

TRACE ELEMENTS IN RELATION TO

CARDIOVASCULAR DISEASES

(Status of the Joint WHO/IAEA  
Research Programme)

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WORLD HEALTH ORGANIZATION

Geneva

1974



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## PREFACE

This document is based on the reports of three meetings of investigators on Trace Elements in Relation to Cardiovascular Diseases, which were held jointly by the International Atomic Energy Agency (IAEA) and the World Health Organization (WHO) in Geneva, 8-13 February 1971, in Vienna, 19-23 February 1973 and in Geneva, 2-6 April 1973.

The IAEA and WHO have been cooperating in this field since 1969 and have established a network of collaborating laboratories in several countries for the collection of biological specimens and for trace element analysis. The present report contains a description of the scope and progress of the investigations, a summary of the results so far obtained, and recommendations for the future conduct of the programme.



## INTRODUCTION

Cardiovascular diseases are the leading cause of death in the highly industrialized countries where they account for roughly 50% of all causes of death. Death rates from coronary heart disease, in particular, have been steadily increasing over the past few decades. In the developing countries coronary heart disease is less common, but death rates from this condition are increasing there too.

Attempts to explain these geographical differences and alarming secular trends on the basis of dietary habits, blood lipid levels, physical activity patterns, genetic factors, smoking, and psychosocial stress have not yet yielded consistent results. Myocardial infarction and atherosclerosis seem to be related to technological development but their etiology and pathogenesis are still incompletely known.

Attention is now being focused on another factor, namely, trace element imbalance as possibly being of etiological importance in cardiovascular diseases. This factor, directly or indirectly, is related to the geographical localization of the various population groups, to their technological progress, dietary habits, and environmental conditions.

This point of view is substantiated by three arguments.

1. It is well established that certain trace elements play a decisive role in a number of biological processes through their action as activators or inhibitors of enzymatic reactions, by competing with other elements and proteins for binding sites, by influencing the permeability of cell membranes, or through other mechanisms. It is therefore reasonable to assume that these minerals would also exert an action, either directly or indirectly, on the cardiac cell, on the blood vessel walls, on the blood pressure regulating centres, or on other systems related to cardiovascular function such as, for example, the lipid and carbohydrate metabolism.

2. Dietary practices are different from country to country and are changing in time within the same country. While much is known relative to items like protein content, vitamin levels, calories, etc., that can be adjusted to meet optimal criteria, much less is known on the content, availability, and nutritional significance of trace elements in food.

3. Man-made alterations of the environment through the use of fertilizers, food additives, food processing and canning, treatment and softening of drinking-water, and through industrial pollution of air and water, may bring about changes in the mineral balance and, as a consequence, in some biological functions, including the cardio-circulatory function. Over hundreds of thousands of years man became adjusted to the mineral balance of the natural environment. Rocks are the primary source of minerals, which pass from the rock into the soil, the water, into plants, animals and man. The natural balance is now perhaps being disrupted by man himself, whose organism has not yet had time to adjust biochemically to these rapid, technologically-induced changes.

It must be borne in mind that a slight difference in environmental or in dietary trace element balance, either naturally occurring or artificially produced, usually does not give rise to any dramatic outbreak of diseases and, therefore, it escapes medical attention. Nevertheless, a life-long exposure to marginal deficiencies or excesses in the availability of trace elements may lead to the development of chronic diseases.

In recent years epidemiological, clinical, pathological and experimental evidence has accumulated which justified undertaking deeper studies of the role of trace elements in the pathogenesis of cardiovascular diseases. This has been reviewed.<sup>1,2</sup>

In the framework of its activities aiming at a better understanding of the etiology and pathogenesis of cardiovascular diseases the World Health Organization (WHO) is carrying out, in collaboration with the International

Atomic Energy Agency (IAEA), internationally coordinated investigations on the role of trace elements in cardiovascular diseases.

Following a planning meeting that was held in Geneva, 8-13 February 1971,<sup>2</sup> when research needs, protocols and organization of activities were established, collaborative studies were implemented in six areas of research:

1. Comparative trace element analyses of heart, liver, and kidney obtained at autopsy from apparently healthy subjects who died accidentally but were otherwise free from disease (controls) and from subjects who died of coronary heart disease;
2. Comparison of cadmium and zinc content and cadmium/zinc ratios in the liver and the kidneys obtained at autopsy from normotensive and hypertensive subjects;
3. Epidemiological study of blood pressure, electrocardiographic findings and blood cholesterol levels in nonindustrialized groups and correlation with trace element content of blood, toe-nails, hair, as well as of food and water;
4. Trace elements in nutrition: collection of samples of different types of rice, sugar and other staple food from several countries and determination of trace element content in relation to refining and processing;
5. Study of hardness or softness of the water supplies in relation to cardiovascular mortality; and
6. Preliminary investigations of rock and soil content of trace elements in relation to cardiovascular parameters and death rates.

This programme involves the collaboration of pathology departments, epidemiological teams, and analytical laboratories located in the following countries and territories: Argentina, Bulgaria, Canada, Czechoslovakia, Federal Republic of Germany, Finland,

Greece, Hong Kong, Iran, Italy, Israel, Jamaica, New Zealand, Norway, Philippines, Territory of Papua New Guinea, Sweden, Switzerland, United Kingdom and the United States of America.

WHO coordinates the medically-oriented aspect of these studies, while the IAEA coordinates the analytical aspects, which involve mainly neutron activation analysis.

To evaluate the past activities and to plan the future ones, a meeting of the collaborating analysts was held at IAEA in Vienna, 19-23 February 1973,<sup>3</sup> where the analytical procedures and intercomparability of the results obtained so far were thoroughly discussed, and a subsequent meeting was held in Geneva, 2-6 April 1973<sup>4</sup> where medically-oriented collaborating investigators were invited to discuss the biomedical significance of the results of the six projects under way. The reports are available from WHO on request.

1. TRACE ELEMENT ANALYTICAL METHODOLOGY IN AUTOPSY STUDIES

Based on the recommendations of the first WHO meeting of investigators on Trace Elements in Relation to Cardio-Vascular Diseases,<sup>2</sup> a coordinated group of 12 analysts in nine countries (Argentina, Bulgaria, Federal Republic of Germany, Greece, Norway, Philippines, Sweden, United Kingdom, and the United States of America) has commenced work on two separate but related autopsy projects:

1. a study of trace elements (principally Cd, Cr, Cu, Mo<sup>a</sup> and Zn, which are considered to be of primary interest) in relation to coronary heart disease; and
2. a study of Cd and Zn in human kidney and liver in relation to hypertension.

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<sup>a</sup> Now replaced by selenium (see section 2).

The analytical problems posed by these projects include some which test the limits of present day analytical technology. Some of the trace elements of interest occur in tissues in concentrations of only a few parts in  $10^9$  and are therefore extremely difficult to determine with accuracy. The analytical methodology is further complicated by the requirement to analyse many elements in autopsy samples which, for practical reasons, are often limited in size to a gram or so.

The first research coordination meeting of the analysts concerned with this programme was held in Vienna from 19 to 23 February 1973.<sup>3</sup> Its main aims were to review the analytical methodology now in use, to draw up an agreed programme of quality control, and to present the analytical results so far obtained. At the time of the meeting some of the analysts had already been contributing to the programme for more than three years; many, however, did not start to participate in it until 1972. Therefore, there were great disparities in the amounts of work that were reported. At the time of the meeting, some of the analysts were still developing and testing their methods and had no real results to present.

