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Investigations of Factors Influencing Exposure and Response to Lead, Mercury, and Cadmium in Man and in Animals

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The susceptibility of the heme biosynthetic pathway to lead, as reflected by increased free erythrocyte porphyrin (FEP) concentration, is in humans as well as in rats in the order of young ≥ female > male. The difference between adult male and female rats can be explained at least partially by the interaction of estradiol and progesterone with the FEP response to lead; the hormonal influence on FEP does not seem to be mediated through changes in plasma iron.

The classical "tubular type" proteinuria in workers chronically exposed to cadmium has two not necessarily concomitant components, namely, a tubular type and a glomerular type component characterized by increased excretion of low and high molecular weight proteins, respectively. No synergistic effect of cadmium and lead on the proteinuria of workers simultaneously exposed to both metals was observed.

Mercury (most likely methylmercury) is freely transferred from the mother to the fetus; there is only a slight placental barrier for lead and a rather strong one for cadmium. Compared to maternal blood, placenta does not accumulate lead or mercury but concentrates cadmium about 10-fold.

Influence of Age and Sex on Free Erythrocyte Porphyrin (FEP) Response in Humans and Rats Exposed to Lead

The interference of lead with the heme biosynthetic pathway has not yet been completely elucidated (*1–4*). It is known that lead impairs heme synthesis in the bone marrow and causes an increase of protoporphyrin-IX in erythrocytes (*5*). Because of the role of lead as an environmental and occupational pollutant, much effort is being devoted to arriving at a better definition of its interactions with porphyrin metabolism.

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A human volunteer study (*3, 4*) has suggested that the onset of the increase in erythrocyte protoporphyrin-IX may occur in adult women at a lower blood lead concentration (PbB) than in adult men. In our laboratory a comparison of the FEP response in 64 men and 49 women (*6*) and in 143 children (*7*) has been performed. These population groups were subjected to a slight to moderate chronic lead exposure (steady state PbB < 50 μg/100 ml). In Figure 1 are compared the dose–response relationships between PbB and FEP in those groups, taking as FEP cut-off levels (mean + 2SD as found for control values) 82, 83, and 68 μg/100 ml erythrocytes for children, women, and men, respectively. These curves suggest that during moderate and chronic lead exposure the susceptibility of the heme biosynthetic pathway to lead, as reflected by FEP, is in the order children ≥ women > men. Indeed, 50% of the children already exceed the FEP cut-off value when PbB reaches 25 μg/100 ml, 50%

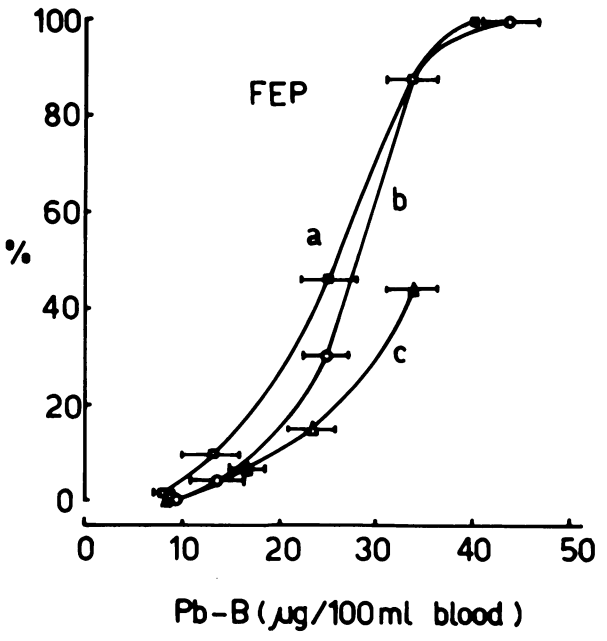

FIGURE 1. Comparison of dose–response curves between PbB and FEP in (a) children; (b) adult females; (c) adult males.

