Organochlorine Compounds in Neoplastic and Adjacent Apparently Normal Gastric Mucosa

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Organochlorine compounds (OCC) (Organochlorine insecticides (OCI) and polychlorinated biphenyls (PCB)) are man-made compounds which have been used on a large scale respectively in preventive medicine, agriculture and industry for several decades.

Beside the expected benefits, they also however contributed to the deterioration of the environment. Their remanence in nature, and their increase in the food chain have resulted in their becoming constituents of the human body in our time (5).

The monitoring of OCC storage in humans revealed their ubiquitousness with quantitative variations related to such parameters as geographical location, age, sex, and physiological and pathological conditions of the population studied.(7)

Radomski et al. found "consistently high concentrations" of pesticides "in patients with cirrhosis, carcinoma and hypertension (4). Dacre and Jennings found that lung tissue from persons in New Zealand, known to have died of lung cancer, contained statistically significant higher concentrations of DDT and dieldrin when compared to deceased persons without lung cancer (2). An increased concentration of OCC, and differences regarding the rate of metabolisation of some OCC compounds, were found by us in extracted lipids of malignant breast tissue in comparison to adjacent apparently normal breast tissue and breast tissue in a control group (8).

In this paper we report on some features of the storage of OCC (OCI and PCBs) in gastric carcinomas, adjacent apparently nor mal gastric mucosa, epiploon and abdominal adipose tissue in cancer patients and in controls.

Material and method

Samples of malignant tissue, adjacent apparently normal gastric mucosa, epiploon and anterior abdominal wall adipose tissue of seventeen patients with gastric carcinoma obtained from the A.C. Camargo Hospital, Foundation Antonio Prudente, Sao Paulo, were studied. Gastric mucosa, epiploon and abdominal adipose tissue of fifteen presumably healthy people who died accidentally, obtained from the Medico-Legal Institute of Sao Paulo, served as controls.

Fragments of 1 - 2 g. of tissue were kept in jars containing a 4% formalin solution, until the OCC levels were assessed. The lipids of the above mentioned tissues were extracted by the method of Folch (3). 0.5 g. of tissue was homogenized with 10 ml. of a mixture 1:1 chloroform - methanol v/v and filtered through a fat free paper into a centrifuge tube. Two ml. of water were added. whole was mixed with a stirring rod, and afterwards (about 15 minutes) until complete separation into two phases was obtained. The upper phase was removed as completely as possible and the lower phase was transfered into a weight tube and evaporated by a nitrogen flow until constant weight was reached. The figure obtained served as a reference point for the calculation of the amount of OCC in extracted lipids. OCI were separated from PCBs using the Armour and Burke method (chromatography on a silicic acid celite column) (1). The extracted lipids were displyed in 20 ml. petroleum ether and allowed to pass through the column. PCBs were obtained in the eluate. OCI 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 levels of the OCC were determined by a gas chromatograph with an electron capture detector and spiral glass column (6 feet/4mm.), containing a mixture of equal parts of 15% QF-1 and 10% DC - 200 on 80 - 100 mesh chromosorb W.H.P. for PCBs and 5% OF-1 on chromosorb W.H.P. 80 - 100 mesh for OCI. Arochlor 1254 and a mixture of pure OCI were used as standards.

The values of OCI and PCBs we refer to in this paper represent the concentrations of the respective compounds in extracted lipids of the above mentioned tissues.

Results and comments.

The concentration of total DDT in the extracted lipids of the gastric carcinoma samples was higher than in those of the adjacent apparently normal gastric mucosa. (16.74 versus 10.39 ppm, p<0.05) The concentration of total DDT in the extracted lipids of the gastric mucosa was higher than in those of the epiploon or the abdominal adipose tissue for the two groups of our study. (10.39 versus 6.44 ppm (p<0.05) or 7.92 ppm and 9.67 versus 6.83 ppm (p<0.10) or 7.35 ppm respectively for the cancer and the control groups) (Tables 1 and 2)

The total p, p'-DDT residue was higher in the extracted lipids of the tumoral tissue than in those of the adjacent gastric mucosa (13.16 versus 7.69 ppm, p < 0.01). The components of total p,p'-DDT (p,p'-DDT and its metabolites p,p'-DDD and p,p'-DDE)were also found in higher concentrations in the extracted lipids of the gastric carcinomas in comparison with those of the adjacent gastric mucosa (3.50 versus 1.88 ppm (p < 0.02), 2.50 versus 1,25 ppm (p = 0.02) and 6.42 versus 4.09 ppm (p < 0.10) respectively). (Table 2) The fact that levels of p,p'-DDT and p,p'-DDD are significantly higher in the extracted lipids of the tumor than in the adjacent mucosa may plead for an increased direct absorption from the gastric lumen, or a delayed metabolisation and/or excretion of these compounds.

