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CONVERSION OF ACETATE TO LIPIDS AND CO_2 BY LIVER OF RATS EXPOSED TO ACCELERATION STRESS[1]

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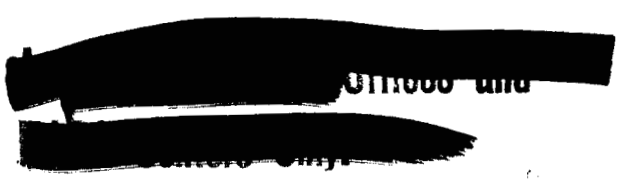
Running Head: Lipid Synthesis After Acceleration



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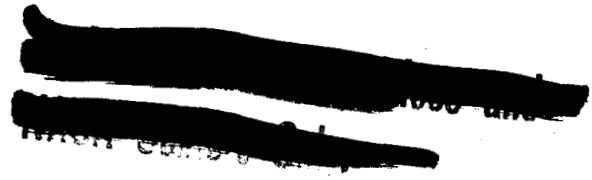
ABSTRACT

Male Sprague-Dawley rats in age groups from 1 to 20 weeks were centrifuged at 4.5 g for periods of 0.5 hr to 14 days. Slices of liver, kidney, and inguinal adipose tissue were incubated with C^{14}-labeled acetate, and the resulting CO_2 and lipids were measured. When compared with controls liver and adipose tissue from nonfasted centrifuged rats showed an increased formation of C^{14}-lipids while kidney showed a decrease. Liver from fasted centrifuged rats also showed an increased formation of C^{14}-lipids when compared to the fasted noncentrifuged controls. The increase in acetate conversion varied with age of animal and duration of exposure. No significant change in C^{14}-acetate oxidation was noted in either fed or fasted centrifuged animals as compared to their corresponding controls. The total lipid content of the tissues from centrifuged rats was generally lower than in the controls and was also a function of age of the animal and time of exposure.

Author

INDEX TERMS

acceleration stress acetate lipogenesis oxidation lipid content



Earlier studies indicated functional deficiencies resulting from exposure to such space stresses as acceleration (2). This result was obtained using devices which sense primarily cardiovascular-hemodynamic information. Although these types of measurements are informative, the more fundamental changes that occur at a cellular level need to be investigated in order to bring about a fuller understanding of the basic mechanisms of control.

Oyama and Platt (5) recently demonstrated an increased liver glycogen deposition in mice centrifuged for varying times and gravity exposures. From studies on adrenalectomized mice, these workers concluded that the glycogen deposition response was the result of an increased elaboration of adrenal corticosterone.

For a better understanding of the biochemical alterations resulting from acceleration stress, a knowledge of its effects on protein, carbohydrate, and fat metabolism, as well as their interrelationships, must be obtained. Thus, some aspects of altered fat metabolism in rats resulting from acceleration stress are the subject of this report.

METHODS

Centrifuge. A centrifuge with 10 radial arms was employed at an effective operating radius of 8.5 feet. The cages were mounted in the swing-bucket fashion, allowing for one degree of freedom which placed the resulting g-vector perpendicular to the cage floor. The cages were illuminated with individual fluorescent lights automatically timed to provide on and off cycling at 6 AM and 6 PM, respectively. The temperature in the centrifuge room and animal holding room was maintained at $22^{\circ} \pm 2^{\circ}$ C. The centrifuge was stopped daily for approximately 30 min while the animals were fed and their cages cleaned.

General. Male Sprague-Dawley rats were obtained from Simonsen Laboratory, Gilroy, California, and divided into experimental and control groups according to age in weeks ±2 days. All centrifuged rats were killed by decapitation immediately after removal from the centrifuge, except as noted. Appropriate noncentrifuged controls were sacrificed shortly before and/or after the experimental group. Slices of liver, kidney, and adipose tissue from 9-week-old rats exposed to 4.5 g were incubated in the presence of acetate-2-C^{14}. This group of animals had access to food except during the period of centrifugation not exceeding 6 hr. Rats so treated are referred to here as nonfasted. In subsequent studies utilizing acetate-1-C^{14} the rats, referred to as fasted, were not fed for 18-24 hr including the time in the centrifuge.