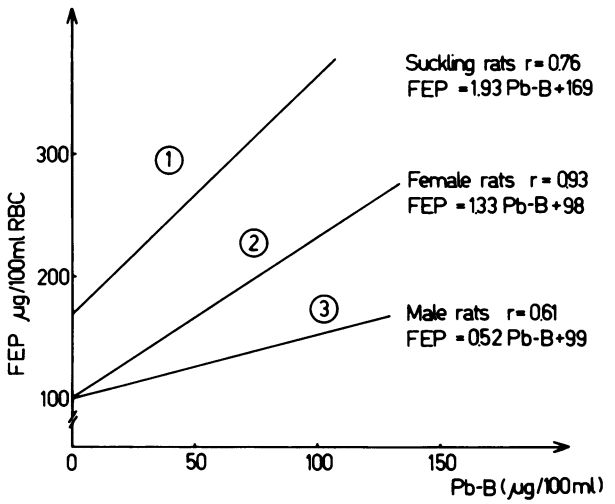

FIGURE 2. Relationship between PbB and FEP in lead-treated rats.

of the women when PbB reaches 28 µg/100 ml, and 50% of the men when PbB reaches 35 µg/100 ml. The reason children and women exhibit an earlier biological response to lead is still unknown. Although serum iron levels were not measured in these studies, the fact that no significant correlations between hemoglobin concentration and log FEP were found in the various population groups suggests the absence of iron deficiency anemia. Recently, in a similar study on 11-year-old children (n = 114) (*8*) and on adult women (n = 61) and men (n = 142) (Roels et al., unpublished data), we did not find significant correlations between log FEP and serum iron or between PbB and serum iron [average PbB ± SD (range): children 16.3 ± 9.1 (4.5–45.8); women 18.4 ± 9.6 (3.9–45.6); men 33.6 ± 10.9 (12.2–76.5); PbB expressed in µgPb/100 ml blood].

To improve our understanding of the mechanisms behind these sex and age differences in FEP response following lead exposure we used an animal model in which lead was administered to Sprague-Dawley rats in their drinking water (*9*). Figure 2 demonstrates that in rats for a PbB range between 5 and 150 µg/100 ml of blood, FEP and PbB show the same pattern of relationships as that found in humans. For suckling rats (n = 200) the correlation coefficient r was 0.76, for adult female rats (n = 55) r = 0.93, and for adult male rats (n = 70) r = 0.61. The FEP response in female rats in this PbB range is 2.5 fold greater than that in male rats and an even greater difference (3.7 fold) is found between suckling rats and adult male rats.

Since the same pattern of FEP response as that found in man could be reproduced in rats, we went on to use animals to study the mechanism behind sex linked differences in response to lead. We have thus studied whether sex hormones could influence FEP response, δ-aminolevulinic acid synthetase (ALA-S) activity of the liver, hemoglobin concentration (Hb), and hematocrit (Hct) in rats exposed to lead. Three-week-old rats were either sham operated or castrated, and after a recovery period of 3 weeks the castrated rats were treated with sex hormones for 5 weeks. Estradiol benzoate, testosterone propionate, or progesterone were administered three times a week intraperitoneally in corn oil at a dose of 1 mg/kg body weight; sham operated animals received only corn oil. During the three last weeks of the hormone treatment lead was given at a dose of 3000 ppm in the drinking water. Animals were starved for 15 hr before sacrifice. After anesthesia by IP injection of 60 mg sodium pentobarbital/kg body weight, blood was quickly withdrawn from the heart and collected in heparin. The liver was homogenized in ice-cold Tris–HCl buffer, pH 7.4, 0.075*M* containing 0.9% NaCl, and a 15% homogenate was used for the assay of ALA-S activity according to Marver, Tschudy, and Perlroth (*10*). Besides the determinations of PbB, Hct, Hb, and FEP, plasma iron was determined to take into account the possible influence of this parameter on the FEP response.

Keeping in mind that the plasma iron level can be influenced by hormones as well as by lead we first tested whether in the male and female rats receiving various hormonal treatments the FEP changes

could be mediated at least partially by changes in plasma iron concentration. Therefore the partial correlation between plasma iron concentration and FEP standardized for PbB was calculated; neither in male nor in female rats was there a significant correlation between those parameters. In another experiment we checked whether an increase in plasma iron concentration brought about by IP injection of iron dextran (Imferon) could modify the FEP level. No effect was found in male or in female rats. We can thus conclude that if hormonal treatment influences the FEP response to lead, it is not through changes in plasma iron concentration.

