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ABSTRACT

The concentration of cadmium in liver hepatoma and in normal liver in filipinos was determined by atomic absorption spectrophotometry. Using NBS Bovine Liver (SRM 1577) as reference material, a value of 0.28 $\pm$ 0.025 ug/g dry weight was obtained for cadmium which is close to the certified NBS value of 0.27 $\pm$ 0.04 ug/g. The mean percentage recovery for cadmium determination by AAS was 98.38%. A mean value of 2.14 $\pm$ 1.58 ug Cd/g liver hepatoma was observed for the 12 cases investigated, showing decreased cadmium levels in the cancerous liver compared to the mean value of 12.62 $\pm$ 3.97 ug Cd/g observed for normal liver obtained from 10 cases of accidental deaths. The values are expressed on a dry weight basis.

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I. INTRODUCTION

Cancer of the liver is one of the malignancies with worst prognosis. Compared to other countries in the world, the Philippines is among those with higher rarios of primary liver cancer to malignancies in the country. Barrera et al. reported that this constitutes 17% of all malignancies in the country (2). Of 48,900 necropsies studied by Edmondsen and Steiner in California, greater frequency of hepatoma among Filipinos and Japanese compared to other ethnic groups was observed (6). In Hawaii, Filipino males were reported to comprise 40% of hepatoma cases within a ten year period (13). Wilbur et al. also reported a high frequency in males and the higher incidence of this disease among Asiatic peoples (18).

In our country, Bulatao-Jayme et al. stated that the incidence of hepatoma is regional, indicating its public health significance (5). Their findings on aflatoxin agree with those of other countries which showed a close relationship between high aflatoxin exposure and a corresponding high incidence of primary liver cancer (13, 8).

Other local factors in the environment may also be associated with the etiology of hepatoma. The role of certain trace elements in primary liver cancer is being investigated at the Philippine Atomic Research Center. It is known that while some elements are considered essential, others are regarded as interesting contaminants. Suggestive but not completely convincing evidence indicate that some of the metals falling under the letter category like cadmium and vanadium may be eventually shown to be essential. Schwartz' review of the role trace elements in cancer makes mention of the often conflicting reports of different authors (15). Earlier investigators encountered technical problems in the determination of trace metals. However, with the establihment of satisfactory techniques for the collection and processing of specimens free from contamination, and the ascertainment of methodologies for neutron activation analysis and atomic absorption spectroscopy for the determination of low concentrations, trace metals in human tissues can now be investigated in various laboratories throughout the world on an intercomparison level, using a biological standard as reference material.

Cadmium is reported to be the most "social" of all heavy metals. It exists both as a natural and industrial contaminant in the environment. It seems to be a normal constituent of plants and can be absorbed through both leaves and roots. The redistribution of this element as a result of technological progress is of great concern especially in industrialized countries. Possible sources of cadmium contamination include industrially polluted rivers, galvanized pipes, cosmetics, cigarettes, phosphate fertilizers and the processing of foods. Animal studies show that cadmium and its compounds can give rise to malignant tumors at the site of injection in rats. Its toxic effecfs include structural and functional damage, induced deficiencies of Zn^{++}, Cu^{++}, Fe^{++} and/or Fe^{+++} and anemia. In humans it is found mostly in the kidney and liver and has been linked to kidney and liver and has been linked to kidney ailments, hypertension and other cardiovasdular conditions. Cadmium from contaminated food, together with other factors as ageing and Ca^{++} deficiency through pregnancy and lactation were found to contribute to the etiology of "itai-itai", a disease characterized by renal tubular dysfunction, proteinuria and osteomalacia.

Cadmium has a long biological half life in mammalian tissues. In man, estimated values vary between 17 and 33 years in the kidney and 7 years in the liver (17). The Panel on Hazardous Trace Substances considered the accumulation of cadmium among people in the general population as substantial while knowledge of margins of safety, other than their adverse renal effects, is inadequare (7). Roe however, believes that the environmental concentrations of cadmium do not constitute a carcinogenic risk for man (9).

This paper presents some data on cadmium concentration in primary liver cancer among Filipinos. The investigation of this toxic metal in cancerous liver (hepatoma) was initiated at the Philippine Atomic Research Center since cadmium reportedly concentrates in the liver and has a long biological half life in this organ. This investigation is part of the study of the concentrations of certain trace elements in hepatoma and in normal liver to find out whether there is any causal relationship between the elements under study and primary liver cancer.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Samples

Liver tissues from apparently normal individuals who met accidental deaths were obtained from autopsy cases of the National Bureau of Investigation in Manila. The liver specimen consisted of a section from the superior anterior surface of the right lobe of the liver after removal of the capsule with the use of a glass knife.

Pathological liver tissues from hepatoma autopsy cases were obtained from the Philippine General Hospital, the Veterans Memorail Hospital and the Rizal Provincial Hospital.

