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Total Mercury and Methylmercury in Hair, Maternal and Umbilical Blood, and Placenta from Women in the Seville Area

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The presence of mercury in organic combination, as methylmercury, in fish, came to light as a consequence of contamination of the water in the bay of Minamata (Japan). It was shown that mercury could methylate in the environment due to the action of methanogenic bacteria (Wood, 1968) passing into marine organisms, where it accumulates, and thence to man and domestic animals via de trophic chain.

Although this process is worse when contamination is high, it also occurs under normal conditions representing danger for the general population and especially for two high risk groups: fishermen and pregnant women; the first due to the importance of fish in their diet and the second because the faetus is the form of life most susceptible to mercury and its compounds (WHO, 1986).

The aim of the present paper is to estimate the situation of exposure to mercury, in our region, of one of the high risk groups: pregnant women at delivery, whose only source of mercury has been ingestion of fish. For better interpretation of transport through the placenta, perfused placenta (exsanguinated) and also, separately, venous and arterial blood from the umbilical cord, have been analysed.

MATERIAL AND METHODS

The samples were obtained from 77 volunteers aged between 20 and 40 years, hospitalized for the end of their gestation. None had received mercurial medication of used hair dyes or bleaches.

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Samples of hair (2g, n=70) were taken from the occipital region with stainless steel scissors and as close as possible to the roots; in seven cases the samples were pubic hair obtained by shaving (0.5 g). The samples were cut in pieces as small as possible and washed successively with acetone-water-water-water-acetone. After the last wash the hair was dried in a current of nitrogen and protected in polyethylene tubes until digestion (UNEP, 1987).

5 mL of blood were extracted from the maternal median/cubital vein at delivery, (n=27). 3 mL of venous and 3 mL of arterial blood were obtained from the umbilical vessels. All the samples were introduced into heparinized tubes. The cotyledons of the placenta (5 g, n=27) were perfused in the laboratory with a 0.9% solution of sodium chloride to obtain blood-free tissue.

The determinations of total mercury (Hg_T) were carried out by cold vapour system in a Perkin-Elmer AAS 2380-MSH 10 instrument, after digestion of the samples in a microwave oven with 4 mL of nitric acid. At the same time, under similar conditions, reference materials and blank samples were treated.

The determinations of methylmercury (MeHg) were carried out by gas chromatography (Hewlett Packard 5890) with electron capture detector, equipped with a diethyleneglycol succinate column, automatic sample injector 7676A and 3392A integrator. The chromatographic conditions were: injector temperature 180°C, column temperature programmed (initial: 80°C, 2 min.; ramp: 5°C per minute; final: 150°C, 10 min.) detector temperature 250°C; flow of carrying gas: hydrogen 10 mL/min. Chemical extraction and purification of the samples was carried out according to Cappon and Smith (1978).

The results were submitted to statistical study with a SIGMA programme in an IBM 5170 personal computer. The regression line which correlates both parameters was calculated and its accuracy established by an ANOVA analysis of variance. For each tissue basic statistical parameters were established. Regression lines were established for each parameter. Mean values for Hg_T, MeHg and percent ratio MeHg-Hg between different tissues were compared by a t-Student test and regression lines analyzed. Linear regression between Hg-MeHg and Hg-percent ratio was also analyzed within each tissue.

Table 1. Hg_T , MeHg and percent ratio of methylated form in hair (µg/g).

	x	σ	Coef. of Variance	Range	n
Hg_T	2.85	3.38	118.59	0.15-20.02	50
MeHg	1.49	1.84	123.48	0.07-9.85	50
Percentage	58.41	25.56	43.76	6.45-100.00	50

Table 2. Hg_T , MeHg and percentage of methylated from in hair (µg/g), maternal blood (µg/L), placenta (µg/Kg), arterial and venous umbilical blood (µg/L).

	x	σ	Coef. of Variance	Range	n
Hair					
Hg_T	1.18	0.56	47.45	0.30-2.16	19
MeHg	1.03	0.51	49.51	0.25-1.95	17
%	90.13	5.96	6.61	78.40-102.98	17
Mat.Bl.					
Hg_T	6.23	1.89	30.33	2.40-9.10	23
MeHg	4.97	1.87	7.62	1.15-7.95	19
%	87.90	12.42	14.13	42.85-96.19	18
Placenta					
Hg_T	5.43	3.13	57.64	2.31-14.31	27
MeHg	4.14	1.47	35.50	2.39-7.85	21
%	93.49	6.27	6.71	84.81-100.00	20
U.Art.Bl.					
Hg_T	6.82	2.95	43.25	2.10-12.70	25
MeHg	5.35	2.21	41.30	1.92-11.32	18
%	85.01	16.34	19.22	53.01-94.79	17
U.Ven.Bl.					
Hg_T	6.43	3.61	56.14	2.00-15.10	24
MeHg	5.25	2.83	53.90	1.74-14.21	17
%	88.23	12.83	14.54	45.34-99.00	19

RESULTS AND DISCUSSION

Hair is considered as an accumulator (WHO, 1990) and indicator of Hg concentration in blood (Sherlock,1982).