Studies on fasted rats were designed to determine effects on lipogenesis of (a) the age of the animal and (b) the exposure time on centrifuge. The animal's age was recorded at time of sacrifice, after centrifugation. The rats were not restrained during the experiment.

Analytical Procedures. The kidney and liver were cut into 0.5mm slices with a Mickle tissue chopper. Inguinal adipose tissue was finely cut with scissors. One gram of the tissue was transferred to a 50 ml screwcap Erlenmeyer flask containing 10 ml of Krebs-bicarbonate buffer at pH 7.4 fortified with 0.011M glucose and 0.01M succinate. The buffer contained 10 μc of C^{14}-sodium acetate with a specific activity of 1 mc/mMole.

A plastic CO_2 collection well was inserted into the flask[2] and the flask aerated with a 95% O_2 - 5% CO_2 gas mixture. The flask was sealed with a rubber diaphragm by means of a screwcap and incubated in a shaker bath at 37.5° C for 3 hr. At the end of this period 1.0 ml of 2.5N NaOH was injected with a syringe into the CO_2 absorption well. The reactions were

stopped by the addition of 0.2 ml of 10N H_2SO_4 to the main compartment. After standing overnight, the flask was opened, the CO_2 fraction diluted to 25 ml, and 0.1 ml taken for radio assay in a liquid scintillation spectrometer.

Lipids were isolated by a modification of the method of Abraham, Matthes, and Chaikoff (1). The tissues were exhaustively washed with water and saponified overnight with 30% alcoholic KOH in a steambath. The saponified material was acidified with 6N HCl using bromocresol green indicator. The lipids were extracted once with 40 ml and once with 20 ml of n-hexane, then combined and washed once with 5 ml of 1% acetic acid and twice with 5 ml of water. The hexane extract was dried over anhydrous sodium sulfate, filtered, and diluted to 100 ml. The C^{14}-lipid was measured in a liquid scintillation spectrometer by assaying 2 ml aliquots in 15 ml of toluene containing 0.5% 2,5-diphenyloxazole and 0.03% 1,4-bis-2-(4-methyl-5-phenyloxazoly)-benzene. To determine the total fat a 50 ml aliquot of the hexane extract was evaporated and the dried residue was weighed.

RESULTS

Initial studies were made on liver, kidney, and inguinal adipose tissues of nonfasted 9-week-old rats weighing approximately 275 gm, which had been exposed to 4.5 g for 5 hr (Fig. 1). The observed differences between control and centrifuged tissue in conversion of acetate-2-C^{14} to lipids were significant for each tissue at the 5% level of probability. Adipose tissue showed the highest conversion of acetate to lipids on an absolute weight basis while liver showed the largest percent increase, namely 145% ($p < 0.003$) above that of the noncentrifuged controls. Kidney of centrifuged rats showed a decreased conversion of acetate into lipids. Results not tabulated show that adipose tissue and liver from centrifuged animals produced approximately 5% less $C^{14}O_2$ than their controls, whereas kidney showed a 5% increase; however, these differences were not statistically significant. The total lipid content

in kidney from experimental animals increased significantly ($p < 0.02$) while that of the liver and adipose tissue remained unaltered. All subsequent experiments were performed on liver.

The effect of the magnitude of g-force and duration of exposure on the liver's conversion of acetate-2-C^{14} to lipids is illustrated in Fig. 2. A significant increase, in comparison to controls, in conversion of the substrate to lipids was noted for exposures of 4.5 g for 1.5 hr (65%), 4.5 g for 5 hr (137%), and 10 g for 5 hr (84%). The 4.5 g for 5 hr exposure showed the greastest response.

No significant change in conversion of the acetate to $C^{14}O_2$ or total lipid content was observed under these experimental conditions.