To enable a better evaluation of the kind of influence the hormones have on the FEP response during lead treatment, we compared groups of lead-treated animals that received different hormonal treatments but had PbB values in the same range. The results found in female rats are summarized in Table 1. Compared to the control animals (no lead, no operation), all the lead-treated groups (sham operated or castrated with or without hormonal treatment) demonstrated an increase in PbB and FEP, and a decrease in plasma iron levels, Hct, Hb, and in liver ALA-S activity. However, among the lead-treated animals the three last parameters were hardly influenced by the hormonal treatment, whereas plasma iron levels were significantly increased by estradiol in castrated animals. The most important findings concern the FEP response to lead in the various treatment groups. Ovariectomy suppressed the lead-induced FEP increase significantly while testosterone treatment hardly modified the FEP level. Treatment with estradiol, and especially with progesterone, brought the FEP concentration back to the level found in the sham operated female rats that received the same lead treatment. In castrated male rats (Table 2), as in castrated female rats, estradiol caused an increase in plasma iron concentration. The increase in the FEP level following lead administration was in contrast to castrated female rats nearly identical in all treatment groups.

In summary, the interaction of sex hormones with the FEP response to lead can be found only in adult female rats and not in male rats. Furthermore, this effect is apparently not mediated through changes in plasma iron concentration and in ALA-S activity. The latter conclusion is only tentative, since ALA-S activity was measured only in liver.

Table 1. Effect of various hormonal treatments on response to lead in female rats.[a]

		Lead in drinking water (3000 ppm)				
			Castrated			
	No lead, no operation (n = 12)	Sham operated (n = 17)	No treatment (n = 10)	Testosterone (n = 9)	+ Estradiol (n = 10)	+ Progesterone (n = 10)
Pb blood, μg/100 ml	5.0 ± 0.3	90.5 ± 4.2	92.8 ± 8.5	93.0 ± 4.5	101.2 ± 7.3	99.2 ± 7.4
FEP, μg/100 ml RBC	85.2 ± 2.1	272.0 ± 19.5	197.9 ± 6.4	188.6 ± 7.1	242.6 ± 10.9	294.3 ± 19.4
Fe plasma, μg/100 ml	198.9 ± 12.2	105.6 ± 5.7	102.5 ± 8.5	132.6 ± 13.5	161.8 ± 14.5	132.0 ± 12.7
ALA-S, nmole ALA/g prot.-hr	161 ± 9	99 ± 6	108 ± 4	133 ± 6	99 ± 6	103 ± 6
Hct, %	41.7 ± 0.4	35.5 ± 0.5	36.7 ± 0.5	37.4 ± 0.8	36.2 ± 0.6	37.2 ± 0.6
Hb, g/100 ml	14.4 ± 0.1	11.2 ± 0.2	11.6 ± 0.2	12.1 ± 0.2	11.5 ± 0.2	11.7 ± 0.2

[a] All values are means ± standard error.

Table 2. Effect of various hormonal treatments on response to lead in male rats.[a]

		Lead in drinking water (3000 ppm)			
			Castrated		
	No lead, no operation (n = 12)	Sham operated (n = 8)	No treatment (n = 5)	+ Estradiol (n = 17)	+ Progesterone (n = 5)
Pb blood, μg/100 ml	5.4 ± 0.1	118.9 ± 6.5	95.7 ± 16.1	105.6 ± 8.0	132.2 ± 4.4
FEP, μg/100 ml RBC	95.6 ± 1.9	250.1 ± 10.9	228.8 ± 5.8	245.6 ± 10.9	257.8 ± 9.6
Fe plasma, μg/100 ml	89.4 ± 4.2	90.0 ± 7.4	86.7 ± 7.1	175.8 ± 14.6	118.7 ± 10.3
ALA-S, nmole ALA/g prot-hr	144 ± 6	84 ± 6	105 ± 6	99 ± 4	120 ± 6
Hct, %	44.5 ± 0.8	34.7 ± 0.9	34.0 ± 1.2	34.8 ± 0.6	35.1 ± 1.8
Hb, g/100 ml	14.8 ± 0.2	11.2 ± 0.3	11.4 ± 0.4	11.7 ± 0.2	10.5 ± 0.5

[a] All values are means ± standard error.

Protein Clearances in Workers Exposed to Cadmium, Lead or Mercury

Workers with different degrees of exposure to lead (n = 19), cadmium (n = 42), mercury (n = 17), or lead + cadmium (n = 17) were examined as well as a control group of workers (n = 77) with a blood lead level below 35 μg/100 ml and a urinary cadmium and mercury concentration below 2 and 5 μg/g creatinine, respectively (Table 3). In order to obtain the renal clearances of β_2-microglobulin and albumin (and also of other proteins like orosomucoid and transferrin) (*11*), the urine of each worker was collected in metal free polyethylene containers over a known period of time during the work shift (from about 7 to 12 a.m.). A blood sample was also taken within this time interval. Immediately after the end of the period of urine collection, the urinary volume was measured and 10 ml urine poured into a plastic tube containing 1 ml phosphate buffer pH 7.6, 0.4*M* supplemented with 1% sodium azide to keep the urine at about pH 7 to 7.2 for storage at −20°C and to preserve it until β_2-microglobulin is measured. The concentration of β_2-microglobulin, albumin, and creatinine in urine and plasma were measured as described previously (*11*). The urinary protein excretion was calculated as the protein/creatinine clearance ratio (C_{pr}/C_{cr}), i.e., the relative protein clearance, according to the formula:

