PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 172, 334–339 (1983)

Biochemical Changes in Rat Liver after 18.5 Days of Spaceflight (41566)

S. ABRAHAM, C. Y. LIN, C. M. VOLKMANN,* AND H. P. KLEIN*

*Children's Hospital Medical Center, 51st and Grove Streets, Oakland, California 94609, and *NASA–Ames Research Center, Moffett Field, California 94035*

Abstract. The effect of "weightlessness" on liver metabolism was examined using tissue from rats flown in earth orbit for 18.5 days aboard the Soviet Cosmos 936 biosatellite. Changes in the activities of certain carbohydrate and lipid enzymes were noted. Of the 28 hepatic enzyme activities assayed, two, palmitoyl-CoA desaturase and lactate dehydrogenase, increased, whereas five, glycogen phosphorylase, 6-phosphogluconate dehydrogenase, both acyltransferases which act on α-glycerolphosphate and diglycerides, and aconitate hydratase decreased. The remaining enzyme activities measured were unchanged. In addition, increased levels of liver glycogen and palmitoleate were noted which probably resulted from the lowered glycogen phosphorylase and increased palmitoyl-CoA desaturase activities, respectively, in those animals that experienced weightlessness. These changes caused by weightlessness were transient since all of the aforementioned alterations returned to normal values when measured in the livers of other rats which had flown in the biosatellite 25 days after recovery.

In 1973, the USSR initiated a series of Cosmos spaceflights in which rats were the principal objects of experimental study. In each of these flights, the animals were flown for approximately 3 weeks and, after recovery, various tissues were subjected to extensive morphological and chemical analyses in order to ascertain whether any significant effects could be attributed to spaceflight. Examination of liver, blood, muscle, and skeletal tissues from rats aboard the early Cosmos flights indicated changes in the lipid and carbohydrate levels of these tissues in response to spaceflight. However, the nature of the reported changes depended upon the type of tissue analyzed and was a function of the techniques used for tissue analyses. For example, when total carbohydrates, lipids, and phospholipids of rat liver were determined after the Cosmos 605 flight, Belitskaya (1) reported that only the phospholipids increased as a result of exposure to weightlessness. However, in another communication, Yakovleva (2) using histochemical techniques, reported that liver tissue from animals aboard Cosmos flights 605 and 782 had significantly more total lipids at recovery than did ground controls. In skeletal tissue, evidence was presented for increased glycolytic rates, and changes were reported in the patterns of the lactate dehydrogenase isoenzymes of this tissue after the Cosmos 690 flight (3). The Cosmos 690 flight rats also showed increased plasma triglycerides and cholestrol (4). As part of this flight, the Soviets and their colleagues also investigated the major lipids of skeletal muscle, and in contrast to the situation in liver (1) reported a decrease in the amount of phospholipids in the microsomal fraction isolated from this tissue (5).

Since virtually all of the data on compositional changes in lipids and carbohydrates of rat tissues were obtained in the absence of any information about the enzyme activities involved, when the opportunity arose to participate in the Cosmos 936 flight (6), we proposed a comprehensive survey of hepatic enzymes of carbohydrate and lipid metabolism. The results of that study form the basis of this report.

Materials and Methods. Cosmos 936 flight was launched on August 3, 1977 and recovery was on August 22, 1977 in a remote area of the USSR (6). Our experimental protocol called for 20 male Wistar (SFP) rats (approximately 62 days old which weighed an average of 215 g at the start of the experiment), individually caged within the spacecraft. These animals were supplied by the Institute of Experimental Endocrinology of the Slovakian Academy of Sciences, Bratislava, Czechoslovakia. Ten animals served as the "weightless" group, and the remaining 10 control rats were placed on a specially constructed centrifuge (35-cm radius) designed to provide artificial gravity (1*g*) by rotating continuously (53.5 ± 3

0037-9727/83 $1.50

rpm) during flight (18.5 days).[1] *For the first time, the availability of an on-board centrifuge made it possible to resolve possible effects of weightlessness from other flight factors.* Since both groups of rats were subjected to similar environments, including caging and diet as well as the stresses of launch, reentry, and recovery, the two groups were considered to be essentially identical except for the gravity component during flight. Five rats from each of the two flight groups were sacrificed within approximately 4.5 hr after recovery of the space module (R_0) and the remaining 10 rats 25 days after recovery (R_{25}). Other experimental details concerning this flight, including the composition of the diet used, the food consumed as well as the body and the liver weights of the individual rats, were all given previously (6).

