bers were destroyed with a median survival time of 10.7 days (S.D. 1.0 day, total number = 121 recipients). Second-set DBA-1/J skin grafts on CFW mice immunized with orthotopic skin grafts, with splenic cells and their fractions(8), or with purified transplantation antigen(9) exhibited gross and histologic stigmata of accelerated reactions by the sixth postoperative day.

The new technique overcomes the principal disadvantage of the bandaging technique of Billingham and Medawar(1), namely inaccessibility to the graft, by sacrificing positive lateral pressure. The excellent results described here, as well as those described by Cannon and Longmire(10), suggest that although positive lateral pressure is undoubtedly beneficial, it is not essential for successful transplantation.

In addition to its value in exposing the early events after skin transplantation, the technique has been of special merit in experiments dealing with the transplantation of fragile embryonic tissues, with the daily application of pharmacologic agents to the graft or its bed, and with the second-set phenomenon where early examination of the test graft is mandatory.

Summary. A simplified chamber technique for bandaging orthotopic murine skin grafts was demonstrated to offer accessibility to the graft and rapid application of the bandages. There were no significant differences between the behavior of orthotopic grafts bandaged with this chamber technique or with the conventional Plaster of Paris method.

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Chemical and Metabolic Changes of Hepatic Lipids from Rats Exposed to Chronic Radial Acceleration. (30226)

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Of the many environments that have come into sharp focus by the recent advances in space exploration, one that needs to be studied in depth is acceleration as it affects human and other mammalian organisms. Studies of acceleration and deceleration are of interest since they are encountered during lift-off and reentry phases of space flights.

Oyama and co-workers(1) have described some general effects on growth, metabolism, body chemistry, and morphology following prolonged exposure of rats to centrifugation. Growth rates and final body weights of rats centrifuged from weaning age to maturity were reduced significantly. Except for minor changes in the relative size of certain organs and some slight changes in hematology, it was observed that rats tolerated and adapted to prolonged exposure of simulated high gravity.

Liver slices from rats subjected to 4.7 *g*

for 3 hours showed an increase in the conversion of acetate to lipids(2). It was of interest to determine whether this alteration in fat metabolism of the acutely exposed rat also occurred in animals exposed for prolonged periods of time. This interest, as well as an attempt to detect changes in metabolism of rats exposed to near tolerance limits (3), has resulted in the study described here.

Methods. A 10 radial-armed centrifuge 8.5 ft in radius was used. Cages (20 × 10 × 10 inches) large enough to hold 4 to 6 rats were suspended by means of a pivotal yoke assembly from each arm positioned at one of 2 fixed distances (6 or 8 ft) from the center of rotation. The cage assembly possessed one degree of freedom moving outwardly during centrifugation. Each cage assumed a final position such that the resultant of the centrifugal and gravitational forces was vertical to the cage floor. The centrifuge was run at a constant 40 rpm. Animals were exposed to a resultant force of either 3.6 or 4.7 *g*. All cages were illuminated with individual fluorescent lights automated to provide on-off cycling at 6 a. m. and 6 p. m., respectively. The centrifuge was run continuously except for daily stoppages of approximately 20 minutes required for the servicing and weighing of the rats. The centrifuge was usually run continuously over the weekends without stopping.

Female Sprague-Dawley rats were used in all studies except for the male rats used in Table I. All rats were fed Purina laboratory chow and water *ad lib.* Noncentrifuged rats (controls) were handled and maintained under conditions as close as possible to those of the centrifuged rats. Since centrifuged rats attained a significantly smaller body mass compared to control rats, weight-controls were also included for comparison. The rats were separated into groups as follows:

Group A: Controls (by age, 15.5 mo. old, 324 ± 23 g)

Group B: Controls (by weight, 3 mo. old, 250 ± 19 g)

Group C: Centrifuged (3.6 *g* from weaning to 12 mo.)

Group D: Centrifuged (3.6 *g* from 3 mo. old to 6 mo., then, 4.7 *g* to 15.5 mo.)

Group E: Centrifuged (4.7 *g* from weaning to 12 mo.)