$$\frac{U_{pr}\,(V/P_{pr})}{U_{cr}\,(V/P_{cr})} = \frac{U_{pr}/P_{pr}}{U_{cr}/P_{cr}}$$

where U_{pr} and P_{pr} denote the urinary and plasma concentration of the specific protein, U_{cr} and P_{cr} the urinary and plasma concentration of creatinine, and V, the urine volume per unit of time. The proteinuria rate (mg/hour) is standardized for a creatinine clearance of 100 ml/min. This standardized proteinuria rate and the relative clearances, except that of β_2-microglobulin, which is not correlated with age, were standardized for 40 years of age.

The specificity of increased urinary excretion of the various proteins has been compared for the different groups. For the range of exposure to metals sustained by the different groups of exposed workers, we can conclude that a significant increase in the urinary excretion of low and/or high molecular weight proteins was found only in the groups exposed to cadmium or to lead + cadmium (see Fig. 3 for β_2-microglobulin and Fig. 4 for albumin).

Our observations suggest that the classical "tubular" type proteinuria induced by cadmium has two not necessarily concomitant components: a tubular type component with increased excretion of low molecular weight proteins and a glomerular

Table 3. Groups of workers with different degrees of exposure to cadmium, lead, and mercury.

Groups	n	Duration of exposure or employment (years)		Metal concentration[a]	
		Mean	Range	Mean	Range
I. Control group	77	11.9	1.4–36.8		
PbB < 35μg/100 ml				16.0	5.5–34.8
HgU < 5 μg/g creatinine				1.49	0.08–4.96
CdU < 2μg/g creatinine				0.81	0.07–1.93
II. Pb-Group	19	13.0	3.1–29.6		
PbB ≥ 35 μg/100 ml				45.6	35.0–59.2
HgU < 5 μg/g creatinine				1.07	0.44–2.53
CdU ≥ 2 μg/g creatinine				1.29	0.19–1.85
III. Cd-group	42	24.5	2.3–47.1		
PbB < 35 μg/100 ml				22.7	8.8–33.5
HgU < 5 μg/g creatinine				1.51	0.29–4.50
CdU ≥ 2 μg/g creatinine				11.4	2.00–59.3
IV. Pb + Cd group	17	24.5	4.8–42.6		
PbB ≥ 35 μg/100 ml				43.5	35.0–53.9
HgU < 5 μg/g creatinine				1.05	0.54–2.24
CdU < 2 μg/g creatinine				6.57	2.02–30.9
V. Hg-group	17	5.4	0.6–9.3		
PbB < 35 μg/100 ml				16.4	7.0–29.8
HgU ≥ 5 μg/g creatinine				34.2	5.65–97.4
CdU < 2 μg/g creatinine				0.80	0.46–1.82

[a] PbB expressed in μg/100 ml; HgCl and CdU expressed in μg/g creatinine.

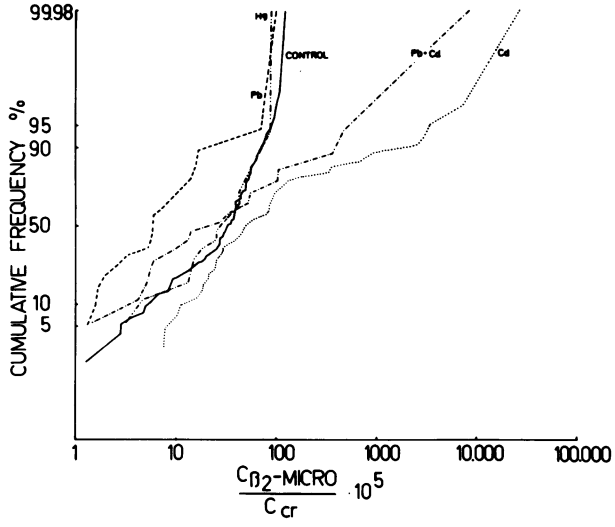

FIGURE 3. Cumulative frequency distributions of relative clearance of β_2-microglobulin in groups of workers with different degrees of exposure to lead, mercury, and cadmium: control (n = 77), Hg (n = 17), Pb (n = 19), Cd + Pb (n = 17), and Cd (n = 42).

