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Relative Mitochondrial Volume in Liver Cells of A/Jax Mice Under Influence of Carbon Tetrachloride

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Relative Mitochondrial Volume in Liver Cells of A/Jax Mice Under Influence of Carbon Tetrachloride[1]

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Abstract. The purpose of this study was to determine the relative volume of mitochondria *in situ* in hepatic cells adjacent to the interlobular veins in the livers of normal A-Jax mice and of A-Jax mice fed carbon tetrachloride dosages for varying periods of time.

The liver tissues were fixed in Regaud's fluid, sectioned at 3 microns, and stained with iron haematoxylin. A procedure developed by Meglitsch et al. was utilized in determining the relative mitochondrial volume of the hepatic cells.

The following were determined in the study: (1) normal mice older than six weeks possessed a greater relative mitochondrial volume in both the peripheral and basal regions of the median layer liver lobe than six-week normal mice, and (2) the relative mitochondrial volume of hepatic cells of A/Jax mice fed carbon tetrachloride was significantly less than normal mice of similar age.

In contrast to the detailed studies that have been made on descriptions of mitochonrial size, form, and number within hepatic cells, the investigations have been lacking in details concerning the quantitative estimation of mitochondrial volume within a specific region of the liver.

Utilization of homogenizing processes for estimation of alterations in the mitochondrial population of liver tissue has been based upon measurement of mitochondrial volume per unit weight of tissue (1). While this indirect method is excellent for comparative purposes of masses of tissue, it is important to compare mitochondrial population of specific hepatic cells.

The purpose of this study was to determine the effect of age on the relative volume of mitochondria *in situ* in hepatic cells adjacent to the portal veins in A/Jax mice liver. Preliminary study of mitochondrial population in hepatic cells of A/Jax mice fed carbon tetrachloride dosages for varying periods of time is also presented.

METHOD

A/Jax mice obtained from Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine were utilized throughout this in-

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Table 1. Sex, Age at Death and Number of Feedings of CCl_4 Received by Each A/Jax Mouse in This Study

Mouse No.	No. feedings of CCl_4	Sex	Age (weeks) of mice at Sacrificing
1	Normal	Female	6
2	Normal	Female	6
3	Normal	Female	6
4	Normal	Female	6
5	Normal	Female	11½
6	Normal	Female	16½
7	Normal	Female	24
8	6	Female	9
9	11	Female	10
10	18	Male	13
11	22	Male	13
12	29	Male	19
13	37	Female	32
14	42	Female	30
15	48	Male	30
16	54	Female	30

vestigation. Water and Wayne Lab Blox were fed *ad libitum.* Mice receiving carbon tetrachloride were fed 0.1 cc of 40% carbon tetrachloride (C.P.) in olive oil orally with a medicine dropper on alternating days. The mice utilized in this investigation are summarized in Table 1.

In all cases the mice were killed by striking the back of the head. Carbon tetrachloride-fed mice were killed one day after their last feeding of carbon tetrachloride. Pieces of the median liver lobe removed from the basal and peripheral regions were fixed in Regaud's fluid, dehydrated in ethyl alcohol, embedded in paraffin, and sectioned at 3 microns. The sections were stained with iron haematoxylin.

Hepatic cells immediately adjacent to the interlobular vein were selected. Sites seen in cross section, which revealed an interlobular vein and a bile duct running parallel, constituted a portal area. The cells selected for this study were grouped into lobules. Determination of the cells within a lobule was done by tracing a liver cord from a central vein to a portal area. Cells at the periphery of the liver cords that radiated from a common central vein were grouped into the same lobule. Four lobules, each consisting of ten hepatic cells for each region of the median liver lobe were used.

The method developed by Meglitsch et al. for determination of the relative volume of mitochondria *in situ* in hepatic cells was utilized in this study (2).

A cell, randomly selected from the specific region described above, was observed by use of an oil-immersion objective. An image of the cell was superimposed by means of a camera lucida upon a grid of points made up of 25 rows of periods with 25 periods in each row. The distance between the rows was half the distance between normal lines of type. The grid was prepared by a type writer with elite type upon a piece of dull white paper. Whenever any part of the image of a mitochondrium in sharp focus was superimposed upon a grid point, this was recorded as a mitochondrial hit. A mitochondrial miss was recorded whenever any part of the cytoplasm free of mitochondria was superimposed upon a grid point. Nuclear hits were not recorded. Counts for each cell were made at two different levels of focus and the number of observable nuclei in the cell was recorded.

