N90-26474

EXPERIMENT K-6-21

EFFECT OF MICROGRAVITY ON 1) METABOLIC ENZYMES OF TYPE I AND TYPE 2 MUSCLE FIBERS AND ON 2) METABOLIC ENZYMES, NEUROTRANSMITTER AMINO ACIDS, AND NEUROTRANSMITTER ASSOCIATED ENZYMES IN MOTOR AND SOMATOSENSORY CEREBRAL CORTEX

PART I: METABOLIC ENZYMES OF INDIVIDUAL MUSCLE FIBERS

PART II: METABOLIC ENZYMES OF HIPPOCAMPUS AND SPINAL CORD

Principal Investigator:

O. Lowry
Washington University School of Medicine
St. Louis, Missouri

Co-Investigators:

D. McDougal, Jr.
Patti M. Nemeth
Maggie, M.-Y. Chi,
M. Pusateri
J. Carter,
J. Manchester
Beverly Norris
Washington University School of Medicine
St. Louis, Missouri

I. Krasnov
Institute of Biomedical Problems
Moscow, USSR

EXPERIMENT K-6-21

PART I: METABOLIC ENZYMES OF INDIVIDUAL MUSCLE FIBERS

INTRODUCTION

The individual fibers of any individual muscle vary greatly in enzyme composition, a fact which is obscured when enzyme levels of a whole muscle are measured. The purpose of this study was therefore to assess the changes due to weightless on the enzyme patterns composed by the individual fibers within the flight muscles.

METHODS

Small portions of soleus (slow-twitch) and tibialis anterior (TA, fast-twitch) muscles were freeze-dried at -35 deg. C. Portions of individual fibers, 2-3 mm long, were dissected free, weighed, and stored separately under vacuum at -70 deg. C. Studies were made on 64 soleus and 164 tibialis fibers from 2 synchronous and 2 flight animals. Each fiber was analyzed in duplicate for 2 to 8 different enzymes, and the size (µg/mm) determined. This involved more than 2300 quantitative measurements.

The work was expedited by a preliminary study which showed that most of the enzymes of interest can be extracted and stored without loss at -70 deg. C in a special glycerol-KC1-detergent medium. Each dry sample, weighing about 0.5 µg (0.5 to 1 mm long), was added to 5 µ1 of this special medium under mineral oil. After incubation for 2 hours at room temperature, the samples were transferred to a -70 deg. C freezer. Since each assay required only 0.1 to 0.2 µ1 of extract (equivalent to 10 to 20 ng of dry fiber), the single 5 µ1 extract was sufficient for duplicate assays of a large number of different enzymes.

RESULTS

The enzymes which were measured fall into two groups, four which are usually most active in slow-twitch and fast-twitch-oxidative fibers: hexokinase, and three enzymes of oxidative metabolism, citrate synthase, malate dehydrogenase (MDH), and ß-hydroxyacyl CoA dehydrogenase, and four enzymes which are most active in fast-twitch-glycolytic fibers: glycogen phosphorylase, glycerol phosphate dehydrogenase, pyruvate kinase, and lactate dehydrogenase (LDH).

Control Activities:

In the synchronous muscles the average for three of the four enzymes of the oxidative, hexokinase group were 45 to 50% higher in soleus than in TA muscles (Fig. 1, Table 1). Citrate synthase was the exception in having similar activities in the two muscles. In contrast, in the synchronous animals the fast-twitch-glycolytic group of enzymes were 7- to 12-fold higher in TA than soleus muscles (Fig. 1, Table 2). Average fiber size was almost the same for both types of muscle.

The enzyme variability among the fibers of each muscle type are of some interest. Figure 2 shows that the coefficient of variation (CV) differs markedly among the different enzymes, and between the two muscle types. In the synchronous muscles, all of the CV's were much higher (64% to 900%) for slow-twitch enzymes of TA than for those of soleus muscles, whereas the reverse was true for fast-twitch-glycolytic enzymes. (Analytical errors were in the order of 5% and were, therefore, almost negligible relative to these large C.V.'s). Note the greater variability in TA for ß-hydroxyacyl CoA dehydrogenase (a key enzyme of fatty acid oxidation) than for the two members of the citrate cycle. Variations in fiber size were the same for both muscles.