The discussions of this meeting covered the following topics.

#### 1.1 PRE-ANALYSIS PROCEDURES

The first and most important of the pre-analysis procedures, namely sample collection, has for the most part been arranged by WHO and has thus usually been the responsibility of someone other than the analyst himself. Very often this is a person who may not be fully aware of all the hazards of contamination. Despite the inherent dangers of this situation, however, there is no evidence that it has yet led to significant errors in the present programme, except possibly for the analysis of chromium in blood samples.

The instruments used for handling the samples prior to analysis, and for cutting or breaking them into small pieces, may be a potent source of contamination unless

the proper precautions are taken. Special instruments used in the present programme have included plastic forceps; knives of carbon steel, quartz, titanium, polyethylene (stiffened by cooling in liquid nitrogen), and Perspex; and tungsten carbide bits (for grinding the surface and contamination of toe-nails). One technique developed for preparing soft tissue samples was simply to break the lyophilized sample into two or more pieces, and then to rub two such pieces together to form a powder. Some stainless steel instruments have also been used, but generally not when Cr was to be analysed. The danger of contaminating samples with Cr from stainless steel instruments is now well documented. Further work is still required to develop suitable noncontaminating knives of greater acceptability to the pathologists, as well as noncontaminating blood needles.

## 1.2 ANALYSIS

Activation analysis has been used for the majority of the trace elements under investigation. Atomic absorption, however, seems to be generally preferred for a few elements such as Ca, Mg and Cd.

### 1.2.1 Activation analysis: post-irradiation chemistry

Apart from zinc, which can be determined instrumentally, destructive<sup>a</sup> methods are generally necessary for the determination of the remaining elements of interest in this programme (see section 2).

Following thermal neutron activation the samples have generally been wet ashed using established methods. Most of the elements can be dealt with satisfactorily in this way. However, chromium, and to a lesser extent molybdenum, may require special care to ensure that they are in the required valency state for the subsequent radiochemistry. Dry ashing at high temperatures has not

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<sup>a</sup> This term refers to whether or not the sample undergoes chemical treatment after neutron activation.

been much used and is generally not to be recommended unless it can be conclusively demonstrated that none of the elements of interest (e.g. Cd) is thereby lost.

Ion exchange and solvent extraction have been the most commonly adopted radiochemical techniques. Chromium has, in some instances, been determined by distillation. Two automatic radiochemical systems<sup>3</sup> have been developed, based respectively on ion exchange and solvent extraction principles, but to date neither system has been fully tested for use in this programme and neither can yet be identified as the preferred system.

One of the most urgent tasks remaining is to improve and simplify methods for the determination of chromium. As yet, the only method which appears to give reliable results involves distillation and is time-consuming.

#### 1.2.2 Activation analysis: instrumentation and data processing

Two main types of detector are currently used, (i) the NaI(Tl) detector in the form of a right circular cylinder, with or without well, and (ii) the Ge(Li) detector of coaxial design, with or without well, and in one case with a Compton coincidence shield. Generally the NaI(Tl) detectors are used for uncomplicated spectra of one to a few elements, and the Ge(Li) detectors are used for more complex spectra of several elements.

Multichannel analysers of various kinds are in use in all laboratories, but no one type seems to be preferred. The methods in use for the evaluation of gamma-ray spectra vary from simple manual methods to the use of rather sophisticated computer programmes, such as the method of least squares. These calculations have been made on machines ranging in size from desk-top programmable calculators up to computers as large as the IBM 370/165.

### 1.3 NONACTIVATION METHODS

The only nonactivation method that has so far been used in the present programme is atomic absorption analysis in both its flame and flameless varieties. It appears to offer an attractive alternative to neutron activation analysis in the ppm range and, with few modifications, can be used in the  $pp10^9$  range for a restricted number of elements in certain matrices.

Although the meeting was not competent to review in detail all existing trace analysis techniques, the attention of participants was drawn to alternative methods such as anodic stripping voltametry, pulse polarography, differential pulse anodic stripping, emission spectroscopy, gas chromatography, X-ray fluorescence analysis, spark-source mass spectrometry and isotope dilution mass spectrometry.

For the future, the participants endorsed the recommendation of a previous meeting,<sup>2</sup> that the use of more than one method should in fact be encouraged since useful information on the reliability of the analyses may thereby be obtained.

### 1.4 QUALITY CONTROL

IAEA sees one of its principal roles in this programme as being to assist in the observance of proper quality control. To this end a consultation was held in Vienna in December 1972 to define the aims of quality control and to discuss some of the procedures that should be followed. The principal conclusions were:

1. that an overall accuracy and precision within the limits of  $\pm 10\%$  coefficient of variation should (with certain qualifications) be attained in all analyses;
2. that a number of specific recommendations should be followed concerning the treatment of the samples prior to analysis (principally to ensure the absence of contamination); and

3. that appropriate quality control tests should be conducted on a continuing basis by the use of standard analytical reference materials and duplicate samples of various kinds.

All of these recommendations have been accepted by the WHO/IAEA collaborating analysts, though there has not yet been time to implement them in full. However, one intercomparison has already been carried out using the standard reference material bovine liver supplied by the United States National Bureau of Standards (NBS). The results obtained are given in Table 1, and may generally be taken to demonstrate that the presently used analytical methods are working without significant error in the majority of cases. Many of the results reported are acceptable both as regards accuracy and precision (e.g. Cu and Zn). Others appear to have acceptable accuracy but fail as regards precision (e.g. Cd). Unfortunately, chromium, one of the elements of greatest interest in this programme, appears to be amongst the most difficult of all to analyse. For this element in particular the presently used techniques appear to be generally deficient in both accuracy and precision.

A significant aid to future quality control, which will also have a bearing on the statistical analysis of the results, will be the use of a standardized method for reporting results. In future all analytical results submitted by the WHO/IAEA collaborating analysts will be reported according to a standard form supplied by IAEA.<sup>a</sup>

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<sup>a</sup> The form can be obtained from the Medical Applications Section, Department of Research and Isotopes, IAEA, Vienna.

TABLE 1. RESULTS OF AN IAEA-COORDINATED  
 Mean values of the elemental concentrations  
 The coefficients of variation

Laboratory code No. <sup>b</sup> Element	7	1	1	8	9
Ag					
Al			7.0 19%		
As					0.069 11.6%
Br			7.35 4.5%	13.4 6.1%	
Ca					
Cd		0.283 9.2%			0.27 14.8%
Ce					
Cl			1880 4.3%	11663 6.7%	2590 2.7%
Co	0.34 60%	0.225 7.6%			0.26 5.8%
Cr	0.54 64%	0.49 39%	1.57 25%		0.15 33%
Cs	0.044 73%				
Cu				181 1.7%	180 3.9% 194 6.4%
Fe	132 46%	331 7.0%	293 4.1%	229 7.4% 1433 10.2%	285 20.4%
Hg		0.041 24%			
K			9480 3.1%	7537 2.1%	9040 6.9%
Mg			517 11%		674 15.3%
Mn			9.42 4.3%	10.2 3.4%	9.12 7.5%
Mo			2.5 8.0%		4.9 24%
Na			2570 8.6%	1019 3.0%	2250 5.3%
Pb		0.262 5.3%			
Rb	23.4 31%	23.3 11%	19.9 4.0%		
Sb					
Se		1.00 33%	1.23 16%		1.13 8.4%
Sc					
V			<0.06		
W					
Zn		160 12%	139 4.3%	101 0.7%	131 7.3% 134 5.6%

<sup>a</sup> Some of these results are preliminary in nature and are not necessarily typical of the analytical methods that will finally be used.

