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OCT 23 1985

Subject: Lead, Blood Pressure, and Cardiovascular Disease

To: Lester Grant
Paul Mushak

From: Joel Schwartz

This memo has two purposes. First, to call to your attention several additional articles on lead and blood pressure that probably bear mentioning in chapter 12, and second to discuss the question of drawing inferences to cardiovascular disease, which you will address in your addenda.

I am enclosing two additional human studies on lead and blood pressure. One, from England,(Khera et al. Int J. Environmental Studies 1980 vol 14:309-312) was a case control study that found significantly higher lead levels in the blood of hypertensive and cardiovascular patients than in the blood of hospital controls. Interestingly, the lead levels were highest in the hypertensive patients, and elevated, but not as much, in the patients with ischaemic heart disease. This is what one would expect, since ischaemic heart disease is caused by other factors besides blood pressure. The second human study is of a small sample of men in Zutphen. Again, a significant correlation between blood lead and blood pressure was found.

Two recent animal studies by Meridth et al. have found evidence of increased plasma renin in animals and humans exposed to moderate levels of lead, and they suggest it may be part of a mechanism whereby lead promotes hypertension. I have also enclosed them. In addition, there were a number of papers that I discussed in my memo to CASAC last spring which report the results of animal studies on lead and blood pressure, which I had not located when you were drafting the Corrigenda. The criteria document should at least refer to them, and several, such as Picinnini et al. and Carmignani et al. merit at least some discussion. Revis et al. probably merits mention just because it is in a different species. I have copies of most of the papers, so please let me know if you have any difficulty locating them.

LEAD, BLOOD PRESSURE, AND CARDIOVASCULAR DISEASE

Fortunately, the question of whether higher blood pressure means higher risk of cardiovascular and cerebrovascular disease is not one that EPA needs to settle. Every prospective cardiovascular epidemiological study has found current blood pressure to be a risk factor in future cardiovascular and cerebrovascular events. There is no debate in the medical profession over this question. Table 1 shows the striking nature of these results for the largest study, the Framingham study. Notice in particular that it shows no discontinuity or threshold at the level currently defined as hypertension. Rather, the risk of myocardial infarction is a continuous function of blood pressure.

This issue of whether lowering a persons blood pressure will result in a reduced rate of cardiovascular and cerebrovascular disease is also one that EPA does not have to take the lead on. The National Institutes of Health periodically convenes "consensus panels" of experts on certain diseases to reach conclusions related to those diseases. The 1984 report of the Joint Committee on Detection, Evaluation, and Treatment of High Blood Pressure (Archives of Internal Medicine May 1984, vol 144) concluded that:

"Risk related to hypertension increases continuously as systolic and diastolic blood pressure rise."

"The goal of treating patients with hypertension is to prevent the morbidity and mortality attributable to high BP. This means the reduction of elevated blood pressure to the extent that excess cardiovascular risk is eliminated."

"Reducing blood pressure with drugs decreases cardiovascular mortality and morbidity in patients with moderate and severe hypertension. Trials of antihypertensive drugs in patients with mild hypertension have uniformly shown protection against stroke, left ventricular hypertrophy, congestive heart failure and progression to more severe levels of hypertension."

"It is apparent from prospective epidemiologic studies as well as from pooled actuarial data that the lower the systolic and diastolic blood pressure, even within the normal range (emphasis added), the better the prognosis."

This conclusion is well accepted in the medical community; they have voted with their prescription pads. Over 20 million Americans are no receiving anti-hypertensive medication, at a cost of about $5 billion dollars per year, plus the incidence of undesirable side affects. This medication is being given to reduce the risk of heart disease and strokes. For EPA to conclude that lowering blood pressure would not reduce the risk of heart disease and stroke would be for it to assert that there are 30 million continuing cases of medical malpractice, as well as for it to reject the conclusions of the NIH expert panel.

The NIH committee's recommendation is supported by the epidemiological evidence. The hypertension, Detection, and Followup program found that reducing blood pressure even in people with only slightly elevated blood pressure reduced the occurrence of coronary events. (NEJM 1983;307:976-980). The Australian national trials showed a substantial decrease in cardiovascular morbidity and mortality for patients with diastolic blood pressure from 95-109 mm Hg, who received active treatment compared to a group receiving a placebo. (Lancet 1980,1:1261-1267).

Recently Levy examined the 27% decline in coronary heart

disease deaths between 1972 and 1982 to elucidate the causes. (Am. J. Cardiology 1984 vol 54 7-13c). Noting that the survival rates for 3 years after acute myocardial infarction have not changed in the last 20 years, based on an almost complete ascertainment of all such cases in Baltimore by the Johns Hopkins epidemiology department (Goldberg et al. Johns Hopkins Med. J. 1979 144:73-80); and that the coronary artery surgery study (circulation 1981; 63 supp; I: I1-I81) also failed to find improved survival from bypass operations, he examined the changes in risk factors. He found that inserting the observed changes in smoking, serum cholesterol, and blood pressure in Framingham regression results predicted a 22% drop in mortality over the period, compared to the observed 27%, and concluded that risk factor modification was the major cause. This again supports the belief that reducing blood pressure will reduce the chance of heart disease.

Moreover, the magnitude of the effect of lead on blood pressure, as indicated by the NHANES regressions, is similar to the size of the reduction achieved by hypertensive medication. The Hypertension detection and Followup Program (NEJM 1983;307:976-980) found a 20% decrease in mortality from an intervention program that reduced blood pressure by about 5 mmHg. The NHANES regressions (Pirkle et al. AJ Epid. 1985) shows that a reduction in blood lead levels from 17ug/dl to 6ug/dl would produce a similar drop in blood pressure.

There aren't very many direct studies of lead and cardiovascular mortality, and they are usually occupational, and hence confounded by the healthy worker effect. However, the most recent analysis of Cooper found an SMR for hypertensive heart disease of 128 and 208 in battery workers and smelter workers respectively. More interestingly, a general population autopsy survey (Voors et al. Arch. Env. Health 37;98-102 1982) looked at lead concentrations in aorta and whether the death was due to heart disease. Lead was highly significant as a predictor of cardiovascular death, and explained 12% of the variation. The finding of Khera et al. of an association between blood lead and heart disease in a general population is also confirmatory.