Tissue and homogenate preparation. Rats were sacrificed by decapitation at the recovery site at R_0; their livers removed as quickly as possible and immediately placed into ice-cold 0.25 *M* sucrose solution. Two pieces of liver (about 100 mg each) were taken for glycogen and fatty acid analyses. These were wrapped in tin foil and frozen in dry ice. Four-gram portions of each liver were minced and individually homogenized in a Potter-Elvehjem type tissue grinder with exactly 12.0 ml of fresh ice-cold 0.25 M sucrose solution as given previously (7). All subsequent preparative procedures were carried out at 0–4°C.

Nuclei, cell debris, and mitochondria were sedimented at 4500*g* for 30 min in a refrigerated centrifuge (Beckman). The mixed pelleted fractions were discarded and the supernatant fractions (obtained with the aid of a disposable pipet from under the free floating fat) retained. These fractions were immediately frozen to dry-ice temperatures in plastic tubes and shipped from the recovery site to our laboratories in the United States. Animals sacrificed at R_{25} (25 days after recovery) were processed in the Soviet Union in an identical manner.

Upon arrival, the supernatant fractions (containing the cytosol and microsomal fractions of the liver) were quickly thawed and subjected to centrifugation in a Spinco Ultracentrifuge at 100,000*g* for 60 min at 0–4°C. The cytosol (particle-free supernatant fraction) and the microsomes (pellet) were separated. The cytosol fraction from each rat liver was divided into many small aliquots which were placed into individual plastic test tubes, quick frozen, and stored at −80°C until used for enzymatic analysis. Once thawed, the sample was immediately used for the assay of enzyme activity and was not repeatedly frozen and thawed. The microsomal fractions were treated in a similar manner.

Fatty acid and glycogen analyses. Samples of frozen liver were thawed, weighed (100 mg), and saponified in 1.0 ml of 3 *N* potassium hydroxide in 50% methanol at 90°C for 12 hr under reflux. Isolation of the fatty acid fractions, preparation of the methyl esters, and quantitative separation of each fatty acid methyl esters, and quantitative separation of each fatty acid methyl ester by gas chromatography was performed as previously described (7).

Other samples of liver (ranging from 60 to 110 mg but precisely weighed out) were taken for analysis of glycogen content by the anthrone procedure as published previously (8).

Enzyme assays. All enzyme assays, unless otherwise noted, were performed with either the 100,000*g* supernatant fraction (cytosol) or the pellet (microsomes) obtained from this centrifugation after removal of the mitochondria. Enzyme activities in the cytosol fractions were determined by following the changes in optical density of the reaction mixtures at 30°C with a Gilford automatic recording spectrophotometer. In all enzyme assays, concentrations of substrates and of added enzymes (where needed) were at least 10 times those required to yield maximal activities. Absorbancy changes were measured with reference to reaction mixtures, devoid either of substrates or of coenzymes. The reactions were started by additions of substrates after a brief period of preincubation of the enzyme in the reaction mixture. Depending upon the assay, 0.0025 to 0.050 ml of the enzyme-containing fraction was used. Measurements of initial velocities were made under conditions in which kinetics were zero-order and activity was proportional to enzyme concentration.

[1] The actual calculated *g* force during flight was 1.7×10^{-7} to $1.5 \times 10^{-4}g$.

Specific activities of the cytosol enzymes were reported as nanomoles either of pyridine nucleotide, oxidized or reduced, or of substrate converted to product per milligram protein per minute, whereas those for the microsomal enzymes are given in Table I. The following extinction coefficients were used in the calculations: reduced NADP (NADPH) and NADH (340 nm), 6.22×10^3 liter $\times$ mole^{-1} $\times$ cm^{-1} (9); *cis*-aconitate (240 nm), 3.54×10^3 liter $\times$ mole^{-1} $\times$ cm^{-1} (10). Protein was determined by the method of Gornall *et al.* (11) or Lowry *et al.* (12).

Enzymes were measured according to well-established methods or modifications of currently used techniques as indicated below:

(a) Cytosolic enzymes—glycogen synthetase by the spectrophotometric method coupled with pyruvate kinase and lactic dehydrogenase given in (13), glycogen phosphorylase (14), hexokinase and glucokinase (15), phosphoglucomutase (16), glucose-6-phosphate dehydrogenase (17), 6-phosphogluconate dehydrogenase (18), phosphofructokinase (16), fructose-1,6-diphosphatase (19), pyruvate kinase (20), lactate dehydrogenase with L-lactate as substrate (21), α-glycerophosphate dehydrogenase (15), glutamate–oxaloacetate transaminase (22), glutamate–pyruvate transaminase (23), citrate cleavage enzyme (15), malic enzyme (24), malate dehydrogenase with L-malate as substrate (25), fumarase with L-malate as substrate (26), isocitrate dehydrogenase (27), acetyl-CoA carboxylase (15), fatty acid synthetase (15), and aconitate hydratase (28).