Effects of Weightlessness on Average Values:

Since data are available, with one exception, from only two synchronous and two flight muscles, some caution must be observed in interpreting small average differences. However, examination of individual fiber patterns is helpful in this regard.

Tables 1 and 2 and Figures 3-6 compare average enzyme activity and fiber size for each synchronous and flight muscle studied. The average size (weight per unit length) was about 35% lower in flight than in synchronous muscles of both types. All of the enzyme activities are based on dry weight. Therefore the absolute enzyme content of the fibers from flight muscles are on the average 35% lower than would appear from these data. This will be discussed later.

In soleus muscle, the only conclusive enzyme change with flight was in hexokinase which increased an average of 137% on the dry weight basis (Table 1, Figure 3). The three enzymes of oxidative metabolism were clearly unchanged. The agreement between muscles is close and standard errors are small. The four enzymes of glycolysis and glycogenolysis were on average increased 20 to 50%, but the mean differences and standard errors in this case were so large as to make any conclusion about significance uncertain (Table 2, Figure 4).

In TA muscles, hexokinase increased about the same percentage as in soleus, but in addition all the enzymes of oxidative metabolism were increased about 60% (Table 1, Figure 5). The change in MDH is most convincing, whereas the standard errors for the other two enzymes are too large to be sure the differences are meaningful. The glycolytic-glycogenolytic enzymes in TA, in contrast to the soleus muscles, were all somewhat lower (12% to 25%) in the flight muscles. If the data for the two synchronous and the two flight muscles are each pooled, the differences due to flight are statistically significant for phosphorylase ($P<0.05$), for glycerol phosphate dehydrogenase ($P<0.01$), and for LDH ($P<0.001$), but not for pyruvate kinase.

Effect of Weightlessness on Individual Fiber Enzymes:

Figure 7 compares the variability of the 8 enzymes in the control and flight TA muscles. A striking difference is the 3- to 4-fold larger C.V.'s for the glycogenolytic enzymes in the flight muscles, with much smaller effects of flight, if any, in regard to variability among the oxidative enzyme group. In the case of the soleus fibers, the C.V.'s were little changed by flight from the control values shown in Figure 2.

The basis for this increase in glycolytic C.V.'s is illustrated by the example shown in Figure 8. This figure records individual values for LDH and MDH plotted against each other in 60 TA fibers, 30 from a synchronous muscle and 30 from a flight muscle. All but one of the synchronous fibers occupied a domain with a narrow range of LDH values and a wide range of MDH values. In contrast, about half of the flight fibers had moved out of the control domain by a decrease in LDH, and in some cases by a modest increase in MDH. Note that half of the control fibers, but only one flight fiber had an MDH value less than 8 mol kg^{-1} h^{-1}. The enzyme patterns for the other flight and control TA muscles were very similar to these. These LDH-MDH patterns are those expected for a change from a population consisting predominantly of fast-glycolytic fibers, in the control TA, to a mixture of fast-oxidative, fast-glycolytic, and a few slow-twitch fibers in the flight muscle.

Also entered in Figure 8 are data for 6 control and 6 flight soleus muscle fibers from the same two animals. These all fall in a single relatively small area with moderate MDH levels and very low LDH.

Figure 9 is a similar plot against each other of two enzymes of oxidative metabolism, citrate synthase and ß-hydroxyacyl CoA dehydrogenase. The data from the two TA controls and the two TA flight muscles have been pooled, since they appeared to occupy the same domain. Note the striking degree

of correlation between the two enzymes. The only obvious difference between flight and control is that 5 of the 12 fibers have been raised out of the control domain. The patterns of these enzymes for the other flight and control TA muscles were quite similar.

Included in Figure 9 is the joint domain of 32 soleus fibers, 16 each from a flight and a control muscle. These were similarly distributed over the area outlined. Individual values have been omitted to avoid confusion.