The relative mitochondrial volume, expressed as a percent, was calculated by the following means:

$$\text{mitochondrial percentage} = \frac{\text{mitochondrial hits}}{\text{mitochondrial hits} + \text{cytoplasmic hits}}$$

Results

A comparison of mitochondrial volume of the portal region of lobules in the basal and peripheral regions of the median liver lobe for each normal mouse indicated a greater range in mitochondrial volume among the six-week normal mice. The older normal mice had a greater relative mitochondrial volume in both the peripheral and basal regions than the six-week normal mice. Comparison of peripheral and basal regions for each normal mouse by use of chi-square test indicated larger values for chi-square among the six-week mice than among the older mice. The probability of the chi-square obtained when the older mice were compared with each other was consistently greater than 0.05 while the probability among the six-week mice ranged from 0.05 to less than 0.001. (See Tables 2 and 3.)

Preliminary study of the mice fed carbon tetrachloride indicated the following: (1) the relative mitochondrial volume of hepatic cells of A/Jax mice fed carbon tetrachloride was significantly less than normal mice of similar age, (2) the decline in relative mitochondrial volume among the carbon tetrachloride-fed mice was present through 37 feedings of carbon tetrachloride, and (3) an increase in relative mitochondrial volume occurred during 42, 48, and 54 feedings. (See Tables 2 and 3.)

Discussion

In each mouse the possibility of significant differences in relative mitochondrial volume among cells in the same region of the lobules, cells in the same region of the lobe with none, one, or two observable nuclei, lobules within the same region of the lobe, and regions of the same lobe were considered as variables.

Table 2. Mitochondrial Hits and Misses of the Portal Region of Four Lobules in the Basal and Peripheral Regions of the Median Lixer Lobe for Each Mouse

Mouse No.	Feedings	Age (Wks.)	Basal Region of Lobe Lobules Total	Hits	Misses	Peripheral Region of Lobe Lobules Total	Hits	Misses
1	Normal	6	470	301	169	656	454	202
			685	449	236	600	408	192
			669	437	232	766	519	247
			467	314	153	622	431	191
		Total	2291	1501	790	2644	1812	832
2	Normal	6	787	462	325	781	527	254
			828	496	332	773	491	282
			756	450	306	806	487	319
			834	486	348	726	447	279
		Total	3205	1894	1311	3086	1952	1134
3	Normal	6	657	454	203	581	421	160
			674	476	198	705	498	207
			774	533	241	639	455	184
			725	518	207	540	395	145
		Total	2830	1918	849	2465	1769	696
4	Normal	6	670	448	222	741	504	237
			658	474	184	617	393	224
			684	473	211	588	403	185
			601	444	157	539	391	148
		Total	2613	1839	774	2485	1691	794
5	Normal	11½	573	427	146	531	390	141
			401	312	89	362	292	70
			403	328	75	485	383	102
			584	463	121	448	342	106
		Total	1961	1530	431	1826	1407	419
6	Normal	16½	675	516	159	632	473	159
			542	411	131	636	487	149
			732	537	195	729	575	154
			598	473	125	641	490	151
		Total	2547	1937	610	2638	2025	613
7	Normal	24	819	621	198	887	637	250
			720	540	180	872	646	226
			789	589	200	689	541	148
			775	543	232	616	463	153
		Total	3103	2293	810	3064	2287	777
8	6	9	975	582	393	982	592	390
			1192	697	495	853	527	326
			775	485	290	840	539	301
			979	608	371	750	481	269
		Total	3921	2372	1549	3425	2139	1286
9	11	10	830	474	356	1036	596	440
			916	540	376	1052	364	418
			797	463	334	834	484	350
			1011	617	394	779	508	271
		Total	3554	2094	1460	3701	2222	1479
10	18	13	957	457	500	1384	627	757
			1199	565	634	1292	647	645
			1398	615	783	1432	701	731
			1338	563	775	1213	572	641
		Total	4892	2200	2692	5321	2547	2774

11	22	13		944	320	624	922	338	584
				1082	408	674	1340	374	966
				1199	476	723	761	256	505
				1615	581	1034	822	273	549
			Total	4840	1785	3055	3845	1241	2604
12	29	19		1187	421	766	751	267	484
				922	352	570	1020	358	662
				1160	368	792	845	283	562
				1380	475	905	928	299	629
			Total	4649	1616	3033	3544	1207	2337
13	37	32		733	240	493	1254	347	907
				1211	411	800	930	240	690
				766	235	531	1087	276	811
				977	327	650	1077	277	800
			Total	3687	1213	2474	4348	1140	3208
14	42	30		779	351	428	1002	443	559
				983	384	599	908	459	449
				806	336	470	891	400	491
				774	327	447	939	434	505
			Total	3342	1398	1944	3740	1736	2004
15	48	30		1090	465	625	962	396	566
				1270	471	799	960	401	559
				1026	389	637	1125	489	636
				1087	386	701	852	354	498
			Total	4473	1711	2762	3899	1640	2259
16	54	30		1046	430	616	940	355	585
				1040	456	584	1091	481	610
				1140	508	632	729	318	411
				1052	403	649	777	306	471
			Total	4278	1797	2481	3537	1460	2077