<sup>b</sup> The list of collaborating laboratories is in reference 3.

INTERCOMPARISON CONDUCTED WITH NBS BOVINE LIVER (SRM 1577)<sup>a</sup>

are quoted as  $\mu\text{g/g}$  of the original dry standard  
are also quoted where known

3	4	11	5	6	10	NBS <sup>c</sup> value
	0.194 31.6%		~0.005			(0.06)
				0.064		(0.055)
			4.3 23%			
			104 29%			(123)
	0.29 16.5%	0.27 9.0%	0.24 25%	0.29 17.3%		0.27 $\pm$ 0.04
			0.013 54%			
	2610 4.2%				2480 5.7%	(2600)
	0.39 7.0%		0.12 13%	0.174		(0.18)
1.3	<0.005	0.051 12.9%	0.13 52%	0.14		
	0.0192 11.7%		0.015 20%			
213	173 10.0%	175 2.4%	194 10%	167		193 $\pm$ 10
	364 7.1%	242 2.4%	240 6%	226		270 $\pm$ 20
				0.020		0.016 $\pm$ 0.002
	8210 10.7%	9875 0.51%	9800 8%		9480 5.2%	9700 $\pm$ 600
				516		(605)
	10.2 11.2%	9.9 3.2%	11 9%	11.5	9.5 5.2%	10.3 $\pm$ 1.0
			2.8 28%	2.97		(3.2)
	2340 6.1%	2425 6.0%	2370 5%		2220 5.0%	2430 $\pm$ 130
0.52						0.34 $\pm$ 0.08
	28.0 6.5%		18.7 9%			18.3 $\pm$ 1.0
	0.0229 52.6%		0.018 50%			
	1.26 9.5%			1.02		1.1 $\pm$ 0.1
	<0.0001		4x10 <sup>-4</sup> 125%			
				0.056		
			~0.005	~0.03		
160	136 7.0%	120 0.42%	135 8%	124 2.4%		130 $\pm$ 10

<sup>c</sup> Values in this column define the 95% confidence interval as quoted by NBS. Values in parentheses not yet certified and are given by NBS for information only.

2. BIOMEDICAL SIGNIFICANCE OF RESULTS OF PROJECTS 1-6

Interim findings were reviewed and considered in relation to other recent evidence pertaining to dietary and environmental factors and cardiovascular diseases.

The various protocols that were issued at the 1971 WHO meeting on Trace Elements in Relation to Cardiovascular Diseases<sup>2</sup> were re-evaluated and found acceptable with only minor modifications.

The priority choice of elements to be studied, which was agreed upon at the 1971 meeting, was also re-evaluated. According to the 1971 choice, which was based on the evidence available at that time on trace element-cardiovascular disease relationships as well as on the capability of available analytical methods, the trace elements of primary interest to be studied were: cadmium, chromium, copper, molybdenum and zinc, plus calcium and magnesium. Iodine, lead, lithium, nickel, selenium and vanadium were considered also of interest, but their importance relative to these studies was of secondary priority.

The present re-evaluation of priorities, as outlined in Table 2 is based mainly on data acquired by WHO/IAEA collaborating investigators during the past two years, as well as on literature data on metabolic pathways and nutritional factors that are controlled by trace elements. In spite of this new evaluation approach, the consensus of the group is practically identical to the 1971 decision. Only selenium was now added to the elements of primary interest, while molybdenum could be considered of secondary interest. Thus, the first priority elements to be studied by all collaborating investigators in relation to cardiovascular diseases are: Cd, Cr, Cu, Se, Zn, plus Ca and Mg. The elements: Li, F, Na, Si, V, Mn, Mo, Sr, I, Hg and Pb could also be studied by the collaborating investigators but are not part of the minimum requirements.

TABLE 2. ELEMENTS FOR WHICH ASSOCIATIONS WITH  
CARDIOVASCULAR DISEASES WERE SUGGESTED\*

	Autopsy and living population studies	Water quality studies	Role in metabolic pathways	Influence through nutrition
Li		+		
F		x		
Na		-		
Mg		+	+	+
Si		x		
Ca		+	+	+
V			+	
Cr	+	x	+	+
Mn			+	
Cu	-	x	+	
Zn	+	x	+	+
Se	x	x	+	+
Mo	x			
Sr		x		
Cd	-	-	-	-
I		x		
Hg	x			
Pb	x	-		

\* This tentative table is based on evidence obtained from WHO/IAEA-coordinated studies as well as from independent studies reported in the literature. Details on such evidence are contained in reference 4. The table does not purport to be exhaustive and should only be considered as a guideline for the identification of those elements which merit further investigation.

+ Allegedly beneficial associations reported on cardiovascular parameters.

- Allegedly harmful associations.

+ - Both beneficial and harmful effects hypothesized.

x Associations have been found, but their biomedical significance has not yet been ascertained.

Blank: elements not studied, or no associations with cardiovascular parameters were found.

Despite considerable progress in the several WHO studies, final interpretation and conclusions must await completion of collection of data and subsequent statistical studies. The evaluation of the six research projects is given below.

2.1 Project 1: TRACE ELEMENTS IN HUMAN TISSUES IN RELATION TO ISCHAEMIC HEART DISEASE

Pioneer work<sup>5</sup> has revealed the particular behaviour of chromium and cadmium in tissues of people from different geographical areas. Generally speaking, populations which are highly prone to atherosclerosis and myocardial infarction, as in the United States of America, showed significantly lower tissue chromium concentration than the less "coronary-prone" African and Asiatic populations.

Other autopsy studies carried out on subjects who died with myocardial infarction or atherosclerosis have revealed that trace element concentration in the infarcted areas of the heart, in the aorta, in the coronary arteries as well as in other tissues and in serum, is different from that of control subjects. Some changes, such as the increase of nickel and manganese levels in serum that were observed in patients with ischaemic heart disease are so sharp and rapid that they might be used as indicators for an early diagnosis of myocardial infarction. These findings were reviewed.<sup>1,2</sup>

The aim of the present WHO/IAEA project is therefore to ascertain whether trace element concentration in autopsy samples of human tissues are related to ischaemic heart disease as a cause of death and whether they differ according to sociocultural and the geochemical environment in which the person had lived, as well as according to the ethnic origin of the subjects.

The subjects under study comprised both males and females of comparable age who:

- (a) died accidentally but were otherwise free from disease (controls); and

(b) died of ischaemic heart disease (atherosclerotic and degenerative heart diseases: International Classification of Diseases, 1965, 410-414).

The samples selected for analysis include: heart (sampled from the anterior wall of the left ventricle), liver (sampled from the superior anterior surface of the right lobe), kidney (samples of cortex and medulla from the lower pole of the left kidney), and toe-nails. In the future head hair should also be collected.

The organizational details, as well as a full description of the results obtained in this study are in reference 4. The protocol followed by the collaborating pathologists is available in WHO and can be obtained on request.<sup>a</sup>

#### Summary of results

Autopsy material related to coronary heart diseases (CHD) have so far been collected in five countries (Czechoslovakia, Israel, Philippines, Sweden and the United States of America). Samples of heart, liver and kidney cortex have been analysed from a total of about 70 subjects, though only for a small proportion of these are the data complete.