Figure 10 plots single fiber values against each other for two fast-twitch enzymes, phosphorylase and pyruvate kinase, from one control and one flight TA muscle. Of the 12 flight fibers, 3 were raised somewhat above the control domain whereas 5 fell far below it. Similar results were observed for the other control and flight TA muscle. Apparently weightlessness can extend the range of these fast-twitch enzymes in both directions, although the biggest trend is downward.

In soleus muscle, single fiber plots of phosphorylase against pyruvate kinase also show a close correlation between the two enzymes, but at much lower absolute levels (Figure 11). Although the effect of flight was to systematically increase pyruvate kinase, only 2 of 10 fibers showed a large move away from the control zone, and results for the other flight soleus muscle did not show a major increase in the range for either enzyme.

A plot of pyruvate kinase against glycerol-P dehydrogenase in TA fibers (Figure 12) is very similar to that of pyruvate kinase against phosphorylase for the same fibers shown in Figure 9. Figure 12 is presented because data have also been obtained for these two enzymes in fibers that were typed by the myofibrillar ATPase staining reaction. Frozen cross sections were made from one synchronous and one flight TA muscle. Alternate sections were stained for ATPase, or freeze-dried for quantitative enzyme assay. The stained sections were used as a guide for selection of fibers for these assays. Although type IIB fibers predominated in the synchronous muscle, and IIA fibers predominated in the flight muscle, it was possible to select an adequate number of fibers of both types from both muscles, and in addition in the flight muscle a few type I fibers were found and analyzed (Table 3).

The results indicate that the flight fibers in Figure 12 with low values for both enzymes are type IIA, with an occasional type I fiber, and the remainder are type IIB.

Absolute Changes in Enzyme Content With Weightlessness:

As mentioned above, the data so far presented are all based on dry weight, but the flight muscle fibers consistently lost about a third of their dry weight per unit length. Since fiber length can hardly change except with skeletal growth (and synchronous animals served as controls), basing enzyme contents on fiber length should provide a valid measure of the absolute changes in amount of enzyme. Figures 13 and 14 compare average levels of enzymes in soleus and TA muscles, respectively, when calculated on either a dry weight or fiber length basis. In soleus muscle, (Figure 13), the dry weight basis seems to indicate that flight caused a very large increase in hexokinase, with the other seven enzymes either unchanged or increased. In contrast, on a fiber length basis, it is apparent that although hexokinase increased in absolute terms, the increase was no more than 50%, and that six of the other enzymes decreased by 10 to 40%. Similarly in TA muscle (Figure 14), on a dry weight basis, hexokinase and the three enzymes of oxidative metabolism appeared to increase with flight by 60 to 95%, with the glycolytic, glycogenolytic enzymes falling by at most 25%, whereas in absolute terms (fiber length basis) oxidative enzymes were almost unchanged, hexokinase increased, but only by 25%, and phosphorylase and the glycolytic enzymes decreased about 50%.

CONCLUSION

In spite of the limitation in numbers of muscles examined, it is apparent that 1) the size of individual fibers (i.e. their dry weight) was reduced about a third, 2) that this loss in dry mass was accompanied

by changes in the eight enzymes studied, and 3) that these changes were different for the two muscles, and different for the two enzyme groups. In the soleus muscle the absolute amounts of the three enzymes of oxidative metabolism decreased about in proportion to the dry weight loss, so that their concentration in the atrophic fibers was almost unchanged. In contrast, there was little loss among the four enzymes of glycogenolysis - glycolysis so that their concentrations were substantially increased in the atrophic fibers. In the TA muscle, these seven enzymes were affected in just the opposite direction. There appeared to be no absolute loss among the oxidative enzymes, whereas the glycogenolytic enzymes were reduced by nearly half, so that the concentrations of the first metabolic group were increased within the atrophic fibers and the concentrations of the second group were only marginally decreased.

The behavior of hexokinase was exceptional in that it did not decrease in absolute terms in either type of muscle and probably increased as much as 50% in soleus. Thus, their was a large increase in concentration of this enzyme in the atrophied fibers of both muscles.

Another clear-cut finding was the large increase in the range of activities of the glycolytic enzymes among individual fibers of TA muscles. This was due to the emergence of TA fibers with activities for enzymes of this group extending down to levels as low as those found in control soleus muscles. It would be interesting to know if this represents a transition stage, and whether with prolonged weightlessness most of the fibers would be transformed into a low glycogenolytic type.