I believe that these results indicate that accepted medical judgment finds that decreases in blood pressure are expected to result in decreases in cardiovascular and cerebrovascular disease, and that the limited data available directly relating lead and cardiovascular disease in the general population supports this expectation.

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TRACE METALS AND CORONARY HEART DISEASE RISK INDICATORS IN 152 ELDERLY MEN (THE ZUTPHEN STUDY)

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Kromhout, D. (Institute of Social Medicine, State University of Leiden, 2300 RC Leiden, The Netherlands), A. A. E. Wibowo, R. F. M. Herber, L. M. Dalderup, H. Heerdink, C. de Lezenne Coulander, and R. L. Zielhuis. Trace metals and coronary heart disease risk indicators in 152 elderly men (The Zutphen Study). *Am J Epidemiol* 1985;122:378–85.

Information about trace metals and coronary heart disease risk indicators was collected in 1977 among 152 men aged 57–76 years in the town of Zutphen, the Netherlands. Serum zinc, serum copper, blood cadmium, and blood lead were determined by atomic absorption spectrometry and serum lithium by flame emission spectrometry. After uni- and multivariate regression analysis, the following statistically significant relations were found: serum zinc was inversely related to resting heart rate; serum copper was positively related to cigarette smoking and inversely to high density lipoprotein cholesterol; blood cadmium was strongly positively related to cigarette smoking and inversely to Quetelet index; the positive relation between blood lead and cigarette smoking was of borderline significance; and blood lead was related to blood pressure, with the relation being stronger for systolic than for diastolic blood pressure.

blood pressure; cadmium; cholesterol; copper; coronary disease; lead; smoking; zinc

Descriptive data on serum and blood trace metal distributions in elderly populations are scarce. This type of information is of interest because cadmium and lead have been suggested to play a role in the etiology of hypertension (1–4). Animal experimental evidence (5) suggests that the dietary zinc/copper ratio may be a determinant of coronary heart disease, and, based on descriptive epidemiologic data, a protective effect of lithium on coronary heart disease has been postulated (6). To our knowledge, with the exception of the notion that blood cadmium and lead levels are higher in smokers than in non-smokers (7), the relationships between serum and blood trace metals and established coronary heart disease risk indicators have not been studied in elderly populations.

It was therefore decided to collect information about lithium, copper, and zinc in serum and cadmium and lead in blood in a

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subgroup of 152 men aged 57–76 years who participated in the 15th round of the Zutphen Study. Also the following coronary heart disease risk indicators were measured: total and high density lipoprotein cholesterol, systolic and diastolic blood pressure, smoking habits, and Quetelet index (weight/height^2). This paper reports the results of the descriptive analyses of trace metals and coronary heart disease risk indicators and discusses the relations between them.

Materials and Methods

Since 1960, a longitudinal investigation of the relations between diet, other risk indicators, and coronary heart disease has been carried out among middle-aged men from the town of Zutphen, the Netherlands. The Zutphen Study forms the Dutch contribution to the Seven Countries Study (8–10). In 1960, a random sample of 1,088 men born between 1900 and 1919 who had lived for at least five years in Zutphen was selected for the study, and, of these 1,088 men, 919 (84.5 per cent) were medically examined.

Between 1960 and 1973, the men underwent a medical examination yearly. In 1977 and 1978, the 15th round took place, and, of all the men who were still alive, 611 (92 per cent) were re-examined. Among the 473 men examined in 1977, a sample was selected of 152 men aged 57–76 years who had lived since 1960 in the same house. In this sample, relations were investigated between the type of waterpipes (e.g., lead or copper) and some trace metal levels in blood. Information about coronary heart disease risk indicators was also collected. Statistically significant differences in coronary heart disease risk indicator levels were not found between the men who participated in the trace metal study and all other men examined in 1977.

Information about smoking habits was obtained by a questionnaire completed by the men at home. Height and weight were determined according to a standardized protocol by a trained staff (8). Blood pressure was measured according to a standardized protocol by one internist using a mercury sphygmomanometer (8). Blood pressures were taken at the right arm with the men in a supine position. The first recording was done at the beginning and the second and third at the end of the medical examination. Only the systolic and diastolic (fifth phase) values of the third measurement were recorded. Resting heart rate was calculated from the electrocardiogram.

Non-fasting serum total and high density lipoprotein cholesterol determinations were carried out at the Lipid Laboratory of the Department of Human Nutrition, Agricultural University Wageningen, the Netherlands. Serum total cholesterol was determined automatically according to Huang et al. (39) using serum standards in which total cholesterol was determined according to Abell-Kendall (11, 12). High density lipoproteins were isolated by precipitation of the apo-B containing lipoproteins with heparin-manganese (13). The cholesterol content of the high density lipoprotein fraction was determined in the same way as described for total cholesterol (14). During the time that the cholesterol determinations were carried out for the present study, the Lipid Laboratory was standardized according to the criteria of the Lipid Standardization Laboratory of the Centers for Disease Control, Atlanta, GA.

To prevent contamination, cadmium- and lead-free disposable syringes and polyethylene tubes were used for blood collection. Lead-free heparin was used to prevent blood clotting and the blood samples were deep-frozen till analysis took place. Blood lead, blood cadmium, serum copper, and serum zinc were determined by electrothermal atomization atomic absorption spectrometry with improvements in precision by using a peakshape monitoring device (15). Serum lithium was determined by flame emission spectrometry. The accuracy of blood lead and blood cadmium was checked by participation in interna-

tional round robin studies. The inaccuracy was ≤50 μg/liter for blood lead and ≤0.7 μg/liter for blood cadmium. The accuracy of serum copper and serum zinc was checked in a comparison between the inductively coupled plasma spectrometry and the electrothermal atomization atomic absorption spectrometry method (16). The inaccuracy for zinc and copper was for both ≤0.1 mg/liter. For lithium, no comparison could be made.

For the statistical analyses, SPSS package programs were used (17). Besides descriptive statistics, means and standard deviations, correlation coefficients were calculated for continuous variables. Analysis of variance was used if the dependent variable was continuous and the independent variable categorical. Multiple comparisons were tested by the Scheffé method (17). Multiple regression analyses were carried out using coronary heart disease risk indicators as dependent variables and blood trace metals and other determinants of these risk indicators as independent variables. For variables with skewed distributions, log transformations were used in uni- and multivariate regression analyses. No difference was observed whether transformed or untransformed variables were used. Therefore, only the results of untransformed variables will be reported.