(b) Microsomal enzymes—palmitoyl-CoA synthetase was assayed according to the procedure described by Polokoff and Bell (29) and acyl-CoA desaturase activity according to Klein and Volkmann (30). Enzyme activity was calculated as nanomoles of oleate or palmitoleate (depending upon the substrate used) produced per 5 min per mg protein. The glucose-6 phosphatase activity was assayed according to the method described by Nordlie and Arion (31). The orthophosphate liberated from glucose-6-phosphate was measured by a modification of the Berenblum and Chain method as described by Lindberg and Ernster (32).

α-Glycerolphosphate acyltransferase and diglyceride acyltransferase activities of hepatic microsomes were measured according to the procedure reported previously (33, 34). *sn*-[U-^{14}C]Glycero-3-phosphate and palmitoyl-CoA were used as substrates for the former acyltransferase and *sn*-1,2,dilaurin and [1-^{14}C]palmitoyl-CoA were the substrates for the latter acyltransferase. In the case of diglyceride acyltransferase, the synthesized ^{14}C-labeled triglycerides were isolated from thin-layer chromatography plates in which chloroform:acetone, 100:1.5 (v/v) was used as the solvent system (33).

Chemicals. All chemicals, cofactors, and auxilliary enzymes were obtained from commercial sources and were of the highest purity available.

Results and Discussion. Extensive preliminary experiments with rats of the same strain coming from the same source and fed the identical diet demonstrated that all of the activity of each enzyme assayed was stable under the conditions of storage, sample preparation, etc. used (i.e., at −80°C). In addition, these experiments also proved that all activities measured were retained in the proper cellular location, even after freezing of the post-mitochondrial supernatant fractions for as long as 2 weeks. Thus, the data contained in Table I represent enzymatic activities and the amounts of specific constituents in the liver of the rats *at the time of sacrifice.*

When the data on the enzymatic activities of the weightless and centrifugal groups of rats were compared of the 28 enzymes assayed, the following which act in carbohydrate metabolism were unchanged after spaceflight: glycogen synthetase, hexokinase, glucokinase, phosphoglucomutase, glucose-6-phosphate dehydrogenase, phosphofructokinase, fructose-1,6-diphosphatase, pyruvate kinase, glutamate–oxaloacetate transaminase, glutamate–pyruvate transaminase, citrate cleavage enzyme, malic enzyme, malate dehydrogenase, fumarase, isocitrate dehydrogenase, and glucose-6-phosphatase. However, seven of the enzymes surveyed yielded different values for the two groups of animals (Table I); whereas palmitoyl-CoA desaturase and lactate dehydroganase increased, glycogen phosphorylase, 6-phosphogluconate dehydrogenase, aconitate hydratase, α-glycerolphosphate acyltransferase, and diglyceride acyltransferase decreased.

TABLE I. EFFECT OF MICROGRAVITY ON LIVER CONSTITUENTS AND ENZYME ACTIVITIES OF RATS EXPOSED TO SPACEFLIGHT SACRIFICED IMMEDIATELY (R_0) AND AFTER 25 DAYS POST FLIGHT (R_{25})