Table 1. Organochlorine insecticides (ppm) in extracted lipids of gastric mucosa, epiploon and adipose tissue.

	Tissue	Control				
Compound		Mucosa	Epiploon	Abd. fat		
p,p'-DDT	Range	0.43 - 5.80	0.43 - 4.20	0.83 - 4.50		
	M ± SD	1.80 ± 1.42	2.16 ± 1.13	2.52 ± 1.20		
p,p'-DDD	Range	0.09 - 2.50	0.01 - 2.81	0.0 - 1.26		
. *	M ± SD	1.11 ± 0.78	0.51 ± 0.68	0.35 ± 0.30		
p,p'-DDE	Range	1.04 - 9.13	1.61 - 5.16	0.64 - 6.65		
	M ± SD	3.74 ± 2.28	3.04 ± 1.10	3.11 ± 1.48		
o,p'-DDT	Range	0.0 - 2.97	0.0 - 0.52	0.0 - 1.02		
	M ± SD	0.88 ± 0.84	0.34 ± 0.65	0.32 ± 0.29		
o,p'-DDD	Range	0.0 - 2.08	0.0 - 0.36	0.0 - 0.88		
	M ± SD	0.48 ± 0.63	0.04 ± 0.09	0.09 ± 0.22		
o,p'-DDE	Range	0.08 - 2.83	0.0 - 2.12	0.0 - 2.32		
	M ± SD	1.07 ± 0.75	0.50 ± 0.55	0.56 ± 0.58		
Total	Range	2.17 -15.73	2.24 -11.72	2.0 - 9.51		
p,p'-DDT	M ± SD	7.08 ± 4.02	5.84 ± 2.32	6.34 ± 2.46		
Total	Range	0.45 - 5.96	0.13 - 3.87	0.4 - 2.85		
o,p'-DDT	M ± SD	2.59 ± 1.90	0.99 ± 0.99	1.03 ± 0.76		
Total	Range	3.14 -20.54	2.24 -13.11	3.29 -10.32		
DDT	M + SD	9.67 ± 5.40	6.83 ± 1.90	7.35 ± 2.55		
Y-BHC	Range	0.52 - 4.14	0.25 - 1.44	0.41 - 1.64		
	M ± SD	1.99 ± 1.07	0.80 ± 0.47	0.80 ± 0.40		
Dieldrin	Range	0.18 - 1.97	0.11 - 1.07	0.02 - 2.43		
	M ± SD	0.66 ± 0.47	0.42 ± 0.32	0.59 ± 0.58		
Hexachlor	Range	0.0 - 3.41	0.0 - 1.59	0.0 - 0.93		
Epoxide	M ± SD	0.97 + 0.88	0.47 ± 0.43	0.40 ± 0.21		

The p,p'-DDT x 100 / total p,p'-DDT ratio was lower in the extracted lipids of the mucosa, in comparison to that of the epiploon and abdominal adipose tissue in the cancer as well as in the control group (24.49 %, 39 % and 42 % and 25.50 %, 37 % and 39.74 % respectively). The tumoral tissue maintained this metabolic feature of the gastric mucosa (26.50 %). These facts demonstrate that gastric mucosa (tumoral and normal) metabolises p,p'-DDT to a higher rate than the epiploon or the abdominal adipose tissue and/or the rate of excretion of p,p'-DDT from the gastric mucosa is increased.

Table 2. Organochlorine insecticides (ppm) in extracted lipids in malignant and normal gastric mucosa, epiploon and adipose tissue.