RESULTS

The levels of the coronary heart disease risk indicators were generally high in this elderly population (table 1). If a cut-off point of 160/95 mmHg was taken, the prevalence of systolic hypertension amounted to 34.4 per cent and that of diastolic hypertension to 37.1 per cent. The prevalence of obesity (Quetelet index >27 kg/m²) was 26.5 per cent and the prevalence of hypercholesterolemia (serum cholesterol >260 mg/100 ml) was 17.9 per cent. The percentage of current smokers amounted to 64.9 per cent.

The serum concentrations of the essential trace metals, lithium, copper, and zinc were normally distributed (table 2). The means for the nonessential blood metals, cadmium and lead, were higher than the medians, indicating that the distributions were skewed to the right. Blood cadmium levels above 5 μg/liter were not found. Blood lead levels above 300 μg/liter were present among 8.6 per cent and levels above 400 μg/liter among 1.3 per cent of the elderly men in Zutphen.

Serum copper was significantly related to cigarette smoking (table 3). A dose-response relation was present but only the men smoking 10 or more cigarettes/day had significantly higher serum copper levels than nonsmokers. Serum copper was inversely related to high density lipoprotein cholesterol ($r = -0.22$, $p < 0.01$) (table 4). This relation became somewhat stronger (standardized regression coefficient = -0.27, $p < 0.01$) after multiple regression analyses taking other determinants of high density lipoprotein cholesterol, e.g., Quetelet index, age, cigarette smoking, and resting heart rate, into account.

TABLE 1

Coronary heart disease risk indicator distributions of 152 men aged 57–76 years in Zutphen, the Netherlands, 1977

Risk indicator	Mean	Standard deviation	P10	P50	P90
Systolic blood pressure (mmHg)	154.3	22.0	126	149	184
Diastolic blood pressure (mmHg)	91.8	14.0	75	91	107
Total cholesterol (mg/100 ml)	226.9	38.2	180	223	276
High density lipoprotein cholesterol (mg/100 ml)	46.3	11.2	33	45	59
Quetelet index (kg/m²)	25.4	2.8	22	26	29
Resting heart rate (b/min)	75.4	12.3	59	74	93

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Trace metal
Serum lithium (μg/lit
Serum copper (mg/lit
Serum zinc (mg/liter)
Blood cadmium (μg/li
Blood lead (μg/liter)

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No. of cigarettes smoked/day	No. of men
None	53
<10	58
≥10	40

* Contrast, $p < 0.0$

Correlations betwee

Trace metal
Serum lithium
Serum copper
Serum zinc
Blood cadmium
Blood lead

* $0.01 \leq p < 0.05$.
** $0.001 \leq p < 0.0$
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TABLE 2

Trace metal distributions of 152 men aged 57–76 years in Zutphen, the Netherlands, 1977

Trace metal	Mean	Standard deviation	P10	P50	P90
Serum lithium (μg/liter)	18.3	2.1	16.3	18.5	20.8
Serum copper (mg/liter)	1.21	0.22	0.91	1.18	1.52
Serum zinc (mg/liter)	0.79	0.12	0.61	0.78	0.94
Blood cadmium (μg/liter)	1.55	0.89	0.68	1.26	2.81
Blood lead (μg/liter)	183	74	108	167	280

TABLE 3

Analyses of variance of serum and blood trace metals on cigarette smoking in 151 men aged 57–76 years in Zutphen, the Netherlands, 1977

No. of cigarettes smoked/day	No. of men	Trace element (mean ± standard deviation)		
		Serum copper (mg/liter)	Blood cadmium (μg/liter)	Blood lead (μg/liter)
None	53	1.14 ± 0.22	1.05 ± 0.41	172 ± 63
<10	58	1.21 ± 0.20 *	1.48 ± 0.79 *	176 ± 64
≥10	40	1.31 ± 0.23	2.32 ± 1.00	209 ± 93
		$F = 7.3$, $p = 0.001$	$F = 33.5$, $p < 0.001$	$F = 3.5$, $p = 0.032$

* Contrast, $p < 0.05$.

TABLE 4

Correlations between trace metals and coronary heart disease risk indicators in 151 men aged 57–76 years in Zutphen, the Netherlands, 1977

Trace metal	Risk indicator						
	Age	Systolic blood pressure	Diastolic blood pressure	Total cholesterol	High density lipoprotein cholesterol	Quetelet index	Resting heart rate
Serum lithium	−0.02	0.09	0.06	−0.10	−0.09	−0.06	0.12
Serum copper	0.08	0.04	0.07	0.09	−0.22**	−0.07	0.15*
Serum zinc	−0.05	−0.02	−0.00	0.00	−0.06	0.09	−0.16*
Blood cadmium	−0.17*	−0.12	−0.10	0.07	−0.02	−0.24**	0.13
Blood lead	0.03	0.24**	0.18*	0.07	0.08	0.11	−0.02

* $0.01 \leq p < 0.05$.
** $0.001 \leq p < 0.01$.
*** $p < 0.001$.

A dose-response relation was found between blood cadmium and cigarette smoking (table 3). The greater the number of cigarettes that were smoked, the higher the blood cadmium level. Blood cadmium was also significantly inversely related to age and Quetelet index (table 4). In a multiple regression model using blood cadmium as the dependent variable and cigarette smoking, age, and Quetelet index as independent variables, only cigarette smoking and Quetelet index remained significantly related to blood cadmium. The inverse relation between blood cadmium and age ($r = -0.17$, $p < 0.05$) observed in univariate analysis disappeared after multiple regression analyses due to the inverse relation between cigarette smoking and age ($r = -0.27$, $p < 0.001$).

Blood cadmium and serum copper were significantly related ($r = 0.29$, $p < 0.001$). These trace metals were both significantly related to cigarette smoking (table 3). Therefore a partial correlation was calculated controlling for cigarette smoking. This partial correlation coefficient amounted to 0.15 ($p < 0.05$).

Blood lead was borderline significantly

related to cigarette smoking (table 3). A dose-response relation was not present and the differences between the different categories of smokers, tested by Scheffé, were not statistically significant. Blood lead was significantly positively related to systolic and diastolic blood pressure (table 4). Multiple regression analyses with systolic blood pressure as the dependent variable and blood lead, age and Quetelet index as independent variables showed that the standardized regression coefficient for blood lead was reduced from 0.24 ($p < 0.01$) to 0.21 ($p < 0.01$). When a similar model was used for diastolic blood pressure, the standardized regression coefficient for blood lead was reduced from 0.18 ($p < 0.05$) to 0.15 ($p = 0.05$).