	Group sacrificed at[a]:					
	R_0			R_{25}		
Measurement	Weightless	Centrifuged	*P* Value[b]	Weightless	Centrifuged	*P* Value[b]
Glycogen[c]	7.4 ± 2.0	3.5 ± 0.8	0.01	1.8 ± 0.5	2.5 ± 0.7	NS
Glycogen phosphorylase[d]	31.1 ± 3.6	37.4 ± 2.9	0.05	44.4 ± 3.8	43.5 ± 2.0	NS
Glycogen synthetase[d]	11.2 ± 1.0	12.4 ± 2.2	NS	14.0 ± 2.5	16.9 ± 3.5	NS
Diglyceride acyltransferase[d]	0.69 ± 0.25	1.69 ± 0.63	0.01	2.04 ± 0.31	1.62 ± 0.36	NS
α-Glycerol phosphate acyltransferase[d]	0.10 ± 0.07	0.24 ± 0.12	NS[f]	0.23 ± 0.0	0.17 ± 0.05	NS
Palmitic acid/ palmitoleic acid	7.1 ± 2.5	11.1 ± 1.1	0.01	15.2 ± 9.1	11.6 ± 3.5	NS
Palmitoyl-CoA[e] desaturase	3.7 ± 0.8	2.1 ± 0.4	0.02	0.9 ± 0.1	1.1 ± 0.1	NS
6-Phosphogluconate dehydrogenase[d]	39.1 ± 4.5	51.0 ± 5.9	0.02	59.6 ± 10.2	68.0 ± 7.2	NS
Lactic acid dehydrogenase[d]	11,052 ± 601	9932 ± 530	0.05	10,200 ± 1,453	10,521 ± 513	NS
Aconitate hydratase[d]	80.0 ± 8.8	92.5 ± 2.4	0.05	47.2 ± 1.0	46.6 ± 3.9	NS

[a] Mean values per group of five rats ± SD.
[b] Level of significance of differences between "weightless" and centrifuged rats calculated from Students *t* distribution (38). NS = not statistically significant, i.e., $P > 0.05$.
[c] Given as percentage wet weight of liver.
[d] Given as nanomoles of product produced per min per milligram protein.
[e] Given as nanomoles of palmitoleate formed per 5 min per milligram protein.
[f] This value, although not statistically significant, gave a *P* value between 0.05 and 0.10.

In most cases, the reasons for and the consequences of the observed differences in enzyme activities are unknown. However, the decreased activity of *glycogen phosphorylase* in flight rats may be related to another striking difference observed between the two sets of flight animals. The livers of the weightless group at R_0 contained, on the average, more than twice the amount of glycogen than that of the centrifuge group.[2] The difference in glycogen levels between the two groups may be explained by the finding that *glycogen synthetase* levels were unaffected during spaceflight while *glycogen phosphorylase* showed a 20% decrease in activity. Of interest is the fact that as with all of the enzymes shown to be affected, 25 days after recovery, the glycogen content of the livers of the weightless animals was now no longer statistically different from that of the centrifuged controls and, at this time, the *phosphorylase* activity of the weightless rats was at the same level found in the centrifuged animals (Table I).

Another correlation between a change in enzyme activity and liver constitutents was noted in the case of *palmitoyl-CoA desaturase* and lipid composition. Although we did not attempt a complete inventory of hepatic lipids, we did measure the pattern of fatty acids. Gas chromatographic analyses revealed a remarkable shift in the ratio of palmitic to palmitoleic acids,[3] the weightless (uncentrifuged) group having a higher fraction of the unsaturated acid (Table I). This change is undoubtedly related to the activity of the *palmitoyl-CoA desaturase*, which was found to have 75% more activity in the weightless animals than in the centrifuged ones (Table I). Once again, analyses at R_{25} revealed that the livers of the stationary and the centrifuged animals had similar fatty acid patterns and equivalent *desaturase* activities.

[2] Large increases in liver glycogen of flight rats are also reported by Nemeth *et al.* (35).

[3] No palmitoleic acid was present in the rat diets during the entire experimental period.

Several other key enzymes of lipid biosynthesis, many of which are known to be subject to regulation, were also assayed in this study. These included *acetyl-CoA caboxylase, fatty acid synthetase, palmitoyl-CoA synthetase,* and *stearoyl-CoA desaturase.* All of these enzymes, which are involved in the conversion of acetyl-CoA into long-chain fatty acids and their CoA esters, were found not to be affected by the weightless condition. However, the ability of the liver to complex, long-chain fatty esters may have been significantly altered in the weightless group since the *acyltransferases* for both *sn-glycero-3-phosphate* and *diglyceride* were reduced in activity by 60 and 70%, respectively, when compared with the centrifuged animals (Table I). The decreased activity of these two enzymes suggests an impaired ability of the liver to form triglycerides and phospholipids, although in the earlier Cosmos 605 flight Belitskaya reported finding increases in the phospholipid content of rat liver postflight (1).

The results of these studies, taken as a whole, indicate that certain constituents and enzymes of liver metabolism are indeed altered by exposure to microgravity. The alterations we have uncovered, particularly those that involve increased glycogen levels and decreased activities of the *acyltransferases*, appear to be unique to the weightless condition.[4] Heretofore, the correlation between the glycogen level and the *acyltransferase* activity in liver has been found to be parallel. Under physiological conditions where conditions were manipulated to produce low glycogen levels (e.g., in fasted animals), the transferase enzymes were also reduced in activity (36, 37). However, our data indicate a negative correlation under spaceflight conditions. It would be interesting to know whether similar biochemical events occur in humans exposed to weightlessness and, if so, what overall effects these might produce, particularly since some humans have now been exposed to microgravity for periods of up to 7 months.