The data, when classified according to geographic origin of the subjects, sex, type of tissue, and element analysed, unfortunately yield numbers of cases in each of the above categories which are too small to permit a very detailed statistical analysis. The reason for this relative scarcity of data, as already alluded to, is simply that most of the collaborating laboratories started operating at full or almost full capacity for the analysis of autopsy samples only very recently. Most of them have

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<sup>a</sup> This and all other protocols mentioned in this report, can be obtained from the Cardiovascular Diseases Unit, WHO, Geneva.

devoted more time and effort instead to developing their analytical techniques and to taking first steps to establish adequate quality control.

Unless otherwise mentioned below, the results may be taken to agree satisfactorily with the literature values reported in similar studies.

Results are tabulated in references 3 and 4. The evaluation of these preliminary data may be outlined as follows:

- Cd: The CHD group from Sweden appears to have low levels in heart in comparison with groups from other countries, but otherwise there is a lack of significant differences. Influence, if any, of smoking habits on these results is not known.
- Cr: Reasonable agreement with literature values except for kidney cortex for which the values found in the present project appear low.
- Cu: Data from the Philippines suggest that CHD patients may have elevated levels in heart and kidney cortex. Geographical differences in opposite directions for liver and kidney cortex were found which are difficult to interpret. Data for liver from the Philippines appear low in comparison with literature values, while data for heart samples from Czechoslovakia are high.
- Mo: Some suggestion that CHD patients may have lower levels in liver. Philippine values are generally high, while Swedish values generally low in comparison with literature data.
- Se: Significant geographical differences. Samples from Sweden have low Se concentrations (even in comparison with literature data); samples from other countries have high concentrations.

Zn: In the Philippines, CHD patients have reduced levels in heart and liver but elevated levels in kidney cortex. This pattern, however, is not repeated in the other countries. Geographical differences are numerous and highly significant. Heart-Zn is low in samples from the United States of America in comparison with literature data.

Cd/Zn: Data are too scanty to permit significant conclusions to be drawn.

Results relative to additional elements of secondary importance in relation to cardiovascular diseases showed significant geographical differences, with tissue specimens from Sweden having usually low values for Co, Cs, Fe, Hg, and Rb, while samples from the Philippines, Israel and Czechoslovakia usually have high values.

### Conclusions

General conclusions are not easily drawn at this early stage of the programme. The data so far available seem to indicate various changes in the concentrations of certain elements, e.g. Cu, Mo, and Zn in the heart, liver, and/or kidneys of subjects who died from coronary heart disease, as compared to control subjects. These indications, however, must be studied more deeply before any conclusion can be drawn.

Differences in the concentrations of several trace elements were also found as a function of the geographic origin of the tissues. These differences are not immediately interpretable, but their presence makes continuation of these studies advisable.

These and other possible associations suggested by the data presented here are, unfortunately, based only on very small numbers of samples and analyses and, at the present stage of this work, do not yet allow any final assessment of a possible association of trace elements with ischaemic heart disease.

2.2 Project 2: CADMIUM AND ZINC CONCENTRATIONS IN  
HUMAN KIDNEY AND LIVER IN RELATION TO HYPERTENSION

Cadmium is present in high concentration in human kidneys. Its pattern of tissue distribution, with kidneys having seven to 25 times as much as liver or other tissues is unique; no other trace metal has such marked organ specificity except for pulmonary aluminum which is presumably inhaled in particulate form and fixed locally. In view of this, a biological effect of cadmium seems likely. Cadmium accumulates largely during the first 20 years of life, with infants having less than one-eighth of the adult concentration.

Various human populations show statistically significant differences in renal cadmium concentration, with Caucasoid Americans, Europeans and Asiatics having more than Negroid Africans and less than Mongoloid Asiatics. In a very general way, this pattern is consistent with the epidemiology of hypertension, namely that both renal cadmium and blood pressure are low in Africa south of the Sahara and high in part of eastern Asia, particularly in Japan and Formosa.

Among people dying sudden, accidental deaths, strikingly more renal cadmium was found in hypertensive than in normotensive subjects, but other studies, however, failed to confirm this.

Two studies on air pollution in United States of America cities showed that the cadmium concentration in air was positively correlated with heart disease, hypertension and arteriosclerosis.

Experimentally it was proved that administration of cadmium to animals causes hypertension. All this evidence from previous investigations has been reviewed in detail.<sup>2</sup>

Three mechanisms have been proposed whereby cadmium might induce hypertension:

by local vasoaction;

by antinatriuretic effect in the kidney;

through increased renin activity in cadmium-challenged animals.

However, if cadmium is related to human hypertension, the apparent lack of any excessive hypertension among cadmium workers with their high renal cadmium level must be explained. Likewise, the currently available data on the chronic poisoning allegedly caused by cadmium pollution in northern Japan (itai-itai disease) also gives no indication of excessive hypertension.

Since the present data relating cadmium and human hypertension are only suggestive, a reasonable next step is to implement the currently very sketchy map of mean renal cadmium concentrations in man. Such information would greatly increase our knowledge of the distribution of cadmium as a function of ethnic and geographic factors, and would permit planning of clinical studies to elucidate the biological significance of this element.

An alternative, more direct and hence more conclusive approach for demonstrating a relationship between cadmium and hypertension would be to measure renal cadmium in subjects whose blood pressures were known and to see whether these two parameters could be correlated on an individual basis.

The aim of the present WHO/IAEA project is therefore to determine mean concentrations of renal and hepatic Cd and Zn as functions of the geographical and ethnic origin of the subjects, and for comparison with geographical and individual patterns of blood pressure and hypertension. In the future Selenium will also be analysed.

The subjects under study (males between 30 and 50 years of age) comprise:

(a) hypertensive subjects (mean arterial blood pressure above 160/95 mm Hg), may have died of hypertension or of another cause including hypertension. In either case the diagnosis of hypertension should be definite;

(b) non-hypertensive subjects (mean arterial blood pressure below 140/90 mm Hg), may have died of any cause unrelated to hypertension and should be known not to have been hypertensive at any time.

The specimens include: samples of cortex and medulla from the lower pole of the left kidney, and a sample from the superior anterior surface of the right lobe of the liver.

The rationale and background information on this study are fully described in reference 2. The protocol is available in WHO.<sup>a</sup> Since the scope of the project is limited, it should be possible, within a relatively short period, to obtain some answers which might have significant public health implications.

To date, material from 44 subjects in three geographic locations (Hong Kong, Malmö and St Louis, Missouri) has been analysed.

The number of cases studied is too small to permit a detailed statistical analysis to be made. Only simple statistical tests have therefore been applied.

In relative terms, the Cd concentrations observed in the Swedish material were generally low, while those in the United States of America material were high. Kidneys

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<sup>a</sup> See footnote page 15.

and liver samples from Hong Kong were high for both cadmium and zinc. The latter were also high in comparison with literature values.

