Phospholipid Composition of Mitochondria and Microsomes of Liver and Kidney in Adrenalectomized, Hypophysectomized, and Diabetic Rats* (33905)

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Phospholipids are found in membranes of mitochondria, nuclei, and microsomes of mammalian tissues (1–3). Liver mitochondria of rats are composed of about 21–28% phospholipids on a dry-weight basis. The phospholipids account for about 90% of the total lipids present (1, 2). A number of enzymes found in mitochondria involved in oxidative phosphorylation contain phospholipids (4, 5).

The concentration of liver mitochondrial phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and sphingomyelin are increased in rats receiving thyroxine (6). There was a corresponding increase in the incorporation of $^{32}P_i$ into the phospholipids following the administration of thyroxine, and a decrease in a hypothyroid state (6). The growth hormone produces an increase in the synthesis of total liver phospholipids in normal or in hypophysectomized rats (7). Fat mobilization fraction of the pituitary gave a 51% increase in the total lipids of the liver of rats in 6 hr and increased the incorporation of $^{32}P_i$ into liver phospholipids (8). This work has now been extended to include the study of the effects of various hormone states on the concentration of the various phospholipid fractions of liver and kidney mitochondria and microsomes.

Methods. Young male albino rats of Sprague-Dawley strain were obtained from Badger Research Corporation. Adrenalectomies were performed by the supplier; completeness of removal of adrenal glands was ascertained by inspection following sacrifice. A 20-μg dose of *d*-aldosterone (CiBA Pharmaceutical Products, Inc., Summit, New Jersey) was injected intraperitoneally into the adrenalectomized animals 28, 16, and 6 hr before sacrifice (9). Animals fasted 24 hr were made diabetic by injecting intraperitoneally 200 mg/kg of body weight of recrystallized alloxan monohydrate (10). After 7 days, animals with blood sugar levels above 350 mg/100 ml were selected for experimentation. Diabetic rats were given 8 units of protamine zinc insulin (Lilly) intraperitoneally 12-hr intervals for 4 days before sacrifice (11). Hypophysectomized rats and their controls of Sprague-Dawley strain were obtained from Hormone Assay Laboratories, Chicago, Illinois. Wire-bottom cages were used to house individual rats. The animals were allowed free access to Purina lab chow and water (or 1% NaCl solution for adrenalectomized animals) *ad libitum*, throughout the duration of the experiments.

The rats were sacrificed by decapitation. The livers were removed, blotted, weighed, and homogenized with ice-cold 0.25 *M* sucrose in a Potter–Elvehjem homogenizer with a Teflon pestle. The mitochondrial and microsomal fractions were isolated by differential centrifugation (12). The nuclear fraction was separated from the homogenate by centrifuging for 10 min at 800. The nuclear pellet was homogenized and the nuclear suspension was centrifuged. The supernatant solutions were combined and centrifuged for 10 min at 14,500 *g* to sediment the mitochondrial fraction. The mitochondrial pellet was washed twice. The combined supernatant solutions from the mitochondrial preparation were centrifuged at 78,450*g* for 45 min to sediment the microsomal pellet. An aliquot was taken from each mitochondrial and microsomal preparation for protein analysis (13). Lipids were extracted from mitochon-

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TABLE I. Concentration of Individual Phospholipids in Liver Mitochondria.[a]

