Intracellular Distribution of Trace Elements in Liver Tissue.* (26546)

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Determinations of metals in whole liver tissue have revealed a general pattern of concentrations(1-3). Attempts to correlate abnormal conditions and changes in trace element concentration have shown promise, but the variations among samples require a detailed statistical study of large numbers of organs(4-10). The present study is an attempt to determine the intracellular distribution of selected metals found in liver tissue fractionated by differential centrifugation. Knowledge of the intracellular location of a trace element is of value in determining its function and alterations in that distribution might be characteristic of pathological states.

Methods. The usual precautions applicable to trace element analyses to minimize contamination were observed. Sucrose (0.25 M) was passed twice through an IR 120 Amberlite resin column to remove metallic impurities. Qualitative spectrographic examination for the elements of interest revealed only a trace of copper. Blank determinations were run concurrently with samples. The wet ashing technic and spectrographic method employed was a modification of those reported elsewhere(11). Nitrogen was determined in an aliquot of the samples after the method of Ma and Zuazaga(12).

Animals were acclimatized for 2 weeks in similar, individual cages and fed Purina Lab Chow from a single batch. Human liver specimens were secured in the operating room and immediately fractionated. There was no trace element evaluation of diets or medications received by the patients.

Procedure. Five groups of 5 normal young male rats of Wistar strain were used to determine mean values. The fifth group was also a control for 5 rats into which Walker rat carcinosarcoma 256 had been transplanted. Animals were killed by decapitation, livers were removed immediately and rinsed with cold, 0.25 M Sucrose. All subsequent operations were performed at a temperature of 0-5°C. Livers were finely minced in the medium with stainless steel scissors and homogenized with a plastic and glass Potter-Elvehjem type homogenizer. An aliquot was taken for analysis as the homogenate sample.

The fractionation procedure followed was essentially that of Hogeboom, Schneider and Pallade(13). The homogenate was centrifuged in a Spinco refrigerated centrifuge for 10 minutes at 500 × g and separated by decantation. The sediment was rehomogenized with 4-5 volumes of sucrose and again centrifuged for 10 minutes at 500 × g. The supernatants were combined. The sediment was collected with distilled water and frozen for later analysis, as the "nuclear" fraction. This fraction also contained some unbroken cells, blood cells, connective tissue and mitochondria in addition to free nuclei.

The combined supernatants from the first step were centrifuged for 10 minutes at 7,000 × g, decanted, and sediment washed with sucrose, collected and analyzed as the "mitochondria" fraction.

The remaining supernatant portion was further centrifuged for 60 minutes at 105,000 × g and sediment collected as the "microsome" fraction.

For analysis, frozen samples were thawed, homogenized and aliquots taken for nitrogen determination. The principle portions of samples were wet ashed in covered beakers with the exception of supernatant samples. The large amount of sucrose in supernatant samples made wet ashing of this fraction impractical. Instead, samples were made acid ($pH<1$) with concentrated hydrochloric acid, precipitated proteins filtered off and metals precipitated directly from the filtrate. The ash from the protein precipitate was examined spectrographically and found to be

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TABLE I. Distribution of Trace Elements in Normal Rat Liver Fractions. Average results of 5 groups with standard errors of means.

	Results expressed as % of reconstituted total						
	N	Fe	Zn	Cu	Mo	Mn	Cr
Nuclei	37 ± .6*	37 ± 4.9	31 ± 3.6	30 ± 7.2	28 ± 8.1	37 ± 4.9	49 ± 9.9
Mitochondria	18 ± .6	10.7 ± 1.2	9.3 ± 1.3	17 ± 3.0	38 ± 5.3	39 ± 3.8	14 ± 3.0
Microsomes	17 ± .4	21 ± 1.6	10.6 ± .6	4.8 ± .8	6.1 ± 1.4	14 ± 2.2	14 ± 2.6
Supernatant	28 ± .7	32 ± 7.2	49 ± 4.8	48 ± 8.3	27 ± 7.1	9.7 ± 2.8	23 ± 10
	Results expressed as μg of element per mg of nitrogen						
	Fe	Zn	Cu	Mo	Mn	Cr	
Nuclei	2.6 ± .27 *	.77 ± .062	.25 ± .12	.011 ± .0006	.029 ± .0079	.0041 ± .00090	
Mitochondria	1.7 ± .071	.42 ± .030	.19 ± .046	.025 ± .0024	.067 ± .031	.0029 ± .00076	
Microsomes	3.6 ± .83	.65 ± .087	.061 ± .020	.0050 ± .00036	.032 ± .017	.0026 ± .00047	
Supernatant	5.0 ± 2.9	2.0 ± .43	.39 ± .065	.019 ± .00061	.0072 ± .0034	.0040 ± .0019	
Reconstituted total	3.3 ± .84	1.05 ± .17	.25 ± .040	.015 ± .0009	.037 ± .015	.0036 ± .00024	

* ± S.E.

free from detectable amounts of metals of interest.