The limited data so far available indicate the existence of geographical differences but do not permit any final conclusion to be drawn on the relevance of Cd concentration or of the Cd/Zn ratio in kidneys in relation to hypertension. In view of the scarcity of data the study should be continued in essentially its present form, with zinc and cadmium being measured in all samples, and selenium whenever possible. It is indeed known that Se may counteract the harmful effect of Cd.<sup>6</sup> Collections should be extended to additional locations, at least six in the developing countries of Africa south of the Sahara, and six in non-coastal regions of South-East Asia. These two settings have so far provided the extremes of renal cadmium concentrations in previous studies.<sup>6</sup> Since no comparable renal cadmium data are available for South America or Australia, these regions should also be included in the survey.

### 2.3 Project 3: LIVING POPULATION STUDIES

Living population studies were mainly aimed at studying population groups in developing areas which are still relatively unexposed to modern technology. This information is essential at this time since spreading of technology may soon change the mineral balance that these groups still have with respect to their natural environment. Differences that could be detected in trace element concentrations in these groups as compared to industrialized population groups could then be related with the geographical distribution of cardiovascular disease. This project involves collection and trace element analysis of serum, red blood cells, and toe-nails (or finger-nails) from population groups living in Jamaica, Manila, the Caspian coast of Iran, Papua New Guinea, New Zealand and certain Polynesian islands, and the United States of America. In the future head hair will also be collected. The analytical data are

correlated with blood pressure, blood cholesterol level, prevalence of hypertension, ECG findings, and anthropometric measurements.

Thus far, specimens obtained from a total of 179 subjects were analysed. Additional data on urinary cadmium in 52 Maoris from the Tokelau islands and on urinary Cd, Zn and Cu in 202 white females from New Zealand were also obtained.<sup>a</sup> The number of subjects, particularly when broken down by place, age, sex, blood pressure level, etc., is too small at the present time to permit any definite conclusion to be drawn. However, certain trends can be identified.

The preliminary results obtained so far seem to show trends of higher Cu in serum and red blood cells in high risk subjects (i.e. subjects with hypertension, arteriosclerotic heart disease, and old myocardial infarction) as compared to healthy controls. Serum Zn was also lower in high risk subjects from Manila, although this trend was not confirmed in hypertensive Maoris from Tiki Tiki (New Zealand). On the whole, these findings are in agreement with the ones reported in the literature in similar studies and therefore merit deeper study in the future. Chromium also tends to be lower in the serum of hypertensive subjects and in finger-nails of atherosclerotic subjects, but the number of analyses is still too limited to draw any firm conclusion. The scarcity of the Cr data is mainly due to the difficulties inherent to the analysis of this element, and it was agreed that concentrated efforts should be made to provide the collaborating analysts with suitable procedures for Cr analysis. Of the other elements analysed, Hg, Fe, Co, did not reveal any trend between high risk subjects

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<sup>a</sup> Personal communication by Joan M. McKenzie and Daphne L. Kay, University of Otago, Dunedin (see also: N. Z. Med. J., 78, 68, 1973).

and healthy controls. Selenium is significantly higher in serum of hypertensive males from Tiki Tiki. In one study (Manila) Molybdenum was analysed and found to be lower in serum and higher in red blood cells of high risk subjects as compared to age matched controls.

Results also show higher urinary excretion of Cd in hypertensive females from New Zealand.

Analysis of toe-nails presents special problems when the specimens come from population groups who walk barefoot. Dirt is so deeply ingrained that it requires a special technique to remove it. Finger-nails, however, seem to be less suitable for trace element analysis due to metal contamination, particularly of the first three fingers of the right hand, obviously due to occupational exposure. Future studies should therefore be carried out on toe-nails, both in living populations and at autopsy.

Differences in trace element concentrations were observed in samples coming from different areas, the meaning of which has not been ascertained yet. On the other hand, no trends were evident between age and the concentrations in serum and toe-nails of any of the elements studied.

The protocol, as described in the report of the previous Geneva meeting<sup>2</sup> was approved again and is available from WHO on request.<sup>a</sup>

#### 2.4 Project 4: TRACE ELEMENTS IN NUTRITION<sup>b</sup>

Numerous "risk factors" associated with one or another cardiovascular syndrome have been identified or postulated. Some of them are dietary, others are not.

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<sup>a</sup> See footnote page 15.

<sup>b</sup> See also: Trace Elements in Human Nutrition. Report of a WHO Expert Committee. Wld Hlth Org. techn. Rep. Ser., 1973, No. 532, Geneva

Among the dietary factors, the most extensive evidence relates to the content and the type of dietary lipids, particularly the ratio polyunsaturated/saturated fatty acids, and dietary cholesterol, as well as the effects of these dietary factors on serum lipids and on the process of atherosclerosis.

Rats have been shown to develop hyperlipidaemia when fed on diets containing high levels of refined sucrose,<sup>7</sup> but the epidemiological evidence relating higher cardiovascular death rates to excessive consumption of refined sugars in man is still unconvincing.<sup>8</sup>

The feeding of high amounts of table salt (NaCl) produces hypertension, vascular and renal changes and, in extreme instances, altered levels of serum lipids in rats, but the epidemiological evidence on an association between cardiovascular diseases and high salt intake by normal man is indecisive. It is established, however, that some patients with hypertensive disease benefit from restriction of salt intake.

Epidemiological evidence suggests also an inverse correlation between fluoride intake by man and the appearance of calcification of the abdominal aorta.<sup>9</sup> Recently a hypothesis had been advanced that relates fibre content of the diet to a wide variety of diseases, including cardiovascular diseases.<sup>10,11</sup>

Parameters such as obesity that reflect nutritional status, and living habits such as physical activity, cigarette smoking, and drinking of alcoholic beverages, may have several physiological effects, including alteration in appetite. These effects complicate the epidemiological study of nutrition and other factors in relation to cardiovascular disease.

These examples illustrate the multiplicity of varied and interrelated dietary influences that may contribute to different cardiovascular disease syndromes and underline the need to examine total dietary information, including trace element intake, when attempting to identify relationships.

#### 2.4.1 Mineral elements in lipid and carbohydrate metabolism

Since some workers have suggested that excessive consumption of fats (and, sometimes, of sucrose) in man is associated with an increased risk of atherosclerosis and ischaemic heart disease, it seems worthwhile to summarize here the available evidence which indicates that certain minerals may play a role in the metabolic pathways of lipids and carbohydrates.

Calcium. A moderate increase of Ca in the diet of experimental animals has resulted in lower levels of circulating and organ cholesterol, and also in a change in the fatty acid pattern of various tissue lipids. The implications for man are not clear, but they may be interesting in relation to the well-known association between water hardness and cardiovascular diseases (see section 2.5).

Vanadium. High doses of V outside of the physiological range in man and experimental animals, as well as environmental exposure of man to V have been shown to reduce serum cholesterol.<sup>12</sup> The mechanism is that of an inhibition of cholesterol synthesis beyond the step of mevalonic acid.<sup>13</sup> However, this effect is seen only in young subjects and not in older people with hypercholesterolaemia.

Vanadium deficiency in chicks was shown to increase serum triglycerides,<sup>14</sup> but results on cholesterol are ambiguous. The vanadium requirement of experimental animals appears to be very low and is supplied by contamination from an ordinary environment.

Chromium. Deficiency results in impaired glucose tolerance in laboratory animals.<sup>15</sup> Chromium acts as a co-factor for insulin,<sup>15</sup> and impaired glucose tolerance in malnourished children improves following Cr administration.<sup>16</sup> A quantitative estimate of the daily chromium requirement cannot be given yet because

of the widely varying degree of physiological availability of chromium compounds, whose biochemical characteristics are still poorly known.