In Table headings we have used the following abreviations: Mat. maternal; Bl. blood; U. umbilical; Art. arterial, Ven. venous.

Table 3. Distribution Coefficients of total mercury and methylmercury in the different tissues of the mother-foetus systems, versus the content in maternal blood.

	x	σ	Coef. of Variance	Range	n
Total Hg					
Hair	218.00	61.00	28.00	137.00-342.00	16
Placenta	0.97	0.55	57.03	0.36-2.13	23
U.Art.Bl.	1.20	0.33	27.59	0.58-2.02	22
U.Ven.Bl.	1.09	0.52	48.06	0.57-2.50	23
MeHg					
Hair	260.00	91.00	35.00	143.00-433.01	10
Placenta	1.05	0.70	66.43	0.35-2.62	19
U.Art.Bl.	1.10	0.23	27.15	0.63-1.67	18
U.Ven.Bl.	1.11	0.51	46.45	0.60-2.50	19

Although the values are distributed in a similar way as in other European populations (WHO, FAO, UNEP, 1987) included in the WHO Long Term Programme, great interindividual variability is very noticeable in this group (Table 1). Variation coefficients of 118.59% and 123.48% for Hg_T and, MeHg respectively were found. In seven samples, concentrations higher than 5 mg/kg of Hg_T were found, value considered by Kjellstrom et al. (1986) as a critical concentration of Hg in maternal hair, beyond which there could be risk for the foetus; (three more individuals presented values which almost reached this limit). Thus, 20% of individuals in this group are under high risk. Furthermore, Cox et al (1989) estimate that with concentrations between 10 - 20 mg/Kg of Hg in maternal hair, there are 5% of foetal abnormalities. In our group we found a woman with 9.99 mg/kg and another with 20.00 mg/kg. Nevertheless, neither toxic effects nor foetal abnormalities were detected in newborns.

The percentage of MeHg in relation to Hg_T in hair was of 58.41±25.56, varying between 6.45 and 100% . Our results present a lower average and a wider interval than those found by Horvat (1990) in 34 pregnant women. This is probably due to the above mentioned variability in our group.

A strong correlation was found between Hg and MeHg content in hair (=0.8999) but no lineal relationship between Hg_T content and percent ratio of methylated form. The highest percent ratios MeHg/Hg habitually coincided with very low values of Hg, and low percentages of the

Table 4.- Statistical comparison of content in Hg_T, MeHg and its percent ratio between the different tissues of the mother-foetus system.

y	x	Linear regression Eq.	r	SL ANOVA
Total Hg				
Hair	Blood	0.16x + 0.34	0.6585	p<0.01
Hair	U.Art.Bl.	0.08x + 0.69	0.4311	p<0.10
Blood	U.Art.Bl.	0.51x + 2.53	0.7226	p<0.01
Blood	U.Ven.Bl.	0.20x + 4.87	0.3680	p<0.10
Plac.	U.Art.Bl.	0.51x + 2.00	0.4631	p<0.05
U.Art.Bl.	U.Ven.Bl.	0.47x + 3.94	0.5680	p<0.01
Methyl Hg				
Hair	Blood	0.20x + 0.21	0.7348	p<0.05
Hair	Placenta	0.04x + 0.92	0.1505	No
Hair	U.Art.Bl.	0.13x + 0.50	0.4171	No
Hair	U.Ven.Bl.	0.05x + 0.84	0.1799	No
Blood	U.Art.Bl.	0.69x + 1.35	0.8114	p<0.01
Blood	U.Ven.Bl.	0.28x + 3.47	0.4313	p<0.10
U.Art.Bl.	U.Ven.Bl.	0.34x + 3.63	0.4319	p<0.10
Percent Ratio				
Blood	U.Art.Bl.	0.74x + 23.69	0.9570	p<0.01
Blood	U.Ven.Bl.	0.55x + 38.01	0.5318	p<0.05
U.Art.Bl.	U.Ven.Bl.	0.77x + 17.02	0.6365	p<0.01
Placenta	U.Ven.Bl.	-0.24x + 114.47	-0.4766	p<0.05

former with intermediate values of the latter; for high concentrations of Hg_T, the percentage of MeHg was valuable.