Exptl. conditions	No. of animals	Total phospholipids (μg)	Total phospholipid/mg of protein N (μg/mg)	Phospholipid fractions (% of total lipid P) Sphingomyelin	Phosphatidylcholine	Phosphatidylinositol	Phosphatidylserine	Phosphatidylethanolamine	Phosphatidic acid + cardiolipid
Controls	6	2266 ± 315	39 ± 8	1.5 ± 1.2	45.0 ± 3.0	7.8 ± 1.2	2.0 ± 1.3	31.8 ± 1.0	12.3 ± 2.4
Adrenalectomized	6	1274 ± 227[b]	29 ± 5	4.0 ± 2.3	43.1 ± 7.1	8.3 ± 3.0	3.2 ± 1.0	32.5 ± 3.9	11.4 ± 5.8
Adrenalectomized + aldosterone	6	1488 ± 233[b]	60 ± 7[c]	3.2 ± 1.8	43.8 ± 3.2	8.0 ± 1.4	3.7 ± 1.3	31.5 ± 3.5	9.9 ± 4.7
Alloxan diabetic	4	2075 ± 513	30 ± 6	5.7 ± 5	39.1 ± 3.1	9.5 ± 3.7	3.2 ± 1.8	29.5 ± 5.3	12.6 ± 4.4
Alloxan diabetic + insulin	4	2660 ± 496	25 ± 2[b]	4.0 ± 2.2	38.7 ± 3.7	11.5 ± 2.0	3.6 ± 1.4	29.5 ± 3.3	10.6 ± 1.9
Controls	6	2578 ± 864	30 ±12	3.3 ± 2.8	43.2 ± 2.7	10.1 ± 3.6	3.9 ± 2.4	30.2 ± 4.7	9.4 ± 3.1
Hypophysectomized	6	654 ± 264[b]	24 ± 9.0	3.9 ± 1.5	46.1 ± 2.8	2.8 ± 1.7[b]	2.8 ± 1.8	33.0 ± 2.9	10.3 ± 3.5

[a] Numbers following ± are standard deviations. Test of significance was applied to difference between mean value for the experimental and the control rats of that group. The probability for chance occurrence of this difference was: [b] $p < 0.1$; [c] $p < 0.5$.

TABLE II. Concentration of Individual Phospholipids in Liver Microsomes.[a]

Exptl. conditions	No. of animals	Total phospholipids (μg)	Total phospholipids/mg of protein N (μg/mg)	Phospholipids fractions (% of total lipid P) Sphingomyelin	Phosphatidylcholine	Phosphatidylinositol	Phosphatidylserine	Phosphatidylethanolamine
Controls	6	5196 ± 527	161 ± 17	3.4 ± 0.9	48.5 ± 4.4	13.9 ± 3.4	5.3 ± 0.9	26.1 ± 4.8
Adrenalectomized	6	2408 ± 433[b]	55 ± 11[b]	4.3 ± 1.8	55.3 ± 9.3	11.6 ± 3.3	3.9 ± 2.2	23.7 ± 5.7
Adrenalectomized + aldosterone	6	3058 ± 861[c]	81 ± 19[b]	4.3 ± 0.9	52.0 ± 1.9	11.4 ± 1.7	4.3 ± 1.5	26.8 ± 1.1
Alloxan diabetic	4	3903 ± 732[b]	70 ± 18[b]	3.6 ± 0.5	53.5 ± 1.5	13.3 ± 1.5	6.3 ± 2.8	21.7 ± 4.0
Alloxan diabetic + insulin	6	6577 ± 654	79 ± 15[b]	2.5 ± 0.6	57.5 ± 2.7	13.3 ± 1.5	4.7 ± 0.5	20.1 ± 2.5
Controls	6	6105 ± 471	113 ± 35	3.9 ± 1.2	56.7 ± 2.6	9.4 ± 6.5	8.4 ± 1.8	23.0 ±10.3
Hypophysectomized	6	4072 ± 938[b]	73 ± 12[b]	3.4 ± 0.4	55.6 ± 2.1	9.0 ± 1.2	4.6 ± 0.8	26.1 ± 2.5

[a] Numbers following ± are standard deviations. Test of significance was applied to difference between mean value for the experimental and the control rats of that group. The probability for chance occurrence of this difference was: [b] $p < 0.1$; [c] $p < 0.5$.