Since the metabolism of a growing animal is a function of age (3), a study was made of the effects of acceleration on conversion of acetate to lipids and CO_2 in fasted rats of different ages, and the results are shown in Table 1. Acceleration stress significantly increased the conversion of acetate-1-C^{14} to lipids in age groups from 2 to 9 weeks and for the 20-week-old group. The most significant increased conversion occurred at 5 weeks of age and was preceded by a marked lipogenic response at 3 and 4 weeks. After the increased rate of lipogenesis at weaning, the conversion of acetate-1-C^{14} to lipids maintained a uniform value of 0.3 to 0.5% per gm of tissue per 3 hr through the 20-week age group.

Table 1 shows the conversion of acetate-1-C^{14} to $C^{14}O_2$ by liver of fasted rats exposed to 4.5 g for 5 hr. The basal oxidation varied with age; however, centrifugation had no significant effect on the oxidation rate of the substrate.

The total lipid content of livers from animals of different ages is shown in Table 2. Significant decreases are noted for the 3- and 12-week-old groups. The amount of liver lipids was lower in those groups subjected to centrifugation;

however, in the majority of the age groups, the differences between control and centrifuged groups were insignificant.

Since 5-week-old centrifuged rats showed the most significant response (230% increase, $p < 0.001$), a time exposure series was performed on this age group and the results are shown in Fig. 3. The figure also shows three exposure times for 9-week-old rats. All animals centrifuged more than 2 hr showed increases in hepatic lipogenesis. All increases noted were significant at the 5% level of probability, with the exception of the 8- and 24-hr time period in the 5-week age group. The greatest net increase noted was at 192 hr when conversion of acetate-1-C^{14} to lipid rose from a control value of 0.4% per gm of liver to 1.2% ($p < 0.001$). Although not shown in the figure, a group of 5-week-old rats exposed to 4.5 g for 336 hr was removed from the centrifuge for 4 hr before being sacrificed. Conversion of acetate to fatty acids by liver in these animals was found to be 0.84% compared to the value found for the noncentrifuged rats of 0.39%. The value for hepatic lipogenesis of rats exposed for 336 hr and sacrificed immediately was 1.0% ($p < 0.001$).

Effects noted for the 9-week-old group are similar to those obtained in the 5-week-old group.

Oxidation of acetate to CO_2 was measured in the livers of rats exposed for various durations to centrifugation. Although some significant changes were noted for the first 2 hr of centrifugation, no significant change in oxidation was noted after 2.5 hr exposure, and no pattern of response was established.

Changes in liver lipid content from 5-week-old rats exposed for various periods of time at 4.5 g are shown in Fig. 4. No significant changes were observed up to 5 hr of acceleration. At 8 hr, a significant decrease in liver lipids was noted ($p < 0.01$). At this time period the increase in

lipogenesis in response to centrifugation was diminished. Liver lipids returned to near control values after rats were exposed for 24 and 192 hr but again fell significantly below controls after the animals were centrifuged for 336 hr.

DISCUSSION

Exposing animals to chronic acceleration stress in a centrifuge generally retards their growth rate (4,7,8), partly because of endocrine mechanisms evoked by the stress and partly because of the altered food intake.[3]

From the experimental design of this investigation, one cannot assess effects of acceleration on total metabolism of the intact rat to compare with earlier studies (6). Effects on individual tissues by in vitro techniques probably reflect residual hormonal effects produced by the stress. As measured by conversion of acetate to CO_2, acceleration did not appreciably reduce oxidation. We know from the work of Oyama and Platt (5), that acceleration produces profound effects on intermediary carbohydrate metabolism. The present study shows an increase in lipid synthesis from tissues of rats exposed to acceleration. This increase did not occur either in rats younger than 2 weeks of age or in animals exposed for periods of 1 hr or less. For some age groups (12-16 weeks) or some exposure times (8 and 24 hr), lipogenesis in livers from centrifuged rats was not significantly increased over controls. Thus, certain thresholds of exposure time and age of rat need to be reached in order for a response to be elicited.

Lipid synthesis increased and the concentration of lipids decreased as the centrifugation time was extended, indicating that the total lipids leaving the liver exceeded the sum of uptake and synthesis. Thus, the decrease in liver lipids might be related to the increase in lipogenesis. Investigation concerning the mechanisms involved in these changes is the subject of further study.