Later, in a more specific study on 27 women, maternal venous blood, placenta and blood from the umbilical cord were analysed, besides hair; arterial and venous blood from the cord were considered separately in order to study bidirectional transport between mother and foetus.

Values found in hair are shown in table 2. It can be observed that these values are significantly lower ($p<0.01$) than those found in our previous group for Hg_T, although not for MeHg. Significant differences were also observed ($p<0.001$) in the higher percet ratios MeHg/Hg in this group, suggesting a posibility of relating high percentages with low values of Hg_T , although no statistical correlation was found.

These differences could be produced by chance or be

related to the type and quantity of fish ingested. Another cause could be different contamination of the fish consumed, as it is known that there are seasonal differences in the Hg content in fish (Korthatls and Winfrey, 1987); it happens that our first group of women was studied in winter and the second in summer.

The values in maternal blood (table 2) reflect that the average obtained in our study is below 8 µg/L, average value for Hg_T in the population which is nor specially exposed (WHO, 1990), thus our average can be considered acceptable. With regard to other authors (Pitkin et al., 1976), our values are higher. Nevertheless, they are comparable to those from Sweden and Yugoslavia (Horvat et al., 1990) with regard to the interval, although the average in this country is somewhat lower 3.7±1.9 µg/L. In the same way our average in MeHg is higher than that found in these authors 3.1±2.0 µg/L, with a similar interval (0.3 - 7.9). The correlation coefficient between the two parameters is r=0.8540, and no correlation exists between the percent ratios and the concentration of Hg_T.

If we compare the average values of Hg_T and MeHg in venous blood from the mother with those obtained in hair these is a highly significant difference ($p<0.001$), with values in hair about 200-300 times higher than those in maternal total blood (table 3).

The positive correlation ($\sigma=0.6588$, $p<0.01$) between hair and maternal blood confirms the consideration of hair as an indication of exposition, already accepted by the WHO. Some of the samples were pubic hair and the same as other authors (Horvat et al., 1990), we found similar values in pubic hair and hair from the head.

With regard to placental tissue our results for Hg_T (table 2) are lower than those obtained by Capelli et al. (1986) and by Horvat et al. (1990), which are of the order of 12.00 µg/Kg, and they are also below the average value of reference for the population not espeicly exposed, of 10 µg/Kg (WHO, 1990). The percent ratios of the methylated form are higher with less variability than those of the previously cited authors. This difference could be due to having perfused the tissue, because we wanted to eliminate the Hg corresponding to the haematic fraction, abundant in said organ; Kuhnert et al (1981) who also perfused the placentas, obtained values comparable to ours.

No significant differences or linear relationship were observed between levels in placenta and maternal blood

either for Hg_T or for MeHg, confirmed by distribution coefficients close to unity (table 3). However we cannot infer whether the same behaviour, with regard to distribution, would be maintained if the corporal levels of Hg increased.

We analized venous and arterial umbilical blood separately (table 2). There are no significant differences between the content of Hg_T and MeHg in the two vessels. From consideration of the linear regression equations for Hg, MeHg and percent ratios, between the different tissues (table 4), it can be observed that there is positive correlation between maternal and umbilical blood both venous and arterial which reveals an equilibrium between maternal and foetal levels, in agreement with that observed by Pitkin et al (1976). Between umbilical artery and vein there is also linear correlation in levels of both mercury and methylmercury ($p<0.01$ and $p<0.1$ respectively).

Differences are seen in the behaviour of Hg and MeHg on considering the correlation between placenta and umbilical circulation. There is positive linear relationship between placenta and umbilical artery for Hg_T but not for MeHg and the percent ratio MeHg/Hg; however there is no correlation between the contents of placenta and vein, and a negative correlation is seen between them for the percent ratios.

All this indicates that even at low levels a tendency to selective behaviour of the placenta appears with regard to the accumulation of the forms of Hg, in agreement with that pointed out by Berlin (1986) with toxic doses. But with such low levels of body burden there are no significant differences between the average values, consequently we cannot deduce which of the forms of Hg tends to accumulate preferentially.

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