TABLE III. Concentration of Individual Phospholipids in Kidney Mitochondria (mw) and Microsomes (ms).[a]

					Phospholipid fractions (% of total lipid P)					
Exptl. conditions	No. of animals		Phospholipids (μg)	Total phospholipids/mg of protein N (μg/mg)	Sphingomyelin	Phosphatidylcholine	Phosphatidylinositol	Phosphatidylserine	Phosphatidylethanolamine	Phosphatidic acid + cardiolipid
Control	6	mw	898 ± 47	65 ± 2	6.4 ± 2.9	38.7 ± 4.5	6.1 ± 2.7	5.3 ± 3.2	31.2 ± 3.4	10.7 ± 1.5
		ms	1120 ± 345	99 ± 20	13.4 ± 2.5	37.5 ± 5.5	12.7 ± 2.7	10.9 ± 2.3	21.7 ± 3.4	
Adrenalectomy	6	mw	499 ± 95^b	60 ± 13	3.9 ± 1.0	33.9 ± 2.3	7.4 ± 2.1	5.3 ± 2.9	29.7 ± 4.1	15.4 ± 3.4
		ms	626 ± 201^c	96 ± 18	15.2 ± 2.3	35.7 ± 5.5	9.4 ± 4.5	11.1 ± 2.0	25.4 ± 2.1	
Adrenalectomy + aldosterone	6	mw	601 ± 75^b	98 ± 9	4.7 ± 1.6	37.1 ± 3.0	5.8 ± 2.4	7.2 ± 4.1	33.9 ± 7.5	11.0 ± 1.3
		ms	680 ± 128^c	177 ± 34	16.1 ± 2.9	38.2 ± 3.7	8.9 ± 2.6	8.5 ± 2.3	24.5 ± 0.8	
Alloxan diabetic	4	mw	1091 ± 167	57 ± 13	3.5 ± 1.3	39.3 ± 4.0	4.1 ± 1.6	6.9 ± 1.4	33.7 ± 2.7	11.0 ± 3.9
		ms	966 ± 133	60 ± 13^c	15.6 ± 4.6	37.4 ± 6.8	7.7 ± 1.8	11.6 ± 1.0	24.8 ± 4.1	
Alloxan diabetic + insulin	4	mw	1045 ± 105	54 ± 22	4.6 ± 1.1	35.4 ± 4.5	11.1 ± 2.7	5.3 ± 3.1	32.0 ± 1.8	11.3 ± 1.9
		ms	1104 ± 63	81 ± 7	16.2 ± 2.2	35.8 ± 3.5	11.4 ± 2.7	8.9 ± 2.0	20.0 ± 3.5	
Controls	6	mw	777 ± 98	52 ± 5	4.0 ± 1.4	37.6 ± 3.5	5.2 ± 1.4	2.5 ± 1.1	37.6 ± 2.1	13.1 ± 2.2
		ms	1094 ± 112	70 ± 7	19.5 ± 4.4	37.2 ± 3.6	6.3 ± 3.5	9.3 ± 4.2	27.2 ± 3.7	
Hypophysectomized	6	mw	416 ± 49^b	36 ± 6^b	3.7 ± 1.1	41.0 ± 3.2	3.2 ± 1.9	2.9 ± 0.8	34.5 ± 4.8	14.9 ± 1.7
		ms	751 ± 146	51 ± 8^b	23.5 ± 4.0	38.7 ± 4.0	4.7 ± 1.5	9.7 ± 2.0	21.5 ± 1.8	

[a] Numbers following ± are standard deviations. Test of significance was applied to difference between mean value of the experimental and the controls of that group. The probability for chance occurrence of this difference was: [b] $p < 0.1$; [c] $p < 0.05$.

dria and microsomes with chloroform–methanol (2:1, v/v) and were washed with 0.04 $CaCl_2$ (14). The total phospholipid phosphorus was determined on an aliquot of the chloroform solution (15, 16). Individual phospholipids were separated by thin-layer chromatography by the method of Parker and Peterson (17). The chromatogram plates were exposed to iodine vapor (18), and individual phospholipid phosphorus fractions determined (15, 16). The individual phospholipids were identified with synthetic standards and purified lipids as references and staining with rhodamine GG and observing under ultraviolet light (19).