	Tissue		Gastric tumor				
Compound		Tumor	Mucosa	Epiploon	Abd. fat		
p,p'-DDT	Range	1.23 - 7.24	0.77 - 2.98	0.28 - 4.80	0.73 - 6.39		
	M ± SD	3.50 ± 1.79	1.88 ± 0.72	2.32 ± 1.38	3.09 ± 1.71		
p,p'-DDD	Range	0.49 - 5.92	0.17 - 3.08	0.0 - 2.55	0.0 - 1.11		
	M ± SD	2.50 ± 1.54	1.25 ± 0.85	0.59 ± 0.65	0.52 ± 0.46		
p.p'-DDE	Range	1.01 -12.83	0.61 -16.52	0.42 - 6.47	0.99 - 6.63		
	M ± SD	6.42 ± 3.03	4.09 ± 3.84	2.67 ± 1.54	3.31 ± 1.77		
o,p'-DDT	Range	0.10 - 6.09	0.15 - 4.67	0.0 - 0.75	0.0 - 0.96		
	M ± SD	2.00 ± 1.69	1.16 ± 1.05	0.25 ± 0.26	0.29 ± 0.31		
o,p'-DDD	Range	0.0 - 5.75	0.0 - 4.68	-	-		
	M ± SD	0.37 ± 1.35	0.45 ± 1.11	.			
o,p'-DDE	Range	0.0 - 3.77	0.0 - 2.43	0.0 - 1.03	0.0 - 1.57		
	M ± SD	1.21 ± 0.94	0.98 ± 0.82	0.27 ± 0.28	0.30 ± 0.44		
Total	Range	5.86 -23.77	2.56 -22.85	1.48 -12.64	3.014.81		
p,p'-DDT	M ± SD	13.16 ± 5.56	7.69 ± 4.81	5.89 ± 3.25	7.30 ± 3.23		
Total	Range	0.23 -15.64	0.20 - 8.59	0.00 - 1.15	0.03 - 1.90		
o,p'-DDT	M ± SD	3.58 ± 3.57	2.71 ± 2.19	0.55 ± 0.46	0.62 ± 0.49		
Total	Range	7.62 -34.56	3.61 -28.72	1.06 -13.23	3.81 -15.60		
DDT	M ± SD	16.74 ± 7.87	10.39 ± 6.25	6.44 ± 3.52	7.92 ± 3.22		
Y-BHC	Range	1.19 - 7.75	0.43 - 5.23	0.0 - 0.34	0.0 - 1.33		
	M ± SD	2.77 ± 2.64	2.07 ± 1.25	0.15 ± 0.11	0.43 ± 0.36		
Dieldrin	Range	0.36 - 5.81	0.34 - 5.22	0.03 - 2.05	0.0 - 1.65		
	$M \pm SD$	1.18 ± 1.28	1.18 ± 0.64	0.71 ± 0.58	0.76 ± 0.48		
Hexa-	Range	0.0 - 1.50	0.0 - 3.68	0.0 - 1.97	0.0 - 4.67		
chlor Ep.	M ± SD	0.11 ± 0.36	0.59 ± 0.93	0.69 ± 0.57	0.85 ± 1.10		

The level of Y-BHC and dieldrin is higher in the extracted lipids of the gastric mucosa in comparison with the epiploon and the abdominal adipose tissue in the two groups of this study. No difference has been noted in the concentration of these compounds in the extracted lipids of the tumoral tissue from those of the adjacent gastric mucosa.

Hexachlor epoxide was an exception to this in that its concentration in the extracted lipids of the tumoral tissue was lower than in the adjacent gastric mucosa (0.11 versus 0.59 ppm, p < 0.05).

This compound was also found in lower concentration in the extracted lipids of adjacent gastric mucosa of cancer patients than in those of the mucosa of the control group (0.59 versus 0.97 ppm) a finding which suggests that the gastric tumor and the apparently normal gastric mucosa adjacent to the tumoral tissue may share common biochemical properties.

The analysis of total DDT residues, in the cancer group, according to sex showed a statistically significant difference for the level of total DDT in the extracted lipids of the gastric mucosa (13.35 versus 8.23 ppm, p < 0.02, for males and females respectively) and of the gastric tumor tissue (20.39 versus 14.25 ppm, p < 0.05, for males and females respectively). (Table 3)

Table 3. Total DDT in extracted lipids (ppm) according to sex (Mean \pm SD)