Resting heart rate was significantly positively related to serum copper and significantly inversely to serum zinc (table 4). The positive relations between serum lithium and resting heart rate and between blood cadmium and resting heart rate were borderline statistically significant ($p = 0.07$ and $p = 0.05$, respectively). In a multiple regression model using resting heart rate as the dependent variable and the four trace metals as independent variables, ony serum zinc was significantly ($p = 0.04$) inversely related to resting heart rate.

Discussion

The serum lithium levels seen in this study could not be compared with those observed in other studies due to differences in analytic methods. The average serum zinc level of the elderly men in Zutphen was similar to that of men aged 65–94 years in Belfast, Northern Ireland (18). In both studies, no relation was found between serum zinc and age. That may be due to the small age range, because in a study including men aged 20–84 years, a significant inverse relation with age was observed (19). In studies mainly including men aged 60 years or less (19–21), average serum zinc levels of 1.0 mg/liter were found, compared to 0.8 mg/liter in the present study and in the Belfast study (18). This difference may be of physiologic importance but methodologic differences between these studies in the determination of zinc cannot be ruled out as a major explanation.

The average serum copper level of the Zutphen men was 0.1 mg/liter higher than in the elderly men from Belfast (18). No difference was found between the average serum copper level of the elderly men in Zutphen and middle-aged men from Heidelberg, West Germany (21). In the Zutphen men, serum copper was unrelated to age, probably due to the small age range. In men aged 20–69 years from Omaha, Nebraska (22), an increase in serum copper levels was found with age.

A study on blood cadmium levels among men in a comparable age range, 56–72 years, was carried out in Sweden (7). The median cadmium level of these men was considerably lower than the median level of the Zutphen men, 0.8 versus 1.3 μg/liter. This difference may partly be due to the different percentages of smokers, 46 versus 65 per cent. In a carefully standardized international cooperative study among men of an unspecified age range (23, 24), median values of 0.4–0.9 μg/liter were found in Sweden, Yugoslavia, Israel, and the United States, while median values of 1.0–1.8 μg/liter were found in men from Belgium, Peru, Mexico, Japan, and China. These comparisons may also be confounded by differences in the cadmium concentration of the cigarettes and the smoking habits of the men in these countries. The percentage of smokers varied from 20 per cent for Peru to 64 per cent for Japan. It may be concluded that the blood cadmium levels of the elderly men in Zutphen were comparable with those of men in countries with the higher levels, but values above 5 μg/liter were not found.

The median blood lead level of men aged 56–72 years in Sweden (7) amounted to 76 μg/liter, compared to 167 μg/liter among the men in Zutphen. In the above-mentioned cooperative international study (23,

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24), median levels below 100 μg/liter were found among men from Sweden, China, and Japan, while median levels between 100 and 200 μg/liter were observed in men from the United States, Yugoslavia, India, and Peru. The highest median value, 271 μg/liter was found in Mexico City. Middle-aged men from England and West Germany had average values around 150 μg/liter (21, 25). Comparisons between countries are difficult due to the confounding effects of alcohol, smoking, and traffic intensity (7, 23–25) on blood lead levels. In spite of these difficulties, it may be concluded that the blood lead levels of the elderly men in Zutphen ranked high internationally, with a median of 167 μg/liter and 8.6 per cent with levels above 300 μg/liter and 1.3 per cent with levels above 400 μg/liter.

Serum zinc was significantly inversely related to resting heart rate but was unrelated to total and high density lipoprotein cholesterol. In rats (26, 27), a significant positive relation was found between serum zinc and high density lipoprotein cholesterol. A study in 12 healthy young adult men (28), showed a significant reduction in high density lipoprotein cholesterol after the men had ingested 440 mg zinc sulfate per day for five weeks. It may be concluded that the relation between serum zinc and lipoproteins is unclear.

In the present study, an inverse relation was found between serum copper and high density lipoprotein cholesterol after uni- and multivariate regression analyses. Several epidemiologic studies (29–31) have shown that low high density lipoprotein cholesterol levels are associated with an increased risk of coronary heart disease. Serum copper may therefore be related to coronary heart disease. This hypothesis is corroborated by the results of two case-control studies (21, 32) carried out in middle-aged persons, in which persons with coronary heart disease had significantly higher serum copper levels than controls. Also, a statistically significant positive relation was found between serum copper and total cholesterol (21). A similar, but not statistically significant relation was observed in the present study (table 4). Persons with elevated total cholesterol or low density lipoprotein cholesterol levels are at high risk for coronary heart disease (10, 33). From these results, it may be hypothesized that high serum copper levels are related to low levels of the anti-atherogenic high density lipoprotein fraction and high levels of the atherogenic low density lipoprotein fraction.

Based mainly on the results of animal experimental studies (5, 34), the hypothesis has been formulated that a dietary copper deficiency either alone or in combination with an elevated intake of zinc is associated with an increased total cholesterol level. This relation was probably due to extremely low levels of copper in the diet. When copper levels were used comparable to the North American diet, no relation was found (35). It is questionable whether these results are relevant for the relations between serum copper and serum lipids, because serum copper may not be indicative for copper intake (36, 37). Serum copper levels are, however, significantly positively related to cigarette smoking and coronary heart disease (21). Studies on the relations between copper intake and exposure, serum copper and coronary heart disease are urgently needed.

In the present study, an inverse relation was found between blood cadmium and age. This relation disappeared after controlling for cigarette smoking due to the inverse relation between cigarette smoking and age. It may therefore be concluded that at least in this study the decrease in blood cadmium with age is due to the decrease in the percentage of cigarette smokers with increasing age.

The strong positive relation seen between blood cadmium and cigarette smoking in the elderly men in Zutphen was also found among men of the same age in Sweden (7). This relation was also present in several studies carried out in younger men

(23, 24). It may therefore be concluded that cigarette smokers are at high risk for elevated blood cadmium levels.

In the present study, an inverse relation was noted between blood cadmium and Quetelet index. That may be due to the confounding effect of smoking because smoking is related to blood cadmium (table 4) and Quetelet index ($r = -0.15, p < 0.05$). Therefore, a multiple regression analysis was carried out using blood cadmium as the dependent variable and Quetelet index, cigarette smoking, and age as independent variables. After this analysis, blood cadmium remained significantly inversely related to Quetelet index. This means that body leanness is associated with high blood cadmium levels. An explanation for this relation is not available.