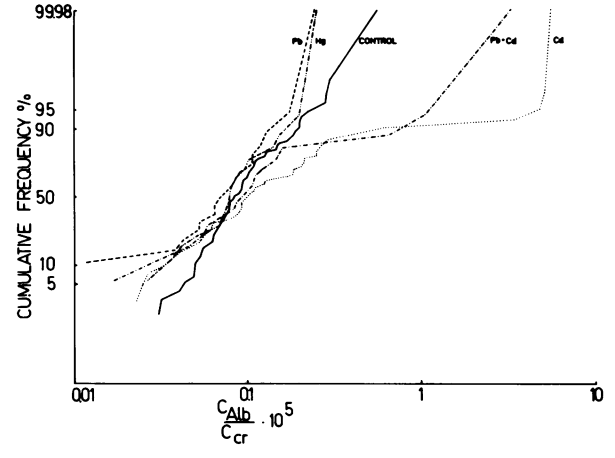

FIGURE 4. Cumulative frequency distributions of standardized relative clearance of albumin in groups of workers with different degrees of exposure to lead, mercury, and cadmium: control (n = 77), Hg (n = 17), Pb (n = 19), Cd + Pb (n = 17), and Cd (n = 42).

type component with increased excretion of high molecular weight proteins. Both changes seem to appear independently and we cannot conclude from this cross-sectional study that one change occurs systematically before the other. This study (*11*) does not suggest a synergistic effect of cadmium and lead on the proteinuria of workers simultaneously exposed to both metals. It should be recognized that the degree of exposure to cadmium, at least on the basis of cadmium concentration in urine (Table 3), appears to be somewhat lower in the group exposed to lead + cadmium than in the group exposed to cadmium only. Furthermore the intensity of exposure to lead was also rather moderate, PbB < 60 μg/100 ml.

Exposure to Lead, Mercury, Cadmium, and Carbon Monoxide during Pregnancy

In 1975 and 1976, we performed a survey on 500 pregnant women living in different areas of Belgium (*12–14*) in order to evaluate the extent of exposure to heavy metals (lead, mercury, cadmium) during fetal life, their possible biological effects, and the factors which may influence the degree of exposure. Carboxyhemoglobin was also determined.

In the mothers, the ranges of the heavy metal concentrations and carboxyhemoglobin (HbCO) in blood were as follows: 3.1–31.0 μg/100 ml for lead (PbB), 0.01–4.70 μg/100 ml for mercury (HgB), 0.01–1.01 μg/100 ml for cadmium (CdB), and 0.12–4.84% for HbCO. The corresponding values found in newborns were 2.7–27.3 μg/100 ml for PbB, 0.01–7.05 μg/100 ml for HgB, 0.01–1.03 μg/100 ml for CdB, and 0.06–8.30% for HbCO. The three heavy metals are thus transferred from the mother to the fetus, but the barrier role of the placenta is different for each of them. There is no barrier for the transfer of mercury (most likely methylmercury), a slight one for lead, and a rather strong one for cadmium. In comparison with maternal blood, the placenta does not concentrate lead or mercury but concentrates cadmium about 10-fold. This explains why the correlation found between the cadmium concentration in maternal and fetal blood is much lower (although statistically significant: r = + 0.38) than that found for lead and mercury ($r > 0.6$). For log-normally distributed values (i.e., cadmium in blood, and lead, mercury, and cadmium concentrations in placenta) the coefficients of correlation were calculated after logarithmic transformation of the corresponding values.

In the mothers, HbCO is slightly correlated with CdB (r = +0.29) which suggests that both pollutants come at least partly from a similar source (smoking). Because of the different barrier capacity of the placenta toward CO and cadmium, no significant correlation between both parameters is found in newborns. Furthermore CdB is also correlated with PbB (r = +0.20 for mother; r = +0.17 for newborn; $p < 0.01$).

In both mothers and newborns, the erythrocyte enzyme δ-aminolevulinic acid dehydratase (ALAD) was negatively correlated with PbB, but in the range

of PbB observed no significant correlation was found between PbB and erythrocyte porphyrin level (FEP).