Results. Tables I and II shows the total phospholipid concentration and the individual phospholipids composition of liver microsomes and mitochondria in adrenalectomized, alloxan diabetic, and hypophysectomized rats. Adrenalectomy resulted in a statistically significant decrease of concentration in the total phospholipids of mitochondria and microsomes. The percentages of the total phospholipid phosphorus for the various phospholipids of liver mitochondria and microsomes of the adrenalectomized rats were similar to those of the controls. In addition to the statistical calculations given in the tables, the microsomes and mitochondria of adrenalectomized rats injected with the aldosterone 28, 16, and 6 hr before sacrifice have a significant increase in concentrations of total phospholipids compared to the adrenalectomized rats. The probability for chance occurrence of this difference was $p<0.01$.

The microsomes from the alloxan diabetic rats have a significant decrease concentration of total phospholipids. The decreased P/O ratio which has been observed in diabetic animals (20) may account for the decrease concentration of total phospholipids of microsome that was observed in diabetic rats. Young and Cornatzer (21, 22) demonstrated that the administration of compounds which decrease oxidative phosphorylation such as oligomycin, bilirubin, dinitrophenol, and arsenate inhibit the individual phospholipids synthesis in liver mitochondria.

Hypophysectomy resulted in a significantly decreased concentration in the total phospholipids of mitochondria and microsomes. A significantly decreased concentration of phosphatidylinositol occurred in liver mitochondria following hypophysectomy. It is of interest that growth hormone stimulates total phospholipid synthesis of mitochondria (23). Phosphatidylinositol which occurs as a minor phospholipid in concentration of mitochondria (10% of total lipid P) has a high and early uptake of $^{32}P_i$ (24, 25). These results (Tables I–III) show that the total phospholipids in the membrane of mitochondria and microsomes of liver and kidney cell are decreased during diabetes, adrenalectomy, and hypophysectomy.

Summary. The individual phospholipid concentration of mitochondria and microsomes of liver and kidney in adrenalectomized, hypophysectomized, and diabetic rats was studied. Adrenalectomy and hypophysectomy resulted in a decreased concentration of total phospholipid of mitochondria and microsomes of liver and kidney. A decreased concentration of total phospholipids occurred in the microsomes of liver and kidney of the diabetic rats. This decrease of liver and kidney mitochondria and microsomes is apparently uniformly distributed in all of the phospholipid fractions except phosphatidylinositol. A decreased concentration of phosphatidylinositol of liver mitochondria occurred in the hypophysectomized rats.

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The "Thompson" Specificity and the HL-A Human Leukocyte System*

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Each gene or chromosomal region of the main human leukocyte antigenic system, HL-A, is manifested by multiple factors which segregate together as units in family studies (1, 2). Within the HL-A region, several subloci have been identified. The factors within each sublocus appear mutually exclusive to one another. For example, only one of the factors HL-A 1, 2, 3, Lc-11, and Dausset 15 and 17 of the first sublocus has been observed to be present on a single chromosome (2). Thus the factors behave as true alleles and no more than two have ever been observed phenotypically in the same person (2,3).

In a prior immunogenetic analysis of the lymphocytes of a large family we detected crossover between a leukocyte specificity designated as "Thompson" and the known HL-A factors (4). We now report the finding of crossover in one of four additional families in which HL-A and "Thompson" segregation were followed. At the same time we have noted that when a population of unrelated donors was carefully and repeatedly typed for four mutually exclusive factors of the HL-A system, namely HL-A 1.0, 2.0, 3.0 and Lc-11, Thompson showed, with a very occasional exception, a mutually exclusive or allelic relationship to these four. Familial and population analyses thus appear paradoxical and together suggest the possibility of gene interaction or epistasis.

Materials and Methods. All typing was done with the Terasaki microcytotoxicity test (5) as modified by us (6). The "Thompson" antiserum was produced by intradermal injection of leukocytes from a donor into a recipient preselected on the basis of similarity to each other with existing antisera (4). Absorption of the "Thompson" antiserum with all of 30 positively reacting cell samples from different donors and of different races completely removed cytotoxic activity. The serum can therefore be considered as monospecific within the limits of the absorption

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