Among the elderly men in Zutphen, no relation was found between blood cadmium and blood pressure. In both animals and humans, consistent relations between blood cadmium and blood pressure have not been observed (1, 2, 4). That may be due to the inverted U-shaped relation between cadmium intake and blood pressure found in rats (38). The maximum increase in blood pressure was found at a cadmium intake of 10 μg/day. This is considerably lower than the average intake among Americans and Europeans of 50–70 μg/day (38). It may therefore be possible that the cadmium intake among humans is too high for showing a relation with blood pressure.

Cigarette smoking was borderline significantly related to blood lead. No dose-response relation was present. Also, in other studies (7, 23, 24), a generally weaker relation was found between cigarette smoking and blood lead than between cigarette smoking and blood cadmium.

In the present study and in studies from Scotland (3, 4), a positive relation was found between blood lead and blood pressure. Among the elderly men in Zutphen, a stronger relation was found for systolic than for diastolic blood pressure. In order to get insight into the stability of this relation, the individual with the highest blood lead level (525 μg/liter) who also had hypertension (218/138 mmHg) was excluded from the analyses. Thereafter, a borderline significant correlation was found between blood lead and systolic blood pressure, and the correlation between blood lead and diastolic blood pressure became insignificant. The relation between blood lead and systolic blood pressure became insignificant after multiple regression analysis with other determinants, e.g., age and Quetelet index, included in the model. The correlations between blood pressure and Quetelet index and age, respectively, did not change after exclusion of the hypertensive man with the elevated blood lead level. It may therefore be concluded that blood lead is probably a less important determinant of blood pressure than age and Quetelet index.

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Additive Statistical Effects of Cadmium and Lead on Heart-Related Disease in a North Carolina Autopsy Series

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ABSTRACT. The association of heart-related mortality with tissue cadmium and lead in a study of autopsies performed on persons who resided in a soft-water, leached-soil area of North Carolina was examined. Liver cadmium concentrations and aortic lead level were indices of these elements. Both cadmium and lead levels had statistically significant correlations with cause of death (heart-related disease vs. non-heart-related disease, excluding cancer). Although cause of death was significantly associated with age, the association with cadmium and lead persisted after statistical adjustment for the effect of age. The combined effects of cadmium and lead provided sufficient information in an additive model to predict cause of death correctly for 80% of the cases, with age contributing insignificantly. These findings indicate the intimate relation of these two trace metals with increased risk of heart-related mortality, even in light of known conventional causes of such deaths.

EXCESS CARDIOVASCULAR MORTALITY on the east coast of the United States (particularly the rural Coastal Plains area)[1] and around Glasgow in Great Britain has not yet been explained definitely.[2] Although the water is soft and the soil is acidic and leached in both areas, a biological link with cardiovascular disease is tenuous. Excess cadmium (Cd) and lead (Pb) have been implicated individually in cardiovascular pathology,[3-6] but their combined effects have not been demonstrated by their accumulation in local autopsy tissues. Previously, Cd level in the liver and Pb level in the aorta have been shown to be positively associated with death resulting from cardiac disease in an autopsy population of North Carolina.[5,6] Presumably, liver and aorta tissues are indicators of the respective body burdens of the two trace metals as they relate to the circulatory system. It has been suggested by several investigators[7-9] that the additive effects of Cd and Pb result in damage to the cardiovascular system. This question is of practical importance because it is likely that these two heavy trace metals often have common sources in contemporary environments.[10-12] In the present analysis these two effects are studied simultaneously to assess the magnitude of their respective roles in an additive statistical model.

MATERIALS AND METHODS

The population studied and methods used have been described previously.[13] Briefly, 106 autopsies were done in 7 hospitals in North Carolina during an uninterrupted period. Patients who died of cancer were excluded because cancer was found to be associated with an increased variability of Cd and Pb levels.[14]

Choice of index organs. Since the heart and aorta share the arterial blood as an immediate environment, the level of Pb in the aorta was used as an index of the heart's exposure to Pb. Cadmium accumulates in the liver and kidney; its concentration in the kidney rises until the age

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Table 1.—Heart-Related Death Causes, by Liver Cadmium and Aortic Lead Level, Excluding Cancer Patients (North Carolina Autopsy Study, 1973)

Pathologic-Anatomical Diagnosis	Age (yr)	Sex	Cd in Liver (ppm ash)	Pb in Aorta (ppm ash)
Myocardial infarction (MI)	76	F	1720	082
Atherosclerosis, renal infarction, MI	37	F	684	084
Congestive heart failure, obstructive lung disease	76	M	605	076
Cardiac arrest, surgery for mitral valve replacement	63	M	460	015
Cardiac arrest, hypertension, arteriolar nephrosis	63	F	449	015
Acute coronary insufficiency	44	M	391	015
MI, fatty liver	54	M	390	015
Renal failure due to arterionephrosclerosis, cardiomyopathy	48	M	375	091
MI	59	M	368	052
Bacteremia and myocarditis, cardiorenal atherosclerosis	60	M	353	107
Atherosclerotic heart disease, cardiac arrest, aortic aneurysma	67	M	350	152
MI	85	M	282	130
Coronary thrombosis, aortic aneurysma	61	M	281	128
Congestive heart failure due to severe kyphoscoliosis	34	M	279	104
Arteriosclerotic occlusion of the iliac arteries, diabetes mellitus, congestive heart failure	74	M	273	015
MI	58	M	273	223
Pulmonary thromboembolic disease, cor pulmonale	67	M	265	067
MI, diabetes mellitus	47	M	236	212
Thrombi, myocardial insufficiency, lung tuberculosis	70	M	214	478
Acute alcohol poisoning with focal fatty infiltration of myocard, fatty liver	42	M	207	465
Acute coronary insufficiency	58	F	181	064
MI, pulmonary congestion, fatty liver	48	M	147	315
Post-operative cholecystitis, congestive heart failure	71	F	111	161
Rheumatic heart disease with valvular and coronary involvement	24	F	097	540
Atherosclerotic coronary insufficiency	47	M	092	unknown
Bronchopneumonia with focal interstitial fibrosis, recent MI, fatty liver	65	M	unknown	226

of 50 yr and then decreases, whereas its concentration in the liver remains more constant after 30 yr of age. Liver concentration of Cd was, therefore, chosen as a more consistent index of relatively recent exposure.[15]

Tissue-processing. The entire thoracic and abdominal aorta and the leftmost peripheral portion of the liver from each subject were cleaned, freeze-dried, and dry-ashed under low temperature to concentrate the trace metals without loss. The material was then examined by atomic absorption spectrometry for Cd and Pb content.