Table 3. Comparison of Mitochondrial Volume of the Portal Region of Lobules, and the Basal and Peripheral Regions of the Median Liver Lobe for Each Mouse

			Basal Region of Lobe			Peripheral Region of Lobe		
Mouse No.	Feedings	Age (Wks.)	Total	Lobules Mit. Vol. %	S.E. %	Total	Lobules Mit. Vol. %	S.E.%
1	Normal	6	470	64.0	2.21	656	69.2	1.80
			685	65.5	1.81	600	68.0	1.97
			669	65.3	1.85	766	67.8	1.68
			467	67.2	2.17	622	69.3	1.85
		Total	2291	65.5	.31	2644	68.5	.29
2	Normal	6	787	58.7	1.93	781	67.5	2.14
			828	60.0	1.79	773	63.5	1.72
			756	59.5	1.78	806	60.4	1.72
			834	58.3	1.70	726	61.6	1.80
		Total	3205	59.1	.27	3086	63.3	.27
3	Normal	6	657	69.1	1.80	581	72.5	1.85
			674	70.6	1.75	705	70.6	1.71
			774	68.9	1.87	639	71.2	1.78
			725	71.5	1.67	540	73.1	1.90
		Total	2830	70.0	.27	2465	71.8	.29
4	Normal	6	670	66.9	1.81	741	68.0	1.71
			658	72.0	1.74	617	63.7	1.93
			684	69.2	1.76	588	68.5	1.91
			601	73.9	1.78	539	72.5	1.92
		Total	2613	70.4	.28	2485	68.0	.30

5	Normal	11½	573	75.5	1.70	531	73.5	1.91
			401	77.8	2.08	362	80.7	2.07
			403	81.4	1.94	485	79.0	1.85
			584	79.3	1.68	448	76.3	2.01
		Total	1961	78.0	.30	1826	77.1	.31
6	Normal	16½	675	76.4	1.63	632	74.8	1.99
			542	75.8	1.83	636	76.6	1.67
			732	73.4	1.63	729	78.9	1.51
			598	79.1	1.66	641	76.4	1.67
		Total	2547	76.1	.27	2638	76.8	.26
7	Normal	24	819	76.8	1.49	887	71.8	1.50
			720	75.0	1.61	872	74.1	1.48
			789	74.7	1.56	689	78.5	1.57
			775	70.1	1.73	616	75.2	1.74
		Total	3103	73.9	.25	3064	74.6	.25
8	6	9	975	59.7	1.56	982	60.3	1.55
			1192	58.5	1.43	853	61.8	1.68
			775	62.6	1.73	840	64.2	1.65
			979	62.1	1.54	750	64.1	1.74
		Total	3921	60.5	.25	3425	62.5	.26
9	11	10	830	57.1	1.71	1036	57.5	1.53
			916	59.0	1.62	1052	60.3	1.50
			797	58.1	1.74	834	58.0	1.70
			1011	61.0	1.53	779	65.2	1.70
		Total	3554	58.9	.26	3701	60.0	.25
10	18	13	957	47.8	1.61	1384	45.3	1.33
			1199	47.1	1.43	1292	50.1	1.38
			1398	44.0	1.32	1432	49.0	1.31
			1338	42.1	1.34	1213	47.2	1.43
		Total	4892	45.0	.22	5321	47.9	.22
11	22	13	944	33.9	1.53	922	36.7	1.58
			1082	37.7	1.47	1340	28.0	1.22
			1199	39.7	1.41	761	33.6	1.71
			1615	36.0	1.19	822	33.2	1.64
		Total	4840	36.9	.22	3845	32.3	.24
12	29	19	1187	35.5	1.38	751	35.6	1.74
			922	38.2	1.60	1020	35.1	1.49
			1160	31.7	1.36	845	33.5	1.62
			1380	34.4	1.27	928	32.2	1.53
		Total	4649	34.8	.22	3544	34.1	.25
13	37	32	733	32.7	1.73	1254	38.3	1.37
			1211	34.0	1.36	930	34.8	1.55
			766	30.7	1.66	1087	25.4	1.31
			977	33.5	1.51	1077	25.7	1.33
		Total	3687	32.9	.24	4348	26.2	.21
14	42	30	779	45.1	1.78	1002	44.2	1.56
			983	39.1	1.55	908	30.6	1.65
			806	41.7	1.73	891	44.9	1.66
			774	42.2	1.77	939	46.2	1.62
		Total	3342	41.8	.27	3740	46.4	.26
15	48	30	1090	42.7	1.49	962	41.2	1.58
			1270	37.1	1.35	960	41.8	1.59
			1026	37.9	1.51	1125	43.5	1.47
			1087	35.5	1.44	852	41.6	1.68
		Total	4473	38.3	.23	3899	42.1	.25
16	54	30	1046	41.1	1.51	940	37.8	1.58
			1040	43.8	1.53	1091	44.1	1.50
			1140	44.6	1.46	729	43.6	1.83
			1052	38.3	1.49	777	39.4	1.75
		Total	4278	42.0	.24	3537	41.3	.26