Results. Mean values for normal rat liver fractions are presented in Table I and include standard errors obtained for the 5 groups of normal rat livers. Values for nitrogen and metals present in determinable quantities are shown both in terms of percent of element in the "reconstituted total" and as micrograms of element per milligram of nitrogen. Metals sought but not found in determinable quantities were Ag, Co, Ni, Pb, Sn, Ti, and V.

Distribution of the metals studied is reproducible and consistent with similar work reported by others(14).

When compared to nitrogen, the "nuclear" fraction is at or near whole tissue level for all of the elements determined with the possible exception of chromium which appears to be concentrated in this fraction. The heterogeneity of this fraction may account in part for this similarity to whole tissue values.

The mitochondrial fraction contains molybdenum and manganese in greater than whole tissue concentration when compared to nitrogen. Copper occurs in approximately equal ratio, and the other elements in less than whole tissue values. Several enzymes have been found to be concentrated in this fraction(15-17). Since the enzymes known to contain molybdenum have been reported to be found mostly in the supernatant fraction (20), the concentration of this element in the mitochodrial fraction suggests the possibility of other molybdenum containing enzymes.

The microsomal fraction contains less than whole tissue concentrations of the metals studied except iron which accounts for the pigmentation of this fraction(18).

The supernatant fluid contains iron, zinc, copper and molybdenum in concentrations greater than whole liver tissue when compared to nitrogen. Many of the metal containing enzymes have been found to be present in this fraction(17,19,20) and the enrichment of these metals would be expected.

Comparison of trace element content of normal and tumor bearing animals. Two previous experiments on large groups of animals with transplanted Walker rat carcinosarcoma 256 tumor disclosed no discernible differences of trace element content in the livers of control and tumor-bearing rats† To determine whether the trace element distribution pattern in rat liver cell fractions might be altered even though whole liver values were unchanged, the tumor was transplanted into the axilla of 5 rats and liver cell fractionation performed after the tumors had reached considerable size about 7 days later. Table II presents the element nitrogen ratios of treated and control animals. No differences in distribution of the metals determined are revealed by comparing the 2 groups.

† Personal data accumulated in U.S.P.H.S. Progress Reports (C-1121).

TABLE II. Distribution of Trace Elements in Rat Liver Fractions. Results expressed as μg of element per mg of nitrogen.

Avg results of 5 normal control animals	Fe	Zn	Cu	Mo	Mn	Cr
Nuclei	3.1	.86	.063	.010	.044	.0024
Mitochondria	1.8	.48	.11	.021	.11	.0015
Microsomes	5.0	.81	.025	.0046	.065	.0034
Supernatant	10.6	2.9	.52	.031	.014	.0079
Reconstituted whole liver	5.3	1.4	.20	.017	.051	.0040
					Total liver sample, 58.0 g	

Avg results of 5 tumor-bearing animals	Fe	Zn	Cu	Mo	Mn	Cr
Nuclei	3.2	.64	.071	.0088	.046	.0034
Mitochondria	1.8	.44	.13	.019	.13	.018
Microsomes	4.8	.71	.024	.0052	.026	.0031
Supernatant	8.0	2.8	.43	.023	.015	.0068
Reconstituted whole liver	4.5	1.2	.20	.014	.049	.0067
					Total liver sample, 54.5 g	

In comparing the controls in Table II with 5 groups of control animals in Table I, there is good agreement of values except for manganese in the supernatant fractions and manganese and copper in the microsomes. These apparent differences are believed to represent variations among groups of normal animals as shown in the standard error values in Table I.