Statistical analysis. A stepwise logistic regression analysis was performed with the cause of death as the dependent variable. Age and the log-transformed trace metal levels (expressed in ppm ash weight) were entered as independent variables. Trace metal levels less than the sensitivity threshold of the chemical analytic method were arbitrarily assigned the value of 3/5 of the threshold level. The authors controlled for the amount of mineralization of the tissues by expressing trace metal concentration per ash weight rather than per dry or wet weight.

RESULTS

No cancer was found in 83 of 106 autopsies. Of those 83, 8 did not yield aorta or liver tissues. The present analysis, therefore, is based on the remaining 75 autopsies. The deaths of a 23-yr-old patient who died of acute bacterial endocarditis and a 64-yr-old patient who died during surgery for an aortic valve replacement were considered non-heart-related disease deaths for this study.

A list of causes of death related to heart disease is given in Table 1.

Liver Cd was placed into the stepwise logistic regression equation first, followed by aorta Pb and age. With all three variables in the equation, both liver Cd ($P = .0033$) and aorta Pb ($P = .0021$) demonstrated statistically significant effects. The independent effect of age did not attain statistical significance at the 5% level ($P = .220$, Table 2).[16]

A plot of aortic Pb by liver Cd for the heart disease deaths and other deaths (Fig. 1) suggests an additive effect on heart disease-related deaths. It should be noted that there is a concentration of aortic Pb values around 3/5 of the threshold level for the chemical analysis, which is an artifact generated by the arbitrary evaluation of below-threshold observations. The five heart disease deaths with the lowest aortic Pb values stand out in Fig. 1. Descriptions additional to the diagnoses of Table 1 are, in order of listing for these 5 cases: (1) post-operative death after mitral valve replacement; (2) hypertension and adrenal cortex

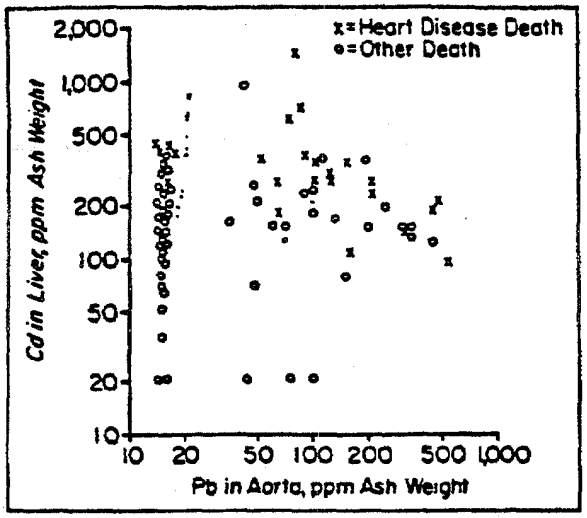

Fig. 1. Proportion of deaths resulting from heart disease, related to hepatic Cd and aortic Pb levels; cancer deaths are excluded. (North Carolina Autopsy Study, 1971)

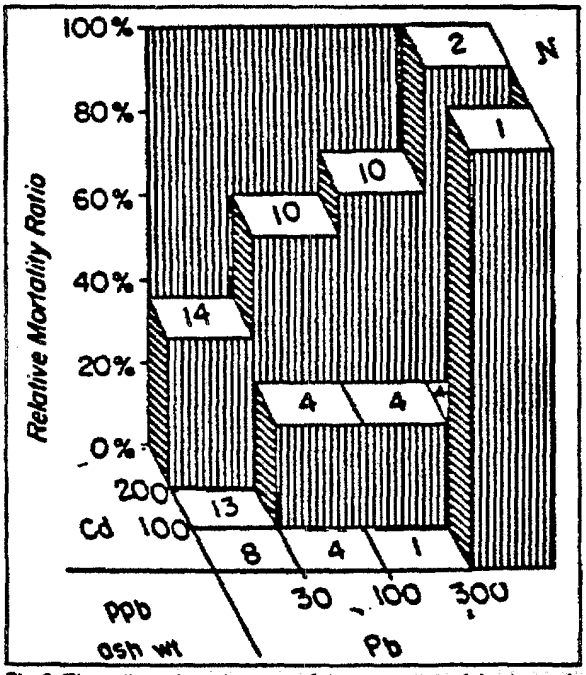

Fig. 2. Three-dimensional bargram of the proportion of deaths resulting from heart disease, related to hepatic Cd and aortic Pb levels. The height of the bars represents this proportional mortality and increases with higher tissue levels for either metal. If a dose-effect relationship for each of these metals exists, then some additive effect seems probable. Cancer deaths are excluded. *N* represents sample size. (North Carolina Autopsy Study, 1971)

adenoma; (3) severe atheromatosis, obesity, acute coronary insufficiency with old coronary thrombus; (4) severe atheromatosis, moderately old and organizing myocardial infarcts, fatty liver; and (5) diabetes mellitus. These 5 cases, mostly with multiple diseases, are exceptions to our finding of the predictive power of an additive model predicting heart-related disease.

Table 3 shows the data of Figure 1 in contingency table format, and Figure 2 presents the same results as a three-dimensional bargram. In general, the proportion of deaths caused by heart-related disease is the lowest when levels of both minerals are low and increases as the combined levels of the two metals increase in a manner compatible with an additive effect for the two metals.

DISCUSSION

The possibility cannot be excluded that the relationships described herein may result, in part, from consumption of moonshine alcohol (aortic Pb) and cigarette smoking (hepatic Cd). The presence of fatty liver, however, did not account for the relation between Pb and heart disease death ratio, nor could amount of cigarette smoking account for the relation between Cd and heart disease death ratio.[5] It is more probable that in the absence of sufficient environmentally determined nutrient protection (calcium, selenium, etc), that Cd and Pb act additively in their effects on atherogenic conditions or on events that cause arteriosclerosis and cardiovascular complications.