Group	Sex		<u>_</u>
	Tissue	M	,F
Control	Abd. fat	8.34 ± 4.75	6.69 ± 2.52
	Epiploon	7.66 ± 3.31	6.27 ± 2.23
	Gastric mucosa	8.84 ± 5.68	10.23 ± 5.30
Cancer	Abd. fat	8.54 ± 2.43	6.91 ± 3.42
	Epiploon	6.57 ± 3.51	6.35 ± 0.40
	Gastric mucosa	13.35 ± 7.96	8.23 ± 3.41
	Gastric tumor	20.39 ± 7.89	14.25 ± 6.76

The highest values for PCBs storage were found in the extracted lipids of the tumoral tissue. A statistically significant difference was found between these values and those of the adjacent gastric mucosa (15.33 versus 6.29 ppm, p < 0.01). (Table 5)

The storage level of total PCBs was higher in the extracted lipids of the tissues obtained from the cancer patients than in those of the control group. (Tables 4 and 5) A statistically significant difference was found for the epiploon (2.00 versus $1.43 \, \text{ppm}$, p < 0.05).

Analysis of these findings by sex, showed that in males the storage of PCBs in extracted lipids of the abdominal wall adipose tissue, epiploon and gastric mucosa was greater in the cancer patients when compared to the controls (2.32 versus 1.39 ppm, p < 0.05, 1.88 versus 1.13 ppm, p < 0.10, and 6.86 versus 4.09 ppm, respectively). The total PCBs concentration was highest in the extracted lipids of the gastric tumor in the male group (19.57 versus 12.36 ppm, p < 0.01). (Table 6)

The statistically significant differences regarding the storage of OCC in cancer patients according to sex raises the question of the role of environmental compounds in affecting the frequency

Table 4. Polychlorinated biphenyls (ppm) in extracted lipids of gastric mucosa, epiploon and adipose tissue.

	Tissue		Control group				
Peaks		Mucosa	Epiploon	Abd. fat			
1	Range	0.0 - 3.04	0.0 - 0.14	0.0 - 0.12			
	M ± SD	0.52 ± 0.79	0.05 ± 0.05	0.04 ± 0.04			
2	Range	0.0 - 1.42	0.0 - 0.26	0.0 - 0.15			
	$M \pm SD$	0.54 ± 0.92	0.08 ± 0.08	0.05 ± 0.05			
3	Range	0.0 - 0.11	0.0 - 0.01	-			
	M ± SD	0.01 ± 0.03	0.0 ± 0.24	· -			
4	Range	0.0 - 0.70	0.0 - 0.25	0.0 - 0.07			
	M ± SD	0.08 ± 0.18	0.03 ± 0.06	0.02 ± 0.02			
5	Range	0.0 - 1.22	0.0 - 0.11	0.0 - 0.14			
	M ± SD	0.45 ± 0.44	0.04 ± 0.03	0.05 ± 0.04			
6	Range	0.99 -10.26	0.43 - 1.20	0.35 - 1.29			
	M ± SD	2.93 ± 2.13	0.82 ± 0.26	0.84 + 0.21			
7	Range	-	-				
	M ± SD	• ·	-	-			
8	Range	0.0 - 1.05	0.0 - 0.24	0.0 - 0.12			
	M ± SD	0.39 ± 0.34	0.07 ± 0.07	0.07 ± 0.05			
9	Range	0.0 - 0.75	0.0 - 0.30	0.0 - 0.23			
	M ± SD	0.20 ± 0.23	0.06 ± 0.07	0.08 ± 0.06			
10	Range	0.0 - 1.21	0.0 - 0.44	0.06 - 0.49			
	M ± SD	0.24 ± 0.32	0.17 ± 0.13	0.23 ± 0.25			
11	Range	-	0.0 - 0.02	0.0 - 0.02			
	M ± SD	-	0.0 ± 0.42	0.0 ± 0.06			
12	Range	-	-	0.0 - 0.11			
	M ± SD	-	-	0.0 ± 0.03			
13	Range	0.0 - 1.07	0.0 - 0.49	0.0 - 0.24			
}	M ± SD	0.08 ± 0.27	0.07 ± 0.12	0.06 ± 0.07			
14	Range	0.0 - 2.90	0.0 - 0.32	0.0 - 0.26			
	M ± SD	0.28 ± 0.76	0.04 ± 0.08	0.08 ± 0.07			
Total	Range	1.44 -11.32	0.71 - 3.22	0.76 - 2.12			
	M + SD	5.72 + 3.51	1.43 + 0.40	1.50 + 0.38			

Table 5. Polychlorinated biphenyls (ppm) in extracted lipids of gastric tumor, gastric mucosa, epiploon and adipose tissue.