Consistency tests administered upon these variables indicated their influence upon the data.

If large significant variations occurred among the hepatic cells in the portal region of the lobule, random selection of cells in this region of the lobule could result in either an underestimation or an overestimation of the relative mitochondrial volume. Random checks upon hepatic cells in the portal region of lobules among normal and experimental mice indicated the cells were not significantly different in the same organism.

Variations among the hepatic cells were not considered significant enough to influence the relative mitochondrial volume obtained for each mouse.

Hepatic cells in a section of liver tissue were cut at different planes, resulting in sections of cells without the nucleus present. In other planes of sectioning the nucleus was included within the cell. Most liver cells had one large round nucleus, although binucleated cells were not uncommon. The possibility of a greater concentration of mitochondria toward the perinuclear zone in hepatic cells could alter the results. A check of the consistency of results for this factor indicated no significant difference in mitochondrial volume among hepatic cells with none, one or two observable nuclei in each region of the median liver lobe.

The cross sections of the liver lobules surrounding the same portal vein did not represent the same level for each lobule. To what extent were the portal regions of these lobules different in relative mitochondrial volume? After a check upon this factor it seemed obvious that the portal regions of the lobules surrounding the same portal vein were not statistically different.

Consistency tests revealed that the relative mitochondrial volume of hepatic cells in the basal region of the median liver lobe for each mouse was generally no different than in the peripheral region.

Preliminary study of the effect of carbon tetrachloride upon the relative mitochondrial volume indicated that the greatest significant decrease occurred between 11 and 22 feedings. It was noteworthy that the lowest relative mitochondrial volumes occured during the period in which tumors appeared in the liver. The significant increase in relative mitochondrial volume between 37 and 42 feedings and the stability of this increase through 54 feedings may be due to adjustment of the hepatic cells in the portal region to the toxic effects of carbon tetrachloride. Eschenbrenner and Miller suggested that during the course of chronic administration of carbon tetrachloride the livers of strain A mice developed either relative or complete resistance or insensitivity to the necrotizing action of the agent (3).

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Gustatory Responses of a Tropical Frugivorous Bat[1]

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Abstract. The electrical activity of the chorda tympani nerve in response to chemical stimulation of the tongue of the tropical frugivorous bat *Artibeus jamaicensis* was compared to that of several rodents and carnivores. The slope of the NaCl response-concentration curve is much steeper than curves for the other animals and the maximum response is reached at a lower concentration (approximately 0.25M NaCl). NaCl is a more adequate stimulus than NH_4Cl, LiCl is approximately equal to NaCl, and KCl response is low. These results are similar to those previously found for the rodents but quite different from those for the carnivores. Response is lower to sucrose stimulation than to quinine or HCl, and response to all three is slightly higher, when compared to the NaCl response, than for most o fthe rodents and carnivores. Responses to monovalent chloride salts and the other taste qualities are quite different from those of the little brown bat, *Myotis lucifugus*.

The NaCl response is not maintained at a steady-state level as in the rat but shows a constant decline after the initial response. A high water response, almost equal to the initial taste response, is obtained after stimulation with 0.01NHCl. Two types of water response are shown.

Differences in gustatory responses of various small mammals to chemical stimulation of the tongue, as measured by the activity of the chorda tympani nerve, have been shown by a number of studies (Beidler, 1953; Beidler, Fishman and Hardiman, 1955; Fishman, 1957, 1959; Pfaffmann, 1953, 1955; Tamar, 1956; and others). Animals belonging to various phylogenetic orders have been included in these studies. The forms ranged from the extremely polyphagous opossum to the relatively stenophagous guinea pig. However, none of these represent groups of closely related forms having entirely different stenophagous diets.

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