ACKNOWLEDGMENT

The excellent technical assistance of Mr. Karl Talarico is gratefully acknowledged.

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TABLE 1. Recovery of C^{14}-lipids and $C^{14}O_2$ after incubation of liver with acetate-1-C^{14}

(1.0 gm of tissue obtained from fasted rats exposed to 4.5 g for 5 hr was incubated for 3 hr at 37.5°in 10.0 ml of buffer. Each value represents the mean ± standard error. Number of experiments are shown in parentheses.)

Age	Lipids (% of added dose)		Change	CO_2 (% of added dose)		Change
Weeks	Control	Centrifuged	%	Control	Centrifuged	%
1 (4)	0.74 ± 0.04	0.68 ± 0.05	-8	64.9 ± 2.0	57.6 ± 4.6	-11
2 (7)	.39 ± .02	.51 ± .05	32*	37.2 ± 1.2	35.9 ± 1.9	-6
3 (10)	1.42 ± .14	1.93 ± .18	36*	43.2 ± 1.1	44.3 ± 1.3	3
4 (8)	.90 ± .12	1.79 ± .27	99*	44.8 ± 1.2	44.3 ± 1.3	1
5 (14)	.30 ± .05	.99 ± .11	230*	45.5 ± 1.2	46.2 ± 1.5	2
7 (7)	.38 ± .08	.95 ± .16	151*	45.8 ± 1.3	49.3 ± 1.3	8
9 (10)	.34 ± .04	.53 ± .06	57*	43.3 ± 1.3	45.9 ± 1.0	6
12 (7)	.49 ± .06	.69 ± .08	41	48.5 ± 1.9	48.1 ± 2.0	-1
14 (4)	.36 ± .01	.76 ± .18	112	43.0 ± 2.2	45.7 ± 1.9	6
16 (4)	.49 ± .20	.45 ± .18	-8	47.7 ± 2.4	42.9 ± 1.8	-10
20 (4)	.34 ± .05	.46 ± .01	35*	45.5 ± 1.8	43.0 ± 2.9	-5

*Values considered significantly different from the control at the 5% probable error level.

TABLE 2. Liver lipids of centrifuged rats of different ages (Experimental conditions as in Table 1).

Age Weeks	Lipids (gm/100 gm)		Change %
	Control	Centrifuged	
1 (4)	2.15 ± 0.05	1.94 ± 0.20	-9.8
2 (7)	2.58 ± .11	3.20 ± .23	24.0*
3 (10)	4.66 ± .15	3.65 ± .17	-21.7*
4 (8)	4.46 ± .30	4.36 ± .21	-2.2
5 (14)	4.08 ± .22	3.74 ± .25	-8.4
7 (7)	4.55 ± .44	3.72 ± .43	-18.2
9 (10)	4.67 ± .19	4.38 ± .26	-6.2
12 (7)	4.23 ± .23	3.32 ± .14	-21.5*
14 (4)	4.91 ± .28	4.10 ± .25	-16.5
16 (4)	5.15 ± .24	4.75 ± .25	-7.8
20 (4)	4.26 ± .14	4.55 ± .26	6.8

*Values considered significantly different from the controls at the 5% probable error level.

FOOTNOTES

[1]Portions of this paper were presented at the 48th Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Illinois, April 14, 1964.

[2]A report on this process is being prepared by E. D. Neville and D. D. Feller.

[3]Report in preparation by J. Oyama and W. T. Platt of NASA, Ames Research Center.

FIGURE TITLES

Fig. 1.- The conversion of acetate-2-C^{14} to lipid by tissues from fed 9-week-old rats exposed to 4.5 g for 5 hours. Each value represents the mean of 5 rats.

Fig. 2.- The effect of different exposures on the total lipid content and the conversion of acetate-2-C^{14} to lipid and CO_2 in liver slices from fed 9-week-old rats. Each value represents the mean of 6 rats expressed as percent of control.