Cr deficiency in experimental animals has been shown by one investigator to increase the incidence of aortic plaques and to raise serum cholesterol,<sup>17</sup> but results of experiments measuring the effect of Cr on lipid metabolism in man have been inconclusive.

Manganese. Manganese deficiency results in decreased glucose tolerance in guinea-pigs,<sup>18</sup> whereas administration of this element was shown to exert a strong hypoglycaemic effect in a small number of people.<sup>19</sup> However, no reports of Mn deficiency in man have been published.

Copper and zinc. An imbalance in the Zn to Cu ratio resulted in increased blood levels of cholesterol, triglycerides and phospholipids in experimental rats,<sup>20</sup> while zinc deficiency by itself has been shown to cause impaired glucose tolerance in rats, probably due to a decrease of insulin synthesis.<sup>21</sup> Zinc deficiency in children did not result in consistent changes in glucose tolerance; however, increased sensitivity to insulin was detected in a substantial proportion of the subjects.

#### 2.4.2 The influence of changing dietary practices on trace element intakes by man

With increasing industrialization and affluence of population groups, the following changes of dietary habits have occurred.

- (i) Reduction of physical activity and of total calorie need.
- (ii) Increased availability of processed foods, and increased consumption of such foods away from the home.

- (iii) Increased consumption of partitioned and refined products.
- (iv) Increased production and consumption of "formulated" products.
- (v) Shifts to new, or to so far less abundant, sources of proteins, such as texturized meat, unicellular organisms, seafood and fish.

These changes have already occurred to different degrees in various countries, depending on traditions and preferences of the populations involved. Therefore it is difficult to predict the rate and extent of such changes. In some cases even their direction may reverse.

These changes can have the following impact on trace element intake.

1. Reduction of total food intake results in a reduced intake of trace elements, if the ratio of individual foods remains the same. For example, the concentration of iron in representative diets in the United States of America (about 6 mg/1000 Kcal) would allow a woman to meet her recommended dietary allowance only if she consumed 3000 Kcal. A lower calorie intake, in line with less physical activity, reduces the iron intake to below the desirable level. It is suspected that a similar situation may exist for zinc, and that the intake of this element may become suboptimal with a reduction of food intake.
2. Tin cans or wraps can add significant amounts of tin to the food although lacquer coating of cans has greatly reduced, but apparently not completely eliminated, contamination in all kinds of closures. It appears that other elements as well can be picked up by acidic foods, for example, chromium by canned tomato juice.
3. Although the addition of cobalt compounds to beer has been stopped, and the use of iodate in the bread making process is declining, industrial production may for purely technical reasons add high or even toxic amounts of

certain trace elements. Cooking and other preparation of foods may alter the level and availability of trace elements, particularly the volatile elements. For example, the selenium content of milk powder has been shown to depend on the method of drying.

4. Partitioning and refining can be nutritionally advantageous, particularly when the result is a reduction in the levels of naturally occurring toxic compounds. However, the practice of refining for convenience, taste preference, or appearance alone, should be examined with a view to its nutritional implications. Before a new technological process is introduced its effect on the nutritional quality of the product should be assessed.

The content of several elements analysed in whole wheat was found to decrease with increasing purity of the flour.<sup>22</sup> It was also shown that the content of trace elements in flour decreased with decreasing extraction grade; in some cases, flour may contain only 20% of the concentration originally present in wheat. On the other hand, the concentration of lead did not decline with the degree of processing.

Before an evaluation on the impact of such losses on health can be made, the question of biological availability of the trace elements in wheat and flour must be better known. Whole wheat contains phytate in concentrations that may interfere with the absorption of certain trace elements, but the milling process reduces phytate content in flour. Zinc and iron are believed to be poorly available in whole grain, although this concept has been challenged (for iron) by recent experiments. On the other hand, whole wheat is a good source of available chromium. It appears from these considerations that knowledge of the availability of individual trace elements must be obtained before the impact of the milling process on trace element intake can be assessed.

Sugar (sucrose) has a very low content of trace elements, particularly when expressed on the basis of its calorie content. For chromium, zinc and copper, at least, the concentrations decline with increasing purity, with refined sucrose having the lowest concentrations.<sup>23,24</sup> In addition to being a poor source of chromium, simple sugars (e.g. glucose) cause an acute loss of this element in the tissues<sup>25</sup> thus increasing the demand for chromium from other foods.

Taking our incomplete knowledge of the biological availability of trace elements in foodstuffs into account, it can still be stated that the consumption of refined foods can have a substantial impact on the trace element intake of man if these foods represent a sizeable proportion of the total food intake.

5. The manufacture of meat analogues from vegetable protein is sharply increasing and can be expected to increase even more in the future. Animal meat is not only a good, reliable source of many essential trace elements, but it also increases the biological availability of poorly available iron from other sources. Unless meat analogues are enriched with trace elements to a level present in the product they displace, a decreased intake of these nutrients will occur.

6. The consumption of other formulated products that make a substantial contribution to the energy intake, but not to that of essential micronutrients, is increasing in affluent populations (for example, distilled liquors, soft drinks and certain snack foods). The impact of these products on trace element intake depends on the proportion that they contribute to the total calorie intake and excessive consumption at the expense of more nutritious foods can lead to a decrease of trace element intake. The possible consequences of these trends on the overall content of trace elements in the diet warrants, therefore, continuous examination.

While it can be concluded from these considerations that changing dietary practices have probably decreased man's intake of some essential trace elements, whether there is a relationship between these changes and cardiovascular diseases will be determined only when considerably more data are available.

With this aim WHO started a pilot investigation on trace element analysis of food whereby 83 samples of refined and unrefined sugar, and 127 samples of polished and unpolished rice were collected from 22 countries, with the help of the WHO Regional Advisers in Nutrition. These samples are being analysed for Cr, Zn, Cu, and Cd.

The analytical difficulties concerning Cr are discussed in section 1. The second aim of this pilot study was, therefore, also to assess the chemical properties of Cr at analysis. The results<sup>4,24</sup> show substantial losses of Cr, Zn, and Cu in refined sugar and in polished rice, the largest proportional loss (over 90%) occurring in chromium.

#### 2.4.3 Trace element analyses following nutritional surveys

The participants agreed that nutritional surveys, which should also include assessment of trace element content of food, should be encouraged by WHO. At the present time a very good opportunity for carrying out such a study exists in an extensive nutritional survey that is being carried out along the Caspian coast of Iran, under the auspices of Iranian public health authorities and of the International Agency for Research on Cancer.

This area supports about 4 million people, and a large population-based study is presently proceeding during which three villages of about 250 people each are being analysed in each of 15 diverse ecological regions, during different seasons of the year.

The broad environmental characteristics, the sociological situation and the individual characteristics have been analysed. Although the main object of the survey is to study factors which underlie the geographical distribution of oesophageal cancer in that area, cardiovascular parameters such as blood pressure and ECG were also collected. Material is also being taken for biochemical, haematological and selected genetic marker tests. A special quantitative food consumption survey has been carried out in six selected households of each village, by weighing and measuring the raw material as prepared for cooking for five consecutive days.

Preliminary, unpublished, results<sup>a</sup> of the survey of the first 14 villages have shown dramatic differences in the staple food intake between the semi-desert area to the east - which has one of the highest incidences of oesophageal cancer - where the staple food consists of unleavened home-made bread of high extraction of locally grown wheat, and the low cancer incidence area in the Caspian Rain Belt to the west. In the latter area, locally grown rice provides the bulk of the calories and the incidence of the disease is very low, particularly in women. The changes in bread-to-rice ratio parallel the changes in the incidence of the disease.