	Tissue	Cancer group			
Peaks		Tumor	Mucosa	Epiploon	Abd. fat
1	Range	_	0.0 - 0.42	0.0 - 0.06	0.0 - 0.51
	M ± SD		0.04 ± 0.05	0.02 ± 0.04	0.04 ± 0.12
2	Range	0.0 - 1.11	0.0 - 0.40	0.0 - 0.21	0.0 - 0.52
	M ± SD	0.07 ± 0.26	0.05 ± 0.13	0.06 ± 0.07	0.08 ± 0.15
3	Range	-	0.0 - 0.51	0.0 - 0.24	0.0 - 0.58
	M ± SD	. -	0.05 ± 0.14	0.06 ± 0.09	0.06 ± 0.15
4	Range	0.0 - 2.99	0.04 - 1.20	0.0 - 0.27	0.0 - 0.31
	M ± SD	1.58 ± 0.86	0.45 ± 0.36	0.06 ± 0.06	0.04 ± 0.07
5	Range	0.0 - 6.66	0.0 - 1.51	0.0 - 0.21	0.0 - 0.09
	M ± SD	2.75 ± 1.68	0.79 ± 0.57	0.04 ± 0.05	0.03 ± 0.03
6	Range	1.13 - 7.52	0.90 - 5.25	0.02 - 1.64	0.36 - 3.42
	M ± SD	4.29 ± 1.87	2.37 ± 1.13	0.96 ± 0.43	0.97 ± 0.68
7	Range	-	<u>-</u>	-	-
}	M + SD	-	-	-	
8	Range	0.22 - 5.01	0.0 - 2.31	0.01 - 0.43	0.0 - 0.52
	M ± SD	1.96 ± 1.30	0.62 ± 0.61	0.17 ± 0.12	0.15 ± 0.12
9	Range	0.09 - 3.08	0.04 - 2.20	0.0 - 0.24	0.0 - 0.17
	M ± SD	1.44 ± 1.11	0.58 ± 0.55	0.10 ± 0.06	0.09 ± 0.06
10	Range	0.65 - 8.41	0.13 - 5.59	0.10 - 0.96	0.10 - 0.85
	M ± SD	3.24 ± 2.09	1.34 ± 1.37	0.48 ± 0.31	0.43 ± 0.18
11	Range	-	-	· -	0.0 - 0.26
	M ± SD	-		-	0.01 ± 0.06
12	Range	-	-	-	-
	M ± SD	- '	-	-	-
13	Range	-	-	0.0 - 0.18	0.0 - 0.13
	M ± SD	-	-	0.05 + 0.06	0.03 ± 0.03
14	Range		-	0.0 - 0.05	0.0 - 0.05
	M ± SD	<u> </u>	-	0.01 + 0.02	0.01 ± 0.01
Total	Range	4.50 -34.70	1.63 -12.66	0.80 - 3.31	0.89 - 3.88
	M ± SD	15.33 ± 7.84	6.29 ± 3.62	2.00 ± 0.75	1.92 ± 0.88

of gastric carcinoma in males. A moderation of the immunological response to foreign antigens found in rats and rabbits exposed to p,p'-DDT (6) may suggest a possible mechanism of action of OCC in carcinogenesis.

Table 6. Total PCBs in extracted lipids (ppm) according to sex (Mean ± SD)

Group	Sex	М	F
	Tissue	II	
Control	Abd. fat	1.39 ± 0.34	1.57 ± 0.38
	Epiploon	1.13 <u>+</u> 0.25	1.63 ± 0.70
	Gastric muc o sa	4.09 ± 5.39	6.81 ± 3.94
Cancer	Abd. fat	2.32 ± 0.90	1.59 ± 0.65
	Epiploon	1.88 ± 0.81	2.08 ± 0.70
	Gastric mucosa	6.86 ± 3.12	5.89 ± 3.87
	Gastric tumor	19.57 ± 8.51	12.36 ± 5.70

Table 7. Percentage of individual PCB compounds related to total PCBs assessed in extracted lipids