Determinations of trace elements in human liver has revealed a wide range of values with different conditions(8). Fractionation of 2 specimens of human liver obtained at surgical operations demonstrate a difference in intracellular distribution as well as in total trace element concentration. The small sample size of one specimen casts some doubt on the reliability of the results. Table III presents values obtained in a human liver fraction obtained at surgery from a patient with carcinoma of the colon. The liver was grossly and microscopically free of disease, but a significant anemia was present prior to operation and undoubtedly accounts for the marked decrease of iron in all fractions. Table IV indicates intracellular distribution of trace metals in a patient with chronic cholecystitis. This liver was histologically normal. There are apparent differences which could reflect disease processes. The paucity of specimens as well as sample size prevents any conclusions, but the results suggest that cellular fractions may reveal more trace element abnormalities than are evident in whole liver analyses.

TABLE III. Distribution of Trace Elements in Human Liver Fractions. Diagnosis: Carcinoma of colon (liver grossly normal).

Results expressed as % of reconstituted total	N	Fe	Zn	Cu	Mo	Mn	Ni	Sn
Nuclei	17	37	25	69	8.1	30	56	62
Mitochondria	19	21	5.2	9.4	27	36	8.8	15
Microsomes	21	21	6.0	6.9	23	14	12	7.0
Supernatant	43	20	64	15	42	20	23	15
						Sample size, 4.1 g		

Results expressed as μg of element/mg of nitrogen	Fe	Zn	Cu	Mo	Mn	Ni	Sn
Nuclei	3.6	3.9	6.7	.011	.091	.39	.091
Mitochondria	1.8	.70	.81	.032	.097	.054	.020
Microsomes	1.6	.71	.52	.024	.034	.067	.0081
Supernatant	.76	3.8	.57	.022	.024	.062	.0088
Rec'nstit'd whole liver	1.6	2.6	1.6	.023	.051	.12	.025

TABLE IV. Distribution of Trace Elements in Human Liver Fractions. Diagnosis: Chronic cholecystitis.

Results expressed as % of reconstituted total	N	Fe	Zn	Cu	Mo	Mn	Cr	Ni
Nuclei	31	30	25	34	26	24	50	81
Mitochondria	11	9.7	5.3	7.4	14	25	10	4.7
Microsomes	16	18	8.8	4.4	11	8.7	12	0
Supernatant	42	42	61	54	49	43	28	14
						Sample size, 24 g		

Results expressed as μg of element/mg of nitrogen	Fe	Zn	Cu	Mo	Mn	Cr	Ni
Nuclei	46	2.5	.57	.011	.038	.065	.12
Mitochondria	41	1.5	.34	.017	.11	.036	.020
Supernatant	48	4.5	.68	.017	.051	.027	.016
Reconstituted whole liver	47	3.1	.52	.014	.050	.040	.047

Summary. Intracellular distribution of 6 metals in liver tissue has been determined for 5 groups of normal rats. Introduction of the Walker rat carcinosarcoma 256 did not change the total level or distribution pattern for the metals determined between treated

and control rats. Two specimens of human liver tissue were fractionated and trace metal distribution determined.

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Patterns of Increase and Spread of Polioviruses in Monkeys after Inoculation into Traumatized Tissue.* (26547)

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If poliomyelitis virus in adequate concentration is inoculated into healing skin wounds of cynomolgus monkeys, an increase of virus in the local site and in other parts of the body follows(1). Data are presented here to show the patterns of increase and spread of 4 different strains of type 1 virus chosen principally for characteristic virulence as measured experimentally or indicated epidemiologically.

Virus strains. Each of the 4 strains of virus used in this study had distinct characteristics of especial interest. Two strains, Mahoney and LSc, had been modified during prolonged passage in tissue culture in other laboratories. The other 2, Chesterfield Inlet and Iberia, were essentially "field" strains; that is, they had undergone only 2 passages in the laboratory. All were identified as type 1 poliovirus by neutralization with homotypic antiserum and by the fact that monkeys infected with each strain developed antibodies to type 1 poliovirus of the Mahoney strain.

The Chesterfield Inlet strain was obtained from feces of a patient who developed poliomyelitis during the epidemic in this arctic community in 1949. This was one of the world's most violent epidemics(2). In a total population of 275 Eskimos living in the village at that time, 18.5% were paralyzed and 5% died. The feces were collected April 27, 1949, and were sent to us by Dr. F. P. Nagler. They were preserved 7 years in the frozen state prior to use. Virus was then freshly isolated in monkey kidney tissue cultures, and second passage material was used

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