Chemical studies of staple foods (mainly bread and rice) in these areas should be carried out in relation to their phytic acid content and in trace element content, particularly zinc. In addition, levels of zinc and of a few other selected trace elements in plasma, hair, and toe-nails should be assessed in population samples in both areas.

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<sup>a</sup> Communicated by Dr H. Shahbazi, Food and Nutrition Institute of Iran, Teheran.

This study, when completed, is expected to yield information on the influence, if any, that certain trace elements in the diet may exert on cardiovascular parameters.

2.5 Project 5: RELATIONSHIPS BETWEEN CARDIOVASCULAR DEATH RATES AND CHEMICAL CHARACTERISTICS OF LOCAL WATER SUPPLIES

There is evidence that cardiovascular death rates are inversely correlated with hardness of drinking water. This trend was detected in England, Canada, Finland, Sweden the United States of America and other countries and has been reviewed.<sup>2,4,26</sup>

The participants noted the general consensus of opinion expressed in the studies published up to the present in this field. There were considerable discrepancies in the published results, but it was considered that inadequacies of data and lack of uniformity of parameters used may have contributed to the discrepancies.

Drinking water is not man's only, or even the major, source of most trace elements. Food is rather more important in this respect. Air may also be an important source of trace elements in some areas, either by direct inhalation, or by contamination of water and food.

In normal circumstances food provides much more of the total daily trace element intake by man than the water or the air. For instance, it was shown that only about 10% of total intake of iodine came from the water supply in goitrous and non-goitrous areas alike. In a study of the concentration of 17 trace elements in the public water supplies located in 44 states of the United States of America, it was calculated that 0.3% to 10.1% of the total daily intake came from the drinking water. Similar conclusions were reached for individuals living in several large cities in the United States of America. Studies of men engaged in strenuous work in Finland consuming 3500 Kcals in the food showed that

consumption of two litres of water per day would provide only 4% of the total intake of iron, 2% of the copper, 1.5% of the manganese and 0.5% of the zinc from the drinking water.<sup>a</sup>

There is nevertheless considerable evidence that the nature and composition of the drinking water can be important to human health. One of the earliest indications of this possibility was the discovery that naturally fluoridated water in certain restricted areas can reduce the incidence of dental caries while at higher levels it can cause mottled enamel and other manifestations of fluorosis in man. As mentioned above, there is also evidence from several countries that the incidence of cardiovascular disease is significantly lower in hard water areas than in areas where the water supplies are soft. Whether this effect on cardiovascular function is due to the presence of some protective factor or factors in the hard water or to some other set of circumstances is unknown. Attempts to identify the nature of the protective effect of hard water or to incriminate a particular deleterious effect of soft water on the cardiovascular system have so far been unsuccessful. It should also be recognized that the influence of waters of differing hardness or chemical composition may be much more complex than the mere quantitative contribution of one or more beneficial trace elements. The comparative effect of hard and soft waters on the mineral composition of foods during cooking is also poorly known. It could be that soft waters remove a much higher proportion of the various trace nutrients from foods in cooking than do hard waters. Hardness may also change the chemical composition of the water standing in the distribution pipes.

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<sup>a</sup> Unpublished data communicated by Dr S. Punsar, Institute of Occupational Health, Helsinki.

Studies are being carried out by WHO on the relationship between hardness (or Ca content) and certain cardiovascular parameters (e.g. blood pressure) in living population groups or at autopsy (extent of atherosclerotic lesion, death rate from hypertension, percentage of myocardial infarction cases).<sup>4</sup> The data show consistent trends of adverse cardiovascular implications as the hardness of local water supplies decreases.

The participants agreed that the WHO-coordinated studies are producing useful information and should therefore be continued so as to yield more extensive data. Besides water hardness, data on Ca, Mg, Na and trace elements should also be collected.

Further mortality studies may be undertaken in some countries and it was thought useful to outline the requirements in a protocol that would facilitate such internationally-coordinated studies.

More studies of water composition and cardiovascular diseases are now needed at the individual level, and studies involving clinical surveys, death certificate and autopsy examinations, such as the 12-town study in England and Wales that is being carried out by the Social Medicine Unit of the Medical Research Council, London,<sup>27</sup> should be encouraged.

Studies in progress in Canada<sup>28</sup> will provide information at the individual level concerning domestic water softening. There is a need to study the mortality experience of whole populations where there has been artificial softening of the community supply, and to study the changes in trace element composition of water caused by the softening process. In these studies the effects of different water softening methods, e.g. base exchange and addition of lime, should be considered.

Besides the studies in progress there are several other situations in which cardiovascular disease data have been or are being collected, and where the addition of data on water quality might give useful information. For instance:

(a) The study on coronary heart disease in seven countries.<sup>29</sup> An attempt was made by one of the WHO collaborating groups to get water analyses from the areas surveyed for cardiovascular parameters in this study, but only a few water data could be obtained. It is likely that ad hoc arrangements would have to be made to collect and analyse the appropriate local water.

(b) The Myocardial Infarction Registers. It is suggested that water hardness data from the 15 or so WHO collaborating centres where these registers are available<sup>a</sup> should be examined to see if there is a reasonable range in hardness between these centres. If so, detailed water analyses, including as many trace elements as possible, could be carried out and related to the incidence of sudden death and other cardiovascular disease parameters being studied. If more registers were being set up, or could be set up in new centres, consideration might be given to differences in local water hardness in choosing the location. This would present a unique opportunity for the study of sudden death and incidence of ischaemic heart disease related to water quality.

The participants also agreed that WHO should ask countries for information on any proposed changes to be made in the water quality of a supply to a large city or area, and should collect or supervise the collection

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<sup>a</sup> A WHO report on the progress of the registers is being prepared.

of relevant statistics before and after the change. Prospective studies of cardiovascular disease parameters could then be set up. It is known that changes in water supply are proposed for some towns. A report by a WHO Temporary Adviser<sup>a</sup> makes several suggestions in this respect.

The possibility of initiating intervention studies was favourably considered. It is of course realized that it would be difficult to get such a proposal accepted before more evidence is available from studies of the effects of changes in water hardness on cardiovascular health. However, if any national authority decided to discontinue artificial softening, this would provide an opportunity to obtain the information still needed. If instead a particular local community wishes to increase the hardness of its drinking water because of the possibility of a beneficial effect upon the age-specific death rate, every effort should be made to obtain the information necessary to evaluate the results of such action. This of course includes the collection of baseline information.

All these water studies should be supplemented with information obtained from other sources, e.g.:

(a) studies on individual trace element content of food as compared to that of drinking water; factors influencing absorption of trace elements, e.g. interrelationships between elements, the effect of bulk ions, the effect of phytates present in food, etc.;

(b) exchange of information with national and international agencies, particularly in localities where water studies are going on. Information about elements which may be absorbed from the air would be specially important, e.g. cadmium, lead, fluoride, etc.;

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<sup>a</sup> See footnote page 15.

## (c) geochemical maps.

Studies should also be undertaken on the effect upon the nutrient composition of foods of boiling in hard and soft waters from areas where the incidence of cardiovascular disease is known to be low and to be high, respectively.

