graph 1, arrow) Again, an example of difference of rate of metabolization of a compound taking place in the malignant and normal mammary gland. Other organochlorine insecticides such as y-BHC, Dieldrin and Heptachlor epoxide show more or less the same tendency (Table 1).

The possible effect of these facts on the evolution of the neoplastic process cannot be estimated. There is some experimental evidence that long term exposure of mice to DDT results not only in an increased incidence of malignant tumors but also in a relative increase of metastases (5, 6). A moderation of the immunological response to foreign antigens found in rats and rabbits exposed to p,p'-DDT (8) may suggest a possible mechanism of action of OCC in carcinogenesis.

In conclusion, the findings of this study reveal, an increased concentration of OCC in extracted lipids of malignant breast tissue when compared to adjacent apparently normal tissue and differences between malignant breast tissue and adjacent apparently normal tissue, regarding the rate of metabolization of p,p'-DDT to p,p'-DDE, o,p'-DDT to o,p'-DDE, o,p'-DDD and that of a chlorinated biphenyl compound.

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