Recent animal experiments and clinical observation have demonstrated low-dose effects, both in vivo and in vitro, of Cd and Pb on the cardiac conduction system.[15,17,18] These low-dose effects are probably not limited to the cardiac conduction tissue; Cd may also have a pressor effect[19] and Pb may also have a central nervous system effect.[20-22] Both may act on cardiac tissue.[9] In vivo, Perry et al.[7] have observed additive effects of Cd and Pb on systolic blood pressure in small rodents. Revis et al.[23] reported that an additive effect of Cd and Pb occurred in pigeons, and was manifested as atherosclerosis and hypertension which were counteracted by calcium. Walker and Moses[8] observed a Pb mobilizing

Table 2.—Logistic Regression of the Proportion* of Deaths Resulting from Heart Disease on Trace Metal Levels and on Age (N = 75) (North Carolina Autopsy Study, 1971)

Variable	BETA	P	D†
Intercept	− 26.01	.0003	
Cadmium in liver, ppm ash weight‡	5.40	.0033	0.108
Lead in aorta, ppm ash weight‡	2.12	.0021	0.118
Age (yr)	0.03	.2204	0.021

NOTES: This model classifies 80% of cases correctly. Cancer deaths have been excluded.
*Probit transformation, Statistical Analysis System (SAS), PROC LOGIST.[14]
†*D* is equivalent to partial R^2 if the distribution would have been Gaussian.
‡Log_{10} transformation. Bivariate correlation coefficients are: age vs. Cd 0.24 (P = .034); age vs. Pb, 0.05; Cd vs. Pb, 0.11.

Table 3.—Proportion of Deaths Resulting from Heart Disease and Total Number of Deaths ($N = 75$) in Categories of Cd and Pb Levels, Cancer Deaths Excluded (North Carolina Autopsy Study, 1971)

Cd in Liver (ppm ash wt.)	Pb in Aorta, ppm ash weight				
	< 30	30–99	100–299	300+	Total
100	0.00 (8)*	0.00 (4)	0.00 (1)	1.00 (1)	0.07 (14)
100–199	0.00 (13)	0.25 (4)	0.25 (4)	0.25 (4)	0.12 (25)
200+	0.36 (14)	0.60 (10)	0.70 (10)	1.00 (2)	0.56 (36)
Total	0.14 (35)	0.39 (18)	0.53 (15)	0.50 (8)	0.32 (75)

*Sample size appears in parentheses.

action of Cd in rats, and Mahaffey et al.[24] observed interactions altering the levels and toxicity of Pb and Cd.

Cadmium is quite dissipated in the western industrialized countries.[25] A main source of entry into the human body is food.[26] Cadmium in the soil is more available to food and fodder plants if the soil is acidic and leached,[27] and if the water is soft.[28] A second source of Cd is cigarettes.[29–32] Crawford and Clayton[33] have demonstrated an association between softness of drinking water and Pb levels in human bones. They attributed this association to the plumbosolvency of soft water in lead-containing pipes. Sauer[34] found a partial correlation between Pb level in drinking water and mortality resulting from cardiovascular-renal disease in 92 Metropolitan State Economic Areas in the United States after controlling for a number of socioeconomic and environmental variables.

The observed relationship between softness of drinking water and sudden heart death[35, 36] and the observed relative concentration of cardiovascular deaths in white males in the southeastern United States[1] point to intimate relations of Cd and Pb uptake with cardiovascular disease, perhaps involving the cardiac neuromuscular impulse conduction system, the arterial tissue, the blood pressure, or a combination of these.[37] Although Cd and Pb each have separate tissue predilections, both Cd and Pb share an affinity to cardiac tissue.[38–40]

CONCLUSION

The data suggest additive statistical effects of tissue Cd and tissue Pb on cause of death (heart-related disease vs. non-heart-related disease) and support the hypothesis that the excessive presence of these heavy metals in crucial tissue components of persons autopsied in the eastern United States has a close relationship with cardiovascular mortality in this autopsy population, even in light of known conventional causes of such deaths. Cardiovascular death in soft-water, leached-soil areas needs further study.

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CADMIUM AND LEAD LEVELS IN BLOOD AND URINE IN A SERIES OF CARDIOVASCULAR AND NORMOTENSIVE PATIENTS

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The mean blood-lead, blood-cadmium, urine-lead and urine-cadmium levels of a series of male Caucasian patients suffering from cardiovascular disease were significantly higher at every age group than the corresponding levels in a control series of normotensive patients.

INTRODUCTION

Despite intensive studies, particularly over the past six years, the question as to whether the levels of lead and cadmium in the blood of hypertensive patients or those with cardiovascular disease, are higher than the corresponding levels in normal subjects remains unsolved. Even more intractable problems concerned with whether such conditions are actually caused by the presence of either of these heavy metals must await this resolution. The higher levels of cardiovascular disease in soft water districts has been cited[1] as a possible reason for implicating lead as a factor and the induction of hypertension in laboratory animals with additional cadmium in their diet as a reason for suggesting that cadmium is a factor.[2] In the case of blood-lead levels the most important contribution has come from the Glasgow team of D. G. Beevers *et al.*[3] who have shown a significant excess of cases with high blood-lead levels among hypertensives in the West of Scotland where tap-water lead levels are notoriously high. The same authors,[4] however, did not detect an appreciable difference in average blood-cadmium levels between two groups of hypertensive and control patients matched for age and sex.

In contrast, S. C. Glauser *et al.*[5] found more than a three-fold difference between average blood-cadmium levels in 17 untreated hypertensive patients and a group of ten somewhat younger normotensive controls.

One of the problems is undoubtedly the considerable difficulty of determining with accuracy and consistency the low levels of the two metals in blood. In the case of cadmium for example, in the last five years, nine different authors have claimed blood-cadmium levels in the normal population of between 0.003μ mole/l (0.4 ng/ml)[6] and 0.33μ mole/l (37 ng/ml).[7]

Lauwerys[8] has claimed that the mean blood-cadmium levels for the general population is 0.04μ mole/l (4.1 ng/ml) and that it is now generally recognised[9] that the normal concentration is below 0.09μ mole/l (10 ng/ml).

PATIENTS

For a period of 15 months from October 1976, 50 patients who attended the General Hospital Birmingham because of a moderate to severe cardiac condition and/or hypertension gave, on the same day, one sample of blood and one of urine which were analysed for cadmium and lead. During the same time similar determinations were made for 75 patients attending the hospital with no known cardiovascular symptoms.