Since the studies on the trace element composition of water in relation to cardiovascular diseases are still at an exploratory stage, the largest possible number of trace elements should be analysed. It is suggested that analysis of composite pooled samples might enable more elements to be studied more economically. However, where the number of elements to be studied must be limited for reasons such as cost or limited laboratory facilities, preference should be given to those listed in Table 2.

The protocol of the previous meeting of investigators on trace elements in relation to cardiovascular diseases<sup>2</sup> was found suitable and no major changes were made. This protocol is also available on request.<sup>a</sup>

#### 2.6 Project 6: GEOCHEMICALLY ORIENTED STUDIES IN RELATION TO CARDIOVASCULAR DISEASES

The role of trace elements in human health is a matter of growing concern to biomedical scientists since there is evidence of a relationship between the chemical characteristics of the natural environment and the occurrence of various diseases.<sup>30</sup>

There are two reasons for suspecting that the chemical composition of the environment may be involved in the etiology of cardiovascular diseases. The first

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<sup>a</sup> See footnote page 15.

is based on the observation, reported by several authors in different countries, that an inverse correlation exists between cardiovascular mortality rates and the hardness of drinking water. This has been discussed above. Second, it has been reported that in some countries the prevalence of cardiovascular and cerebrovascular diseases may be associated with the type of geological substratum.<sup>30,31</sup>

In studying the relationships between environments and cardiovascular diseases, two approaches are possible:

- (a) field studies and analyses of local rock and soil samples, associated with local epidemiological surveys;
- (b) use of geological and soil maps for comparison with cardiovascular disease mortality maps, when available.

The first approach is more precise, but perhaps is not feasible on a large scale at this time; the second approach may indicate contrasting situations but calls for great caution in the interpretation of any apparent association.

Studies in mining areas and in areas neighbouring smelting plants would also be of interest in relation to CVD and other chronic diseases, due to the abnormally high concentration of some elements. The possibility of carrying out such studies in the future should be kept in mind, since they may highlight particular disease patterns associated with the presence of particular elements in certain environments.

While WHO studies on the relationships between water quality and cardiovascular diseases are well advanced (see project 5), other studies dealing with the chemical composition of rocks and soils are, however, only at the very beginning and no data are available yet.

Two such surveys are at present being carried out: along the Caspian coast of Iran (in association with the survey on oesophageal cancer, mentioned above) and in the Tokelau islands in Polynesia.

2.6.1 Influence of chemical composition of soil and of agricultural practices on human mineral balance

Plant materials provide a major source of trace elements to man. The concentration of trace elements in plants, and the consequent levels of dietary intake by man are influenced by the type of soil on which the plants are produced and the type and quantity of fertilizer applied, and by the species and variety of plants grown. Foods of animal origin, notably meat, milk and eggs are, in turn, influenced in their trace element composition by the composition of the plants that the animals consume and the mineral supplements supplied. Changes in fertilizer practice or in the types of plants grown, as a result of advances in agronomy and plant breeding, and in animal husbandry as a result of advances in nutritional knowledge, can therefore influence the trace element composition of the foods that comprise man's daily diet.

Trace element deficiencies and toxicities are more difficult to relate to the soil and to agricultural practices in man than they are in farm stock because man is at the end of the food chain, and modern human dietaries in urbanized communities contain a wide variety of foods obtained from ever widening geographical sources. Regional differences in the intakes of certain trace elements nevertheless exist. The incidence of goitre in relation to soil, plant and water levels of iodine provides the most convincing evidence of a relationship between the composition of rocks, soils and plants and human disease. Regional differences in serum zinc levels in human adults have also been demonstrated in the United States of America, which presumably reflect regional differences in dietary zinc intakes.

It is important to recognize that developments in agricultural technology designed primarily to increase the yield of food crops and animal products can affect the trace element content of these foods in ways which usually cannot be predicted and which therefore require continuous investigation if human health and nutrition are to be safeguarded. For example, a new high yielding strain of rye grass was developed in New Zealand which was subsequently found to contain only one-tenth of the iodine concentration of its parents, whether grown on iodine-low or iodine-high soils. On soils marginal in iodine, the use of this plant could therefore precipitate goitre incidence in cattle and in children consuming the milk from these cattle. In Sweden the use of methyl-mercury compounds as a seed-dressing agent was found to lead to potentially toxic accumulations of methyl-mercury in the eggs of wild fowl and edible flesh of animals consuming the grain. The use of methyl-mercury compounds was discontinued with a consequent marked fall in the levels of methyl-mercury in the foods of that country. In Tasmania the use of iodophor antiseptics in dairies was found to increase the level of iodine in the milk five to 15-fold. This increase, together with the use of iodate in place of bromate as a dough conditioner in bread and of alginates in food, markedly raised the iodine intakes of the population. This resulted in a significant reduction in endemic goitre but was accompanied by a 20-fold increase in the incidence of thyrotoxicosis, mainly in older women with a history of pre-existing goitre.

Although these few examples do not bear any relationship to cardiovascular diseases, they provide clear evidence that changes in agricultural practice can change trace element intakes by man in ways which may be hazardous to human health. It is essential therefore that all changes in agricultural practice should be monitored to ensure that safe and adequate trace element concentrations in foods are maintained.

Recommended approaches for internationally coordinated investigations on trace elements in relation to cardiovascular diseases

The reports of the joint WHO/IAEA meetings on Trace Elements in Relation to Cardiovascular Diseases<sup>2,4</sup> contain specific recommendations intended for the collaborating investigators to help them plan their future research in this field. These operational recommendations will therefore not be repeated here. Recommendations of a more general nature, outlining fields of research and strategies for international coordination are, instead, given here. The items that the participants to the above-mentioned meetings thought to be more important are:

1. The collection of material, evaluation of the analytical results, and dissemination of information on trace element research in relation to cardiovascular diseases should be continued on an internationally-coordinated basis.
2. Contacts should be established among large national or international cardiovascular-oriented surveys that are already going on or are about to be started, as well as among national and international agencies dealing with the establishment of tissue banks for monitoring possible environmentally-induced changes in tissues mineral composition.
3. On account of the spread of technology and of the increasing man-made alterations of the environment, investigations should be carried out to determine the relationships between environmental trace elements (in food, water, soil and air) and tissue trace elements, and between tissue trace elements and disease.
4. Since evidence is accumulating which indicates an apparent association between cardiovascular mortality and water softness, it is considered advisable that national authorities pay increased attention to research in this field. If changes in the quality of water

supplied to a large city or area are planned, relevant cardiovascular statistics before and after the change should be collected to evaluate the health-related results of such action, if any. If a community wishes to harden its water supply for the possibility that this may have a beneficial effect upon cardiovascular death rates, every appropriate effort should be made to obtain information and to evaluate the results of such action. New studies should also be initiated to assess the effect of cooking food in hard and soft waters on the mineral composition of the food itself. The water-cardiovascular disease studies should be carried out both on an ecological basis and at the individual level.

5. Collaboration should be established among appropriate national and international agencies in monitoring changes in agricultural practices to ensure that safe and adequate trace element concentrations in food are maintained.

6. Reference centres should be established for coordinating the collection of material and the trace element analyses in each of the major fields of the trace element-cardiovascular diseases investigations (pathology, epidemiology, water hardness studies, geochemical surveys, food analyses, etc.).

7. It is advisable that WHO act as a clearing house for exchange of information among those working in the above fields.

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