The excision of the tissues were performed within 12 hours after death by skilled pathologists from the NBI and the above mentioned hospitals. Extreme care was observed in handling the samples to prevent trace elemental contamination. The tissues were kept in the freezer prior to analysis. The frozen samples were divided into suitable pieces, and weighed. These were freeze-dried and stored in a cool dry place.

2.2 Atomic Absorption Spectrophotometry

The lyophilized tissues were pulverized and 0.5 gm samples were analyzed by the direct method of Marks et al. (12), using standard additions. The concentrations of cadmium in the resulting solutions were determined by atomic absorption spectrophotometry using the Varian Techtron 1200 operated at an expansion setting of 2 and a readout mode of integration 3. The flame used was air-acetylene.

Bovine Liver (SRM 1577), a biological standard from the National Bureau of Standards was also analyzed. Recovery studies for normal liver were accomplished.

3. RESULTS AND DISCUSSIONS

The sensitivity of the Varian Atomic Absorption Spectrophotometer for cadmium was 0.01 ug/cc standard solution and the detection limit was 0.0006 ug/cc. The mean percentage recovery was 98.38% using cadmium concentrations of 0.05 and 0.10 ppm added to human liver tissue (Table I).

The Bovine Liver reference material analyzed by AAS Direct Method showed a cadmium concentration of 0.28 $\pm$ 0.25 ug/g dry weight (Table II). This value compared closely with the certified NBS value of 0.27 $\pm$ 0.04 ug/g. Good precision was indicated by the small mean deviation obtained for the four trials made.

Liver autopsy specimens from ten apparently normal subjects who died accidentally were found to contain a mean concentration of 12.62 ug per gram dry weight (Table III). This value lies within the range reported by Ishiyaki et al. and by Koch et al. (10, 11).

The cadmium levels in cancerous liver obtained from the Philippine General Hospital (4 cases), Veterans Memorial Hospital (6 cases) and the Rizal Provincial Hospital (2 cases) are shown in Table IV. The age range of the subjects were from 33 yrs. to 69 yrs. eleven of whom were males and only one was a female. It was noted that 59% of the primary liver cancer cases had associated cirrhosis, 44% had been alcoholic drinkers and 16.7% had been smokers. The mean water content in liver hepatoma was greater than that of the normal liver (75.60% vs. 67.61% respectively). This is characteristic of cancerous tissue. For purposes of comparison, the results for both cancerous and normal liver are expressed on a dry weight basis. The mean concentration of cadmium in liver hepatoma was found to be very much lower compared to cadmium levels in the normal liver (2.14 ug/g vs. 12.62 ug/g, respectively). Figure 1 represents the scattergrams of cadmium in the normal liver and in primary cancer liver.

The decreased cadmium values seem to be secondary to a metabolic disorder involving certain factor(s). Cadmium metabolism is affected by the relative concentrations of zinc, copper and other metals. Preliminary data observed by the authors on other trace elements in liver hepatoma show decreased levels for zinc and molybdenum and elevated levels for copper (1), indicating the existence of metal imbalance in the cancerous liver, implying a causal factor which interferes with normal physiological processes. Copper is antagonistic to cadmium and has a limiting effect on molybdenum retention which is aggravated in the presence of inorganic sulfate in the diet. Webb suggested that the antagonism between Cd^{++} or Zn^{++} may involve competition for transport sites (17). Inadequate amounts of zinc can lead to successful competition of the enzyme ligand by the toxic metal. A high cereal intake can reduce the availability of zinc and iron with the formation of insoluble complexes of the metals with phosphates and phytates. Decreased levels may also be due to poor absorption or increased excretion of the element.

The interactions of trace metals are complex. Since no essential function in biological tissue is known so far for cadmium, the decreased levels observed for this element in cancerous liver from the 12 hepatoma cases investigated indicate that this trace metals may not be directly associated with the etiology of the disease. However, the mechanisms of various interactions between trace metals in liver hepatoma is not yet well understood and more research along this line is needed.