It is also noteworthy that all of the changes observed in rats after 18.5 days of spaceflight disappear by 25 days after flight. At present, one can only speculate about the rates at which the observed changes, both in weightlessness and after reexposure to the Earth gravity, and about the regulatory mechanisms that might be involved.

This work was partially supported by Contract NAS2-9523 from the National Aeronautics and Space Administration, Washington, D.C. to S.A.

[4] We have recently completed analyzing rat liver tissues from Cosmos 1129, flown in 1979 without an onboard centrifuge, and can confirm the findings of increased liver glycogen and decreased acyltransferase activities seen in the Cosmos 936 experiment.

1. Belitskaya RA. Carbohydrate and lipid content of rat liver tissue following a 22-day space flight. Space Biol and Aerospace Med **4**:97–99, 1977.
2. Yakovleva VA. Dynamics of changes in lipid content in the liver of rats exposed aboard the Kosmos 605 and Kosmos 782 biosatellites. Arkh Anat Gistol Embriol **73**:39–44, 1977.
3. Petrova NV. Lactate dehydrogenase isoenzymes of rat skeletal muscles following a space flight and with hypokinesia. Space Biol and Med **5**:80–87, 1977.
4. Ahlers I, Misurova E, Praslicka M, Tigranyan RA. Biochemical changes in rats flown on board the Cosmos 690 biosatellite. In: Sneath PHA, ed. Sciences and Space Research. Berlin Akademie-Verlag, Vol 14: pp185–188, 1976.
5. Belitskaya RA. Changes in amount and composition of phospholipids in rat skeletal muscle microsomal fractions under the influence of a flight aboard the Kosmos 690 biosatellite. Space Biol and Med **1**:23–28, 1979.
6. Rosenzweig SN, Souza KA. Final reports of U.S. experiments flown on the Soviet satellite Cosmos 936. NASA Tech Mem 78526, Sept. 1978.
7. Abraham S, Matthes KJ, Chaikoff IL. Factors involved in synthesis of fatty acids from acetate by a soluble fraction obtained from lactating rat mammary gland. Biochim Biophys Acta **49**:268–285, 1961.
8. Hassid WZ, Abraham S. Chemical procedures for analysis of polysaccharides. I. Determination of glycogen and starch. In: Colowick SP, Kaplan NO, eds. Methods in Enzymology. New York, Academic Press, Vol 3: pp34–38, 1957.
9. Horecker BL, Kornberg A. The extinction co-efficients of the reduced band of pyridine nucleotides. J Biol Chem **175**:385–390, 1948.
10. Rauen HM. Biochemisches Taschenbuch. Berlin, Springer Verlag, p. 988, 1956.
11. Gornall AG, Bardawill CJ, David MM. Determination of serum protein by means of the biuret reaction. J Biol Chem **177**:751–766, 1949.
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J Biol Chem **193**:265–275, 1951.
13. Mendicino J, Abou-Issa H, Medicus R, Kratowich N. Fructose-1,6-diphosphatase, phosphofructoki-