METAL DETERMINATIONS

Blood was drawn from each patient into a heparinised metal-free container and urine collected in a glass metal-free tube. 1g samples were dissolved in soluene (5 ml) and diluted with toluene to 10 ml

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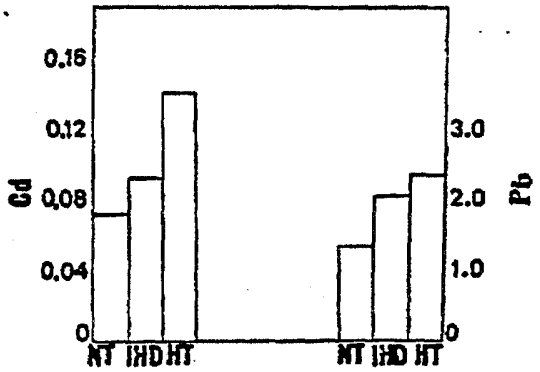
and aliquots (25μl) pipetted into a H.G.A. 74 graphite furnace of a Perkin Elmer 360 A.A.S. modle.

In the case of cadmium the mixture was dried at 60°C for 30 seconds, charred at 550°C for 30 seconds and atomised at 1900°C for 10 seconds. The samples were then burnt off at 2700°C for five seconds. Controls were prepared from blood spiked with cadmium chloride to give blood-cadmium concentrations in the range of 0.009–0.04μ mole/1 (or 1ng/ml–4 ng/ml). For lead, drying, charring and burn-off temperatures and timings were the same as for cadmium. The samples were atomised at 2100°C for ten seconds. The lead standards were in the range of 0.24–2.4μ mole/1.

RESULTS

For all four analytical determinations there was an appreciable difference between the mean heavy metal values in the cardiovascular patients and in the normotensive patients. (Table I). The cardiovascular patients contained small numbers of women (7) Negroid and Asian patients (7) and the normotensive patients included 14 patients less than 30 years of age. When these three groups were removed from consideration because of difficulties in matching, there remained 38 male cardiovascular patients aged over 30 and 48 matched normotensive patients. The results from these matched groups are recorded in Table II. The urine metal levels were extremely variable and other workers[10] have shown that 24 hour samples are necessary to overcome diurnal variations. The blood-cadmium and blood-lead levels are recorded in Table III for four matched age groups. There was some evidence of variations in heavy metal levels with the nature of the cardiovascular disease (Figure 1) but the small numbers in certain categories make definite correlations uncertain.



NT = Normotensives (48 subjects)
IHD = Ischaemic heart disease (25 patients)
HT = Hypertensive (13 patients) of whom three also had ischaemic heart disease.

FIGURE 1 Disease pattern in cardiovascular patients and normotensives in relation to blood-cadmium and blood lead.

TABLE I

Cadmium and lead levels (μ mole/l) in all patients

Patients	Metal level (μ mole/l)			
	Blood-Cd	Urine-Cd	Blood-Pb	Urine-Pb
Cardiovascular	0.10	0.07	2.12	0.37
Normotensive	0.07	0.06	1.35	0.29

TABLE II

Cadmium and lead levels in blood and urine in Caucasian males aged over 30

Patients	Metal level (μ mole/l)					
	Blood-Cd		Urine	Blood-Pb		Urine
	mean	range		mean	range	
Cadiovascular	0.10	0.02–0.35	0.07	2.17	0.43–4.00	0.34
Normotensive	0.07	0.01–0.18	0.05	1.4	0.58–2.2	0.27

TABLE III

Cadmium and lead levels for four matched age groups

	Age group	Average age	No. of patients	Blood-Cd (μ mole/l) mean	Blood-Cd (μ mole/l) range	Blood-Pb (μ mole/l) mean	Blood-Pb (μ mole/l) range
Cardiovascular	30–39	34	5	0.14	0.04–0.27	2.65	1.16–3.96
Normotensive	30–39	35	9	0.06	0.09–0.10	1.35	0.42–1.74
Cardiovascular	40–49	46	8	0.10	0.02–0.25	2.12	0.68–3.23
Normotensive	40–49	45	9	0.07	0.01–0.18	1.45	0.58–1.96
Cardiovascular	50–59	54	16	0.09	0.02–0.35	1.98	1.50–3.04
Normotensive	50–59	51	16	0.07	0.02–0.13	1.30	0.58–1.98
Cardiovascular	> 60	65	8	0.11	0.07–0.21	2.17	0.43–3.96
Normotensive	> 60	67	17	0.07	0.03–0.15	1.40	0.87–2.03

Similarly, smoking habits were not determined with sufficient accuracy for firm conclusions, although overall results showed little change with smoking and lead levels but cadmium levels ca 20 percent higher than normal in normotensive smokers compared with non-smokers. For the cardiovascular patients the group of ex-smokers had the highest blood-cadmium levels but for all three groups the disease state appeared to mask any smoking effect.

DISCUSSION

The results of this pilot study have proved to our satisfaction that average blood-lead and blood-cadmium levels are consistently higher in patients with a wide range of cardiovascular-disease than in matched normal subjects with no such disease symptoms. We have found that the analyses are extremely sensitive to a whole range of factors and are near the limits of sensitivity and reliability of the instrument. The results demonstrate yet again the difficulty of inter-laboratory comparisons which must await validated control work[11] on the same blood samples. The patients were also far from being a cohesive group having a wide range of heart disease varying from severe myocardial infection and ischaemic heart disease to moderate hypertention. Most were also receiving a range of drug therapies and in view of the results found with varying disease states these should be standardised in future trials.

Beevers *et al.*[3] have suggested that chronic exposure to unsatisfactory levels of lead in drinking water can lead to the development of hypertension.

Voors and Shuman[12] have similarly concluded, on the basis of high liver-cadmium levels in North Carolina residents who died of heart disease, that cadmium has a toxic effect on the cardiac conduction system. Our work on placental lead levels did not enable us to clearly implicate lead as a causative factor in stillbirths and neonatal deaths[13] and in our opinion, a similar dilemma exists in the case of cardiovascular disease. Environmental levels of cadmium and lead could be inducing cardiovascular disease in some patients or such disease may result in abnormal metabolism and consequential higher blood-cadmium and blood-lead levels.

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