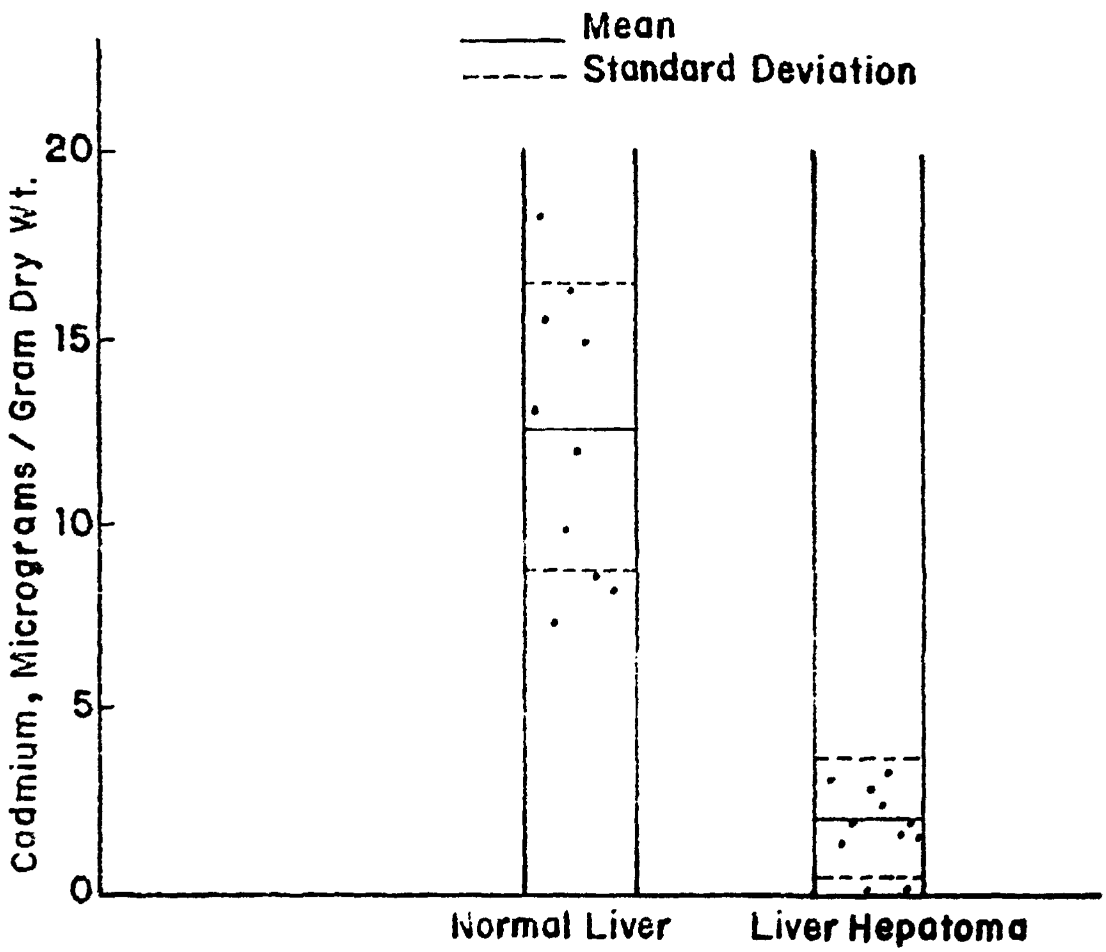

Fig. 1 Scattergrams of cadmium in normal livers and in liver hepatoma.

Table I. Recovery Studies of Cadmium

Method: AAS

Sample weight: 0.5 gm Human Liver (freeze-dried)

Liver tissue		Cadmium added			
		0.05 ppm		0.10 ppm	
Spl. No.	Cd, ug/ml	Cd found ug/ml	% Recovery	Cd found ug/ml	% Recovery
12	0.1350	0.0525	106.0	0.0983	98.3
14	0.2000	0.0440	88.8	6.1000	100.0
17	0.1619	0.0501	100.2	0.0970	97.0
	Mean		98.33		98.43
Total Mean (0.05 and 0.10)			98.38%		
		Range	88 - 106%		

Table II. Analysis of NBS Bovine Liver (SRM 1577)

Method: AAS

Sample Weight: 2 gms. (freeze-dried)

Run No.	Cadmium ug/g dry weight
1	0.28
2	0.34
3	0.26
4	0.26
Mean	0.28
M.D.	0.025

Table III. Cadmium Concentration in Liver of Apparently Normal Subjects.
Method: AAS
Sample weight: 0.5 gm (freeze-dried)

Code No.	Sex	Age Yrs.	% Solid	Cd ug/g tissue (ppm) Dry weight basis
12	M	26	27.78	13.22
13	M	66	34.59	18.42
14	M	70	34.23	16.58
15	M	43	24.04	7.34
16	M	50	34.84	9.96
17	M	48	31.39	16.63
18	M	48	34.14	12.19
36	M	35	31.36	15.00
37	M	35	33.45	8.64
38	M	35	38.08	8.21
Mean		45.60	32.39	12.62
S.D.		14.03	4.00	3.97
Range		26 - 66	24.04 - 38.08	7.34 - 8.42

Table IV. Cadmium Levels in Liver Hepatoma
Method: AAS
Sample weight: 0.5 gm. (freeze-dried)

Code No.	Sex	Age Yrs.	% Solid	Cd ug/g tissue (ppm) Dry weight basis
R-1	F	41	26.85	3.03
R-2	M	34	24.07	1.32
P-3	M	52	32.13	5.90
P-4	M	68	19.06	1.97
P-5	M	41	18.47	N.D.*
P-6	M	33	20.49	2.69
V-2	M	57	31.72	2.23
V-3	M	63	21.74	3.31
V-5	M	49	25.07	1.53
V-6	M	55	23.82	N.D.*
V-7	M	69	28.74	2.01
V-8	M	59	19.67	1.68
Mean		51.75	24.32	2.14
S.D.		19.06	4.74	1.58
Range		33 - 69	18.47 - 32.13	0 - 5.90

* N.D. - Not detectable

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