The rats were fasted for 24 hours before being removed from the centrifuge. They were anesthetized by I.P. injection of nembutal (40 mg/kg body weight) immediately on removal. Approximately 1 g of liver was removed and sliced 0.5 mm thick with a mechanical tissue chopper. Half g samples were placed in a 50 ml screw cap incubation flask (4) containing 5 ml of Krebs bicarbonate buffer(5) at pH 7.4. The buffer medium also had a final concentration of 0.01 M succinate, 0.011 M glucose, and 0.001 M sodium acetate-1-C^{14} (5 μc). Another half g sample of liver slices was treated similarly in a second flask containing 0.001 M sodium acetate-2-C^{14} (5 μc). Incubation was carried out for 3 hours at 37.5°C in a shaker bath under an atmosphere of 95% O_2, 5% CO_2. The reactions were stopped by the addition of 0.2 ml of 10 N H_2SO_4 to the incubation media. The CO_2 fraction was collected overnight with 1 ml of 2.5 N NaOH added to a removable center well.

Lipid extraction was carried out by a modification of the method described elsewhere (5). Nonsaponifiable lipids were extracted 3 times with 8 ml of hexane prior to acidification of the alcoholic-KOH treated tissues. Fatty acids were extracted with fresh aliquots of the same solvent after acidification with 6 N HCl.

The total fatty acid content of the liver was determined by evaporating an aliquot of the extract and weighing the dried residue. The C^{14} content in the fatty acid, nonsaponifiable lipid and CO_2 fractions was determined by liquid scintillation spectrometry.

In an earlier report(2) dealing with exposure of rats to radial acceleration for short durations, the changes in metabolism noted were for total lipids. An experiment was performed here to determine which of 2 lipid fractions, nonsaponifiable lipids or fatty acids, was altered. The data recorded in Table I were obtained from male Sprague-Dawley rats, 9 weeks old, centrifuged at 4.7 *g* for 3 hours. The amount of acetate-1-C^{14} and acetate-2-C^{14} incorporated into these lipid fractions and $C^{14}O_2$ was measured. The

TABLE I. Short-Term Exposure. Recovery of C^{14}-fatty acids, C^{14}-nonsaponifiable lipids and $C^{14}O_2$ after incubation of liver slices with acetate-1-C^{14} and acetate-2-C^{14}.

Fraction	% of acetate-1-C^{14} recovered per g of tissue		% of acetate-2-C^{14} recovered per g of tissue	
	Control	Centrifuged	Control	Centrifuged
Fatty acids	.20	.32	.26	.35
Nonsaponifiable lipids	.21	.29	.28	.36
CO_2	69.7	77.9	23.3	26.5

Half g of tissue obtained from fasted, male Sprague-Dawley rats exposed to 4.7 *g* for 3 hr was incubated for 3 hr at 37.5°C in 5.0 ml of buffer. Each value represents the mean from 6 tissues obtained from 6 individual rats.

data were subjected to analysis of variance and a significant increase in incorporation of acetate into C^{14}-fatty acids was noted in liver from centrifuged rats when compared to noncentrifuged controls ($p<0.001$) for both acetate-1-C^{14} and acetate-2-C^{14}. Although mean values of $C^{14}O_2$ and C^{14}-nonsaponifiable lipids differed for the exposed and control rats, the analysis showed these differences were not statistically significant.

Results. A 2-way analysis of variance, with one variable consisting of 2 control and 3 experimental groups of rats, and the other variable consisting of 2 differently labeled acetates, was performed for each of the 3 fractions analyzed (fatty acids, nonsaponifiable lipids, and CO_2). The means and sample sizes of the experimental data are shown in Table II. Scheffé's(6) method of multiple comparison of the group means was utilized with an overall rejection region of $\alpha = 0.05$.

The analyses show that only the nonsaponifiable lipids significantly differed among the groups. The data in Table II show that the conversion of acetate to nonsaponifiable lipids rose from 0.3% per g of liver for 12-month-old control rats, or from 0.45% for 3-month-old control rats to 0.84% (acetate-1-C^{14}) and 1.20% (acetate-2-C^{14}) for tissue removed from rats exposed to 4.7 *g* for 12 months.

For conversion of acetate to nonsaponifiable lipids, contrasts between means were found to be significant ($p<0.001$) as summarized below:

(a) Group E *vs* Group A
(b) Group E *vs* Group B
(c) Group E *vs* Group C

No significant differences were found between Group D animals which were moved from 3.6 *g* to 4.7 *g* after 3 months, and Group E. Group D was also not found to be significantly different from either of the remaining three groups.

No significant differences were found between groups for conversion of acetate to fatty acid or CO_2.