The median and range values of the heavy metal concentrations in placental tissue (in μg/100 g wet weight) are for lead 7.5 (1.1–39.5), for mercury 1.06 (0.11–10.31), and for cadmium 1.08 (0.25–7.89). A statistically significant correlation was found between cadmium concentration in maternal blood and in placenta ($r = +0.38$). In contrast to the highly significant correlation between HgB-mother and HgB-newborn ($r = +0.62$), the mercury concentrations in placenta are not related to the corresponding levels in maternal and cord blood. This is in agreement with the great permeability of this tissue to methylmercury, the main mercurial derivative to which the general population is exposed. Although the level of lead in the placenta is significantly related to that found in the maternal ($r = +0.22$) and newborn blood ($r = +0.28$), maternal and cord blood lead levels are much better related ($r = +0.81$).

Among the various factors investigated (smoking habit, residence, age, occupation, drinking habits, duration of pregnancy, number of previous pregnancies) only smoking and residence were found to influence at least one of the parameters measured. Smoking has a statistically significant influence on carboxyhemoglobin level in mothers and newborns and on cadmium concentration in maternal blood and in placenta. A slight but statistically significant effect of environmental pollution by lead (urban and industrial > semirural > rural area) on lead uptake by the pregnant mothers and lead transfer to the fetus was demonstrated.

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REFERENCES

1. Granick, S., et al. Assays for porphyrins, δ-aminolevulinic acid dehydratase, and porphyrinogen synthetase in microliter samples of whole blood: applications to metabolic defects involving the heme pathway. Proc. Natl. Acad. Sci. (U.S.) 69: 2381 (1972).
2. Sassa, S., et al. Studies in lead poisoning. I. Microanalysis of erythrocyte protoporphyrin levels by spectrofluorometry in the detection of chronic lead intoxication in the subclinical range. Biochem. Med. 8: 135 (1973).
3. Stuik, E. J. Biological response of male and female volunteers to inorganic lead. Int. Arch. Arbeitsmed. 33: 83 (1974).
4. Stuik, E. J., and Zielhuis, R. L. Increased susceptibility of females to inorganic lead. In: Proceedings of the International Symposium on Recent Advances in the Assessment of the Health Effects of Environmental Pollution, Vol. II. Commission of the European Communities, Luxembourg, 1975, p. 537.
5. Chisolm, J. J., Jr., Hastings, C. W., and Cheung, D. K. K. Microphotofluorometric assay for protoporphyrin in acidified acetone extracts of whole blood. Biochem. Med. 9: 113 (1974).
6. Roels, H. A., et al. Response of free erythrocyte porphyrin and urinary δ-aminolevulinic acid in men and women moderately exposed to lead. Int. Arch. Arbeitsmed. 34: 97 (1975).
7. Roels, H. A., et al. Impact of air pollution by lead on the heme biosynthetic pathway in school-age children. Arch. Environ. Health 31: 310 (1976).
8. Roels, H. A., et al. Lead and cadmium absorption among children near a non-ferrous metal plant. A follow-up study of a test case. Environ. Res. 15: 290 (1978).
9. Buchet J. P., Roels H., and Lauwerys, R. Influence of sex hormones on free erythrocyte protoporphyrin response to lead in rats. Toxicology 9: 361 (1978).
10. Marver, H. S., Tschudy, D. P., Perlroth, M. G. δ-aminolevulinic acid synthetase. I. Studies in liver homogenates. J. Biol. Chem. 241: 2803 (1966).
11. Roels, H., et al. Response of β_2-microglobulin and other proteins in workers exposed to cadmium, lead or mercury. In: β_2-Microglobulin in Proliferative Disorders and Heavy Metal Intoxication, P. A. Peterson and R. Lauwerys, Eds., European Press, Gent, Belgium, 1978, p. 78.
12. Lauwerys, R., et al. Placental transfer of lead, mercury, cadmium, and carbon monoxide in women. I. Comparison of the frequency distributions of the biological indices in maternal and umbilical cord blood. Environ. Res. 15: 278 (1978).
13. Buchet, J. P., et al. Placental transfer of lead, mercury, cadmium, and carbon monoxide in women. II. Influence of some epidemiological factors on the frequency distributions of the biological indices in maternal and umbilical cord blood. Environ. Res., in press.
14. Roels, H., et al. Placental transfer of lead, mercury, cadmium, and carbon monoxide in women. III. Factors influencing the accumulation of heavy metals in placenta and relationship between metal concentration in placenta and in maternal and cord blood. Environ. Res., in press.