Fig. 3.- The effect of exposure time on the conversion of acetate-1-C^{14} to lipid in liver slices from fasted 5- and 9-week-old rats exposed to 4.5 g. Each value represents the mean ± standard error of 7 to 14 rats. Asterisks indicate control values from large common group of rats.

Fig. 4.- The effect of exposure time on the total lipid content in liver slices from fasted 5-week-old rats exposed to 4.5 g. Each value represents the mean ± standard error of 7 to 14 rats.

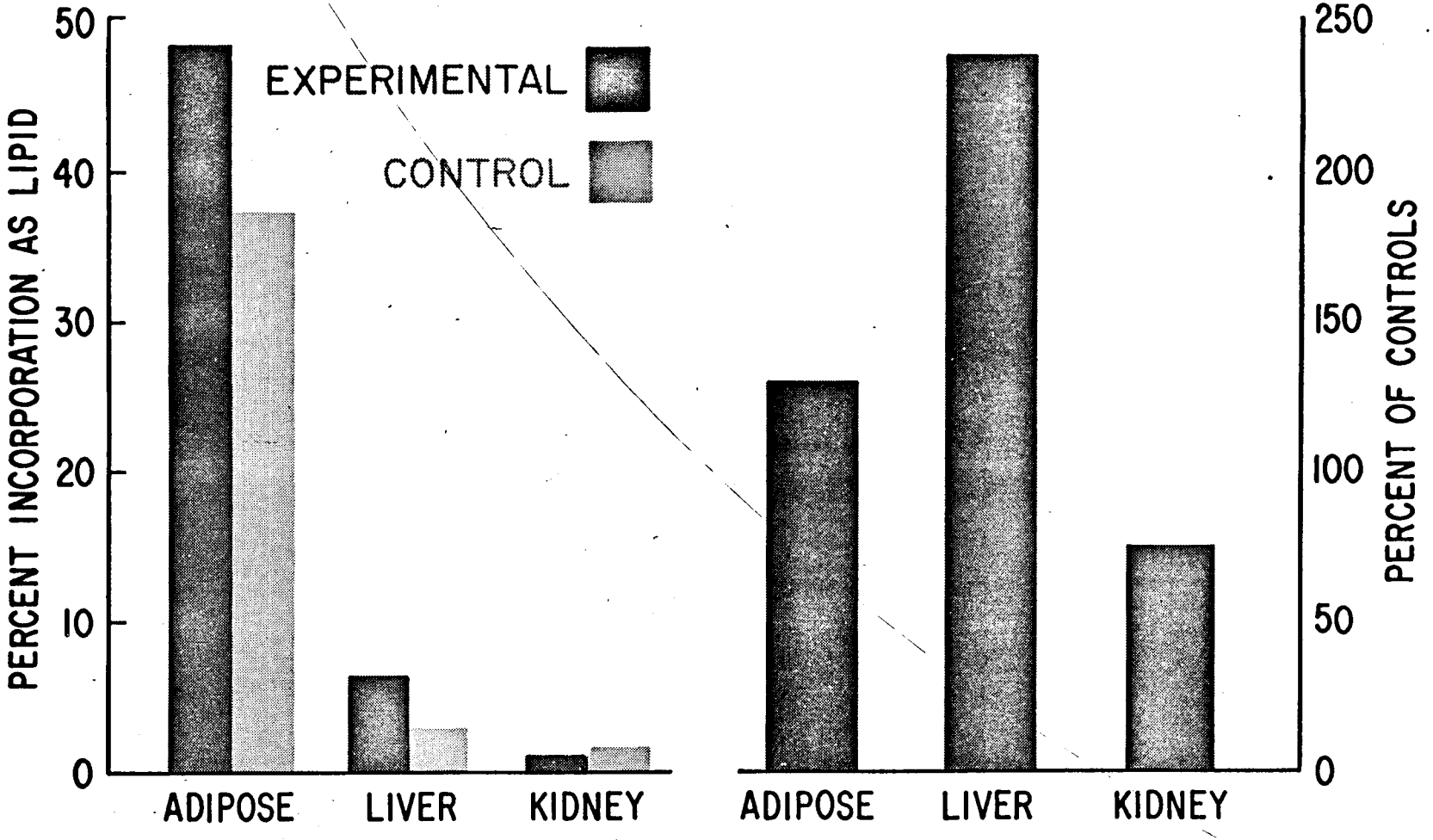

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FIG. 1

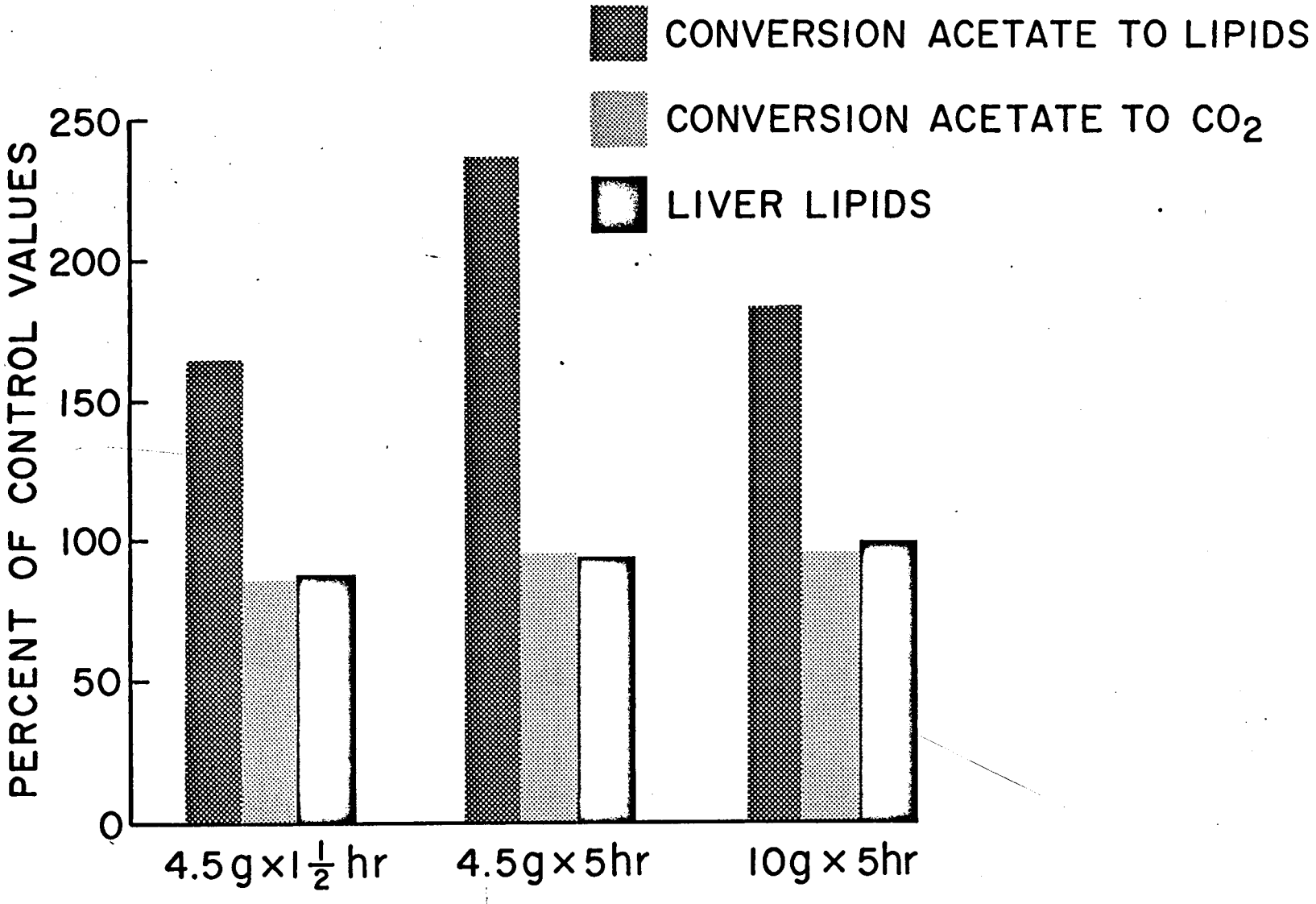

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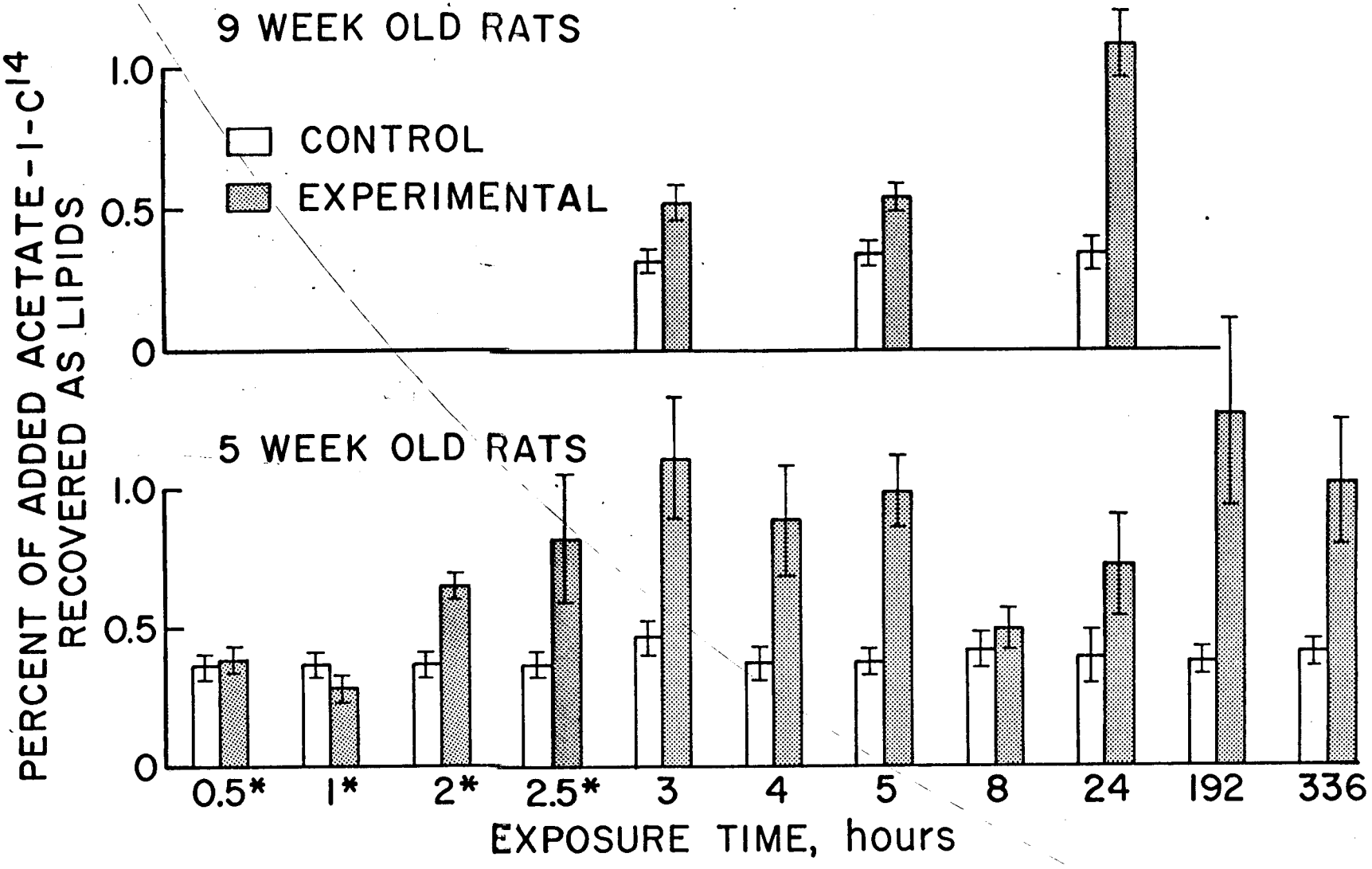
FIG. 2