nase, glycogen synthetase, phosphorylase, and protein kinase from swine kidney. I. Isolation and properties of glycogen synthetase. In: Wood WA, ed. Methods in Enzymology. New York, Academic Press, Vol 42: pp375–381, 1975.
14. Mendicino J, Abou-Issa H, Medicus R, Kratowich N. Fructose-1,6-diphosphatase, phosphofructokinase, glycogen synthetase, phosphorylase, and protein kinase from swine kidney. IV. Purification and properties of phosphorylase. In: Wood WA, ed. Methods in Enzymology. New York, Academic Press, Vol 42: pp389–394, 1975.
15. Smith S, Gagné HT, Pitelka DR, Abraham S. The effect of dietary fat on lipogenesis in mammary gland and liver from lactating and virgin mice. Biochem J **115**:807–815, 1969.
16. Abraham S, Kopelovich L, Kerkof PR, Chaikoff IL. Metabolic characteristics of preparations of isolated sheep thyroid cells. I. Activity levels of enzymes concerned with glycolysis and the tricarboxylic acid cycle. Endocrinology **76**:178–190, 1965.
17. Kornberg A, Horecker BL. Glucose-6-phosphate dehydrogenase. In: Colowick SP, Kaplan NO, eds. Methods in Enzymology. New York, Academic Press, Vol 1, pp323–326, 1955.
18. Horecker BL, Smyrniotis PZ. 6-Phosphogluconic dehydrogenase. In: Colowick SP, Kaplan NO, eds. Methods in Enzymology. New York, Academic Press, Vol 1, pp326–327, 1955.
19. Pontremoli S. Fructose-1,6-diphosphatase. In: Wood WA, ed. Methods in Enzymology. New York, Academic Press, Vol 9, pp625–631, 1966.
20. Bucher T, Pfleiderer G. In: Colowick SP, Kaplan NO, eds. Methods in Enzymology. New York, Academic Press, Vol 1, pp435–440, 1955.
21. Yoshida A, Freese E. Lactate dehydrogenase from *Bacillus subtilis*, In: Wood WA, ed. Methods in Enzymology. New York, Academic Press, Vol 41, pp304–309, 1975.
22. Bergmeyer H, Bernt E. Glutamate–oxaloacetate transaminase. In: Bergmeyer H, ed. Methods of Enzymatic Analysis. New York, Academic Press, pp837–845, 1963.
23. Bergmeyer H, Bernt E. Glutamate–pyruvate transaminase. In: Bergmeyer H, ed. Methods of Enzymatic Analysis. New York, Academic Press, pp846–853, 1963.
24. Ochoa S. Malic enzyme. In: Colowick SP, Kaplan NO, eds. Methods in Enzymology. New York, Academic Press, Vol 1, pp739–753, 1955.
25. Ochoa S. Malic dehydrogenase from pig heart. In: Colowick SP, Kaplan NO, eds. Methods in Enzymology. New York, Academic Press, Vol 1, pp735–739, 1955.
26. Hill RL, Bradshaw RA. Fumarase E.C. 4.2.1.2 L-malate hydrolase. In: Lowenstein JM, ed. Methods in Enzymology. New York, Academic Press, Vol 13, pp91–99, 1969.
27. Ochoa S. Isocitric dehydrogenase system (TPN) from pig heart. In: Colowick SP, Kaplan NO, eds. Methods in Enzymology. New York, Academic Press, Vol 1, pp699–704, 1955.
28. Racker E. Spectrophotometric measurements of the enzymatic formation of fumaric and cis-aconitic acids. Biochim Biophys Acta **4**:211–214, 1950.
29. Polokoff MA, Bell RM. Millipore filter assay for long-chain fatty acid:CoASH ligase activity using ^{3}H-labeled coenzyme A. J Lipid Res **16**:344–345, 1975.
30. Klein HP, Volkmann CM. Factors affecting the palmitoyl coenzymes and desaturases of *Saccharomyces cerevisiae*. J Bacteriol **124**:185–188, 1975.
31. Nordlie RC, Arion WJ. Glucose-6-phosphatase. In: Colowick SP, Kaplan NO, eds. Methods in Enzymology. New York, Academic Press, Vol 11, pp619–625, 1966.
32. Lindberg O, Ernster L. Determination of organic phosphorus compounds by phosphate analysis. In: Glick D, ed. Methods of Biochemical Analysis. New York, Interscience, 1960.
33. Lin CY, Smith S, Abraham S. Acyl specificity in triglyceride synthesis by lactating rat mammary gland. J Lipid Res **17**:647–656, 1976.
34. Tanioka H, Lin CY, Smith S, Abraham S. Acyl specificity in glyceride synthesis by lactating rats. Lipids **9**:229–234, 1974.
35. Nemeth S, Macho L, Palkovic M, Skottova N, Tigranyan RA. Activity of some rat liver enzymes following flight aboard Cosmos-936 biosatellite. Adv Space Res **1**:219, 1981.
36. Fallon HJ, Kemp EL. Effects of diet on hepatic triglyceride synthesis. J Clin Invest **47**:712–719, 1968.
37. Numa S, Yamashita S. Regulation of lipogenesis in abnormal tissues. In: Stadtman ER, ed. Current Topics in Cellular Regulation. New York, Academic Press, Vol B, pp197–246, 1973.
38. Snedecor GW, Cochran WG. Student's *t*-distribution. In: Statistical Methods, 6th edition. Ames, Iowa State Univ. Press, pp59–61, 1967.

Received March 23, 1982. P.S.E.B.M. 1983, Vol. 172.