Peaks		Control			Gastri	c tumor	
Septembell-10p.cel 2040.	Mucosa	Epiploon	Abd.fat	Tumor	Mucosa	Epiploon	Abd.fat
1	9.08	3.49	2.52	1	0.63	0.79	1.84
2	9.44	4.89	3.08	0.45	0.84	2.99	4.06
3	0.17	0.04	-	-	0.82	2.89	3.12
4	1.40	2.74	1.22	10.33	7.08	2.87	2.03
5	7.87	2.80	3,26	17.93	12.55	1.94	1.34
6	51.22	57.32	56.28	·27 . 98	37.64	47.97	50.34
7	-	-	~	-	-	~	-
8	6.82	4.89	4.98	12.79	9.95	8.72	7.83
9	3.50	4.19	5.32	9.37	9.26	4.80	4.48
10	4.20	11.88	15.23	21.15	21.23	23.78	22.26
11	-	0.08	0.10	-		-	0.78
12		-	0.49	-	-	-	- }
13	1.40	4.89	4.21	-	-	2.69	1.57
14	4.90	2.79	3.31	-		0.56	0.35

The concentration of individual polychlorinated biphenyl compounds was found to be generally largest in the extracted lipids

of the malignant tissue (peaks 4 through 10, tables 4 and 5 and graph 1). There was also a certain increase of these peaks for the gastric mucosa adjacent to the tumoral tissue, when compared to the gastric mucosa of the control group. The most striking increase was that of peak 10. This peak was very low in the extracted lipids of gastric mucosa of the control group (0.24 versus 1.34 ppm in the cancer group. p < 0.01) an highest in the extracted lipids of the tumoral tissue (3.24 versus 1.34 ppm in the extracted lipids of the adjacent gastric mucosa.

This would mean that the possibilities for removal of the PCB compound, represented by peak 10, are best in the gastric mucosa of healthy persons. The possibilities decrease in gastric mucosa adjacent to the tumor and are lowest in the tumoral tissue.

The percentages of individual PCB compounds related to the total PCBs, assessed in the extracted lipids of the studied tissues, are presented in table 7. The percentages of peak 10 were similar for the tissues in the cancer patients (21.15 % through 23.78 %) and were higher than those found in the control group (4.20 % through 15.23 %). These facts point to another biochemical characteristic of the tissues of the cancer patient, in their behaviour towards environmental toxic compounds.

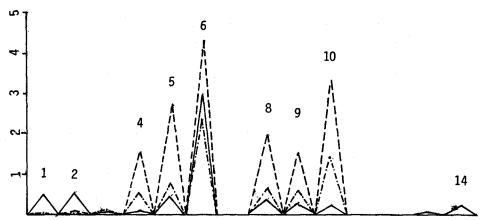
Table 8.	Levels	of	indiv ²	idual	PCB	compounds	in ex-
tracted 1	ipids of	f ga	stric	carci	inoma	s by sex	(ppm).

Sex	Male	Female
Peak	Mean ± SD	Mean + SD
4	2.17 ± 0.99	1.18 ± 0.71
5	3.53 ± 1.80	2.20 ± 1.35
6	5.38 ± 2.01	3.53 ± 1.29
8	2.20 ± 1.37	1.79 ± 1.23
9	1.71 ± 1.41	1.25 ± 1.56
10	4.10 ± 2.02	2.41 ± 1.39

When we analyzed the values of PCB individual compounds by sex (Table 8) it was obvious that there was a difference between their storage level in males and females. The values were higher in males and the differences were statistically significant for peaks 4, 6, and 10, p < 0.01 and for peak 5, p < 0.05.

In conclusion, the findings in this study showed an increased concentration of OCC in the extracted lipids of gastric carcinoma when compared to adjacent apparently normal gastric mucosa and gastric mucosa in the control group.

The analysis of OCC (OCI, PCBs and individual PCB compounds) residues, in the cancer group, according to sex, showed higher levels for males. The role of an increased storage of noxious environmental chemical compounds in male cancer patients was discussed.



Graph 1. Amounts of individual PCB compounds (ppm) in extracted lipids of gastric tumor ---, adjacent gastric mucosa ----, and gastric mucosa in controls ____.

In cancer patients, the OCC levels of gastric mucosa and other tissues showed some characteristics similar to those of the tumoral tissue. This fact points to biochemical characteristics shared by the tumoral tissue and apparently normal tissues in cancer patients.

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