*COMMON CONTROLS

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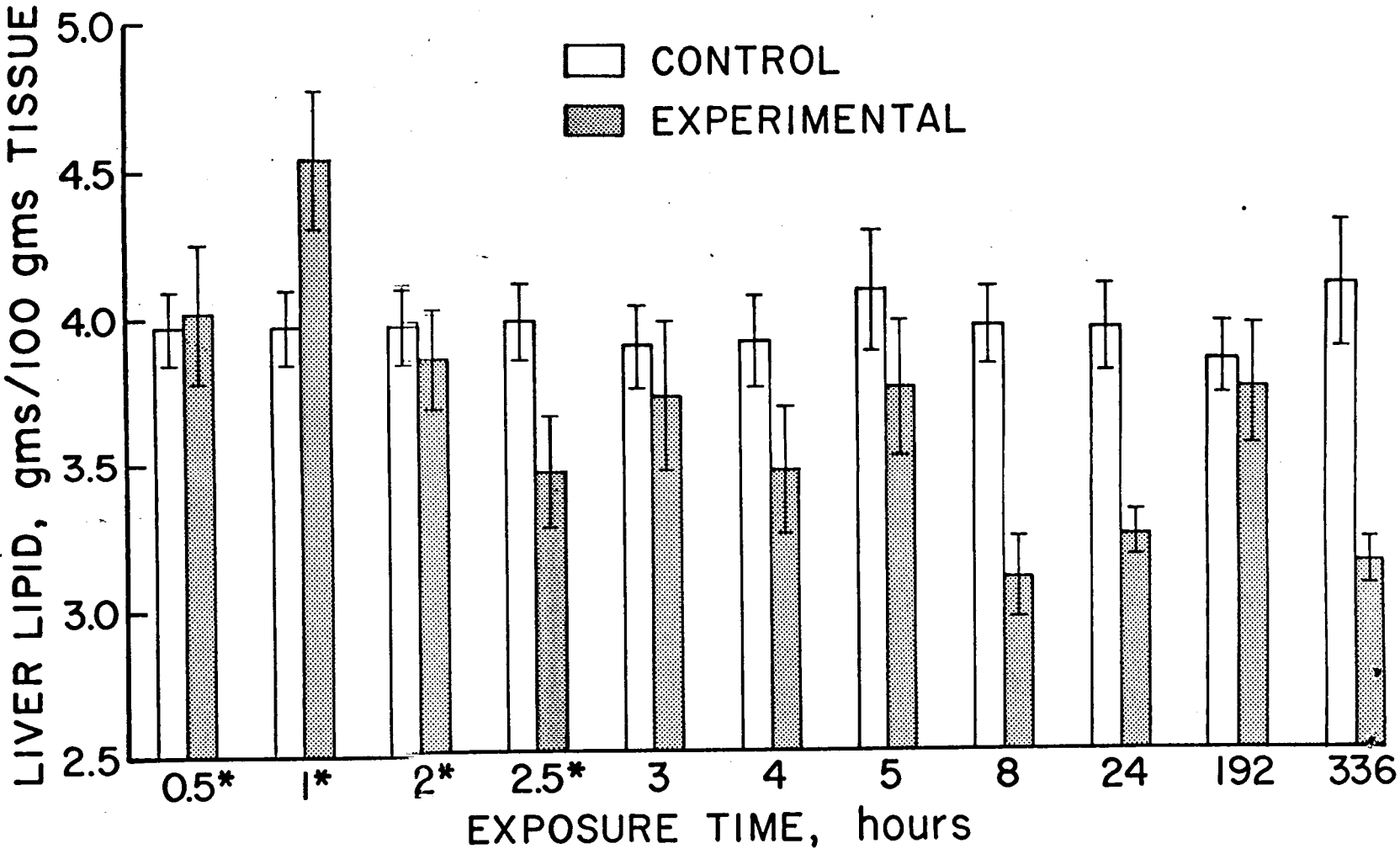
FIG 3



* COMMON CONTROLS

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FIG. 4