Since each liver was sampled twice for analysis of fat content, differences between groups were tested by analysis of variance (nested design). The means for each sample population and means for combined samples per experimental group are recorded in Table III. The results of this analysis show that the group means were significantly different ($p<0.001$). Liver lipid content of rats centrifuged at 4.7 *g* were 3.12 and 3.19% as compared with values obtained from controls of 3.80 and 4.14%.

Discussion. An immediate effect of short-

TABLE II. Long-Term Exposure. Recovery of C^{14}-nonsaponifiable lipids, C^{14}-fatty acids, and $C^{14}O_2$ after incubation of liver slices with acetate-1-C^{14} and acetate-2-C^{14}.

Experimental groups	Sample size	% of acetate-1-C^{14} recovered per g tissue as			% of acetate-2-C^{14} recovered per g tissue as		
		Nonsaponifiable lipids	Fatty acids	CO_2	Nonsaponifiable lipids	Fatty acids	CO_2
A (15.5-mo-old controls: 324 g)	6	.293	.233	75.1	.290	.280	24.8
B (3-mo-old controls: 250 g)	10	.449	.295	65.3	.466	.325	22.8
C (3.6 *g*, weaning to 12 mo)	9	.312	.329	68.2	.359	.438	27.3
D (3.6 *g*, 3 to 6 mo, 4.7 *g* to 15.5 mo)	7	.581	.241	64.1	.687	.289	24.2
E (4.7 *g*, weaning to 12 mo)	9	.844	.379	78.0	1.200	.441	28.2

Liver slices from fasted female Sprague-Dawley rats exposed for long terms were treated as in Table I.

TABLE III. Mean Values for Fatty Acid Content of Liver Obtained from Rats Exposed to Radial Acceleration Listed by Experimental Group and Samples.

Exp group	Sample size	Liver lipids (g per 100 g liver × 100) Sample 1 mean	Sample 2 mean	Group mean
A	6	3.88	3.71	3.80
B	10	4.01	4.27	4.14
C	9	3.75	3.76	3.76
D	7	3.28	3.10	3.19
E	9	3.02	3.22	3.12

term acceleration stress is the dramatic change in metabolism of the 3 primary foodstuffs: carbohydrate, fat, and protein(1,2,7). Oyama(3) has shown that rats exposed to relatively high *g*-fields for prolonged periods readily adapt to their new environment. The animals appeared to be healthy; they gained weight at a somewhat slower rate and if the gravitational field was not too high, they were able to carry on certain normal physiological functions such as reproduction. Investigation of the effects of short-term exposure to increased *g* showed an increased incorporation of C^{14}-acetate to lipids(2). The principal effect in animals so treated was in the amount of acetate incorporated into fatty acids.

In the present study, it was shown that prolonged centrifugation of rats did not affect the incorporation of acetate to fatty acids, but, did affect the incorporation of acetate into nonsaponifiable lipids. This effect was noted only when the gravitational field was at 4.7 *g*, the level that approaches the tolerance limit(3). Current studies indicate that changes occurring as a result of acute acceleration are mediated by the neuroendocrine system(8) which are then modified or adapted after prolonged exposure.

Summary. From weaning until 1 year of age female Sprague-Dawley rats were centrifuged at 3.6 and 4.7 *g*. Liver slices of these rats were incubated with C^{14}-labeled acetate and its incorporation into fatty acids, nonsaponifiable lipids, and CO_2 was measured. When compared with tissue from control rats, liver slices of centrifuged rats exposed to 4.7 *g* showed an increased formation of C^{14}-nonsaponifiable lipids. Comparable changes were not observed in rats exposed to 3.6 *g*. No significant alteration was noted for incorporation of acetate into fatty acids or CO_2. The total lipid content of the liver was decreased significantly in rats exposed to 4.7 *g*. The response evoked from long-term exposure affected synthesis of nonsaponifiable lipids whereas short-term exposure affected fatty acid synthesis.

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Survival of *Plasmodium gallinaceum* Sporozoites in Previously-Excised Skin of Mouse and Chick.* (30227)

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The familiar barnyard fowl is highly susceptible to infection with *Plasmodium gallinaceum* and the laboratory albino mouse is absolutely resistant. Having previously shown (1) that when *Aedes aegypti* mosquitoes introduce the sporozoites of this organism into the skin of the mouse there is an almost im-

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