TABLE 1.

SIZE AND LEVELS OF FOUR ENZYMES CHARACTERISTIC OF SLOW TWITCH MUSCLES IN INDIVIDUAL FIBERS FROM SOLEUS AND ANTERIOR TIBIALIS MUSCLES

	Size	Hexokinase	Citrate synthase	MDH	BOAC
	μg/mm	mol h^{-1}	kg^{-1} (dry) at 20°C		
SOLEUS					
S7	0.84(16) ±0.05	0.468(16) ±0.026	4.47(16) ±0.25	17.7(6) ±1.7	9.4(16) ±0.2
S9	0.92(16) ±0.06	0.507(16) ±0.028		19.7(6) ±1.4	11.5(16) ±0.3
F7	0.61(16) ±0.03	1.38(16) ±0.07	5.04(16) ±0.33	20.6(16) ±0.9	9.2(16) ±0.2
F9	0.50(16) ±0.02	0.93(16) ±0.03		19.9(6) ±1.3	11.4(16) ±0.3
TIB. ANT					
S8	1.04(32) ±0.05	0.212(20) ±0.020	4.71(12) ±0.77	8.5(32) ±0.8	5.2(6) ±2.0
S9	0.91(32) ±0.05	0.295(20) ±0.020	6.42(12) ±1.06	10.4(32) ±1.2	7.1(6) ±2.0
F8	0.55(30) ±0.02	0.472(20) ±0.038	7.81(12) ±1.13	16.1(30) ±1.2	8.9(6) ±2.5
F9	0.67(32) ±0.03	0.526(20) ±0.04	9.54(12) ±1.55	17.3(32) ±1.0	11.9(6) ±3.9

Standard errors are shown for the numbers of fibers in parentheses. Abbreviations: MDH, malate deyhdrogenase; BOAC, ß-hydroxyacyl CoA dehydrogenase; S, synchronous; F, flight.

TABLE 2.

LEVELS OF FOUR ENZYMES CHARACTERISTIC OF FAST-TWICH MUSCLES

	Phosphorylase	Glycerol-P dehydrogenase	Pyruvate kinase	Lactate dehydrogenase
	Mol h^{-1} kg^{-1} (dry) at 20°C			
SOLEUS				
S7	0.60(10) ±0.13	0.47(16) ±0.05	7.72(16) ±0.43	24.0(16) ±1.1
S9	1.04(10) ±0.23		10.1(10) ±1.5	23.0(16) ±1.6
F7	0.70(10) ±0.12	0.62(16) ±0.07	10.3(16) ±0.56	29.4(16) ±0.9
F9	1.46(10) ±0.38		16.2(10) ±2.9	28.0(16) ±2.0
TIB. ANT				
S8	5.39(12) ±0.27	6.13(20) ±0.18	60.0(12) ±2.1	132(25) ±2
S9	7.03(12) ±0.44	5.49(20) ±0.21	64.1(20) ±1.9	136(32) ±3
F8	3.97(12) ±0.59	5.04(18) ±0.40	55.9(12) ±6.8	116(30) ±7
F9	5.30(12) ±0.93	4.13(19) ±0.47	53.9(20) ±5.7	103(32) ±6

Standard errors are shown for the number of fibers in parentheses.

TABLE 3

GLYCEROL PHOSPHATE DEHYDROGENASE (GOPDH) AND PYRUVATE KINASE IN INDIVIDUAL ANTERIOR TIBIALIS MUSCLE FIBERS TYPED ON THE BASIS OF MYOFIBRILLAR ATPASE STAINING

	Type	IIB	IIA	I
GOPDH		Mol kg $^{-1}$ (dry) h $^{-1}$		
Synchronous	S6	6.61(11) ±0.51	2.39(9) ±0.61	
Flight	F6	4.43(10) ±0.71	1.93(4) ±0.88	0.84(4) ±0.03
Pyruvate kinase	S6	38.8(11) ±1.8	14.3(9) ±1.3	
	F6	40.7(10) ±2.2	19.7(4) ±2.6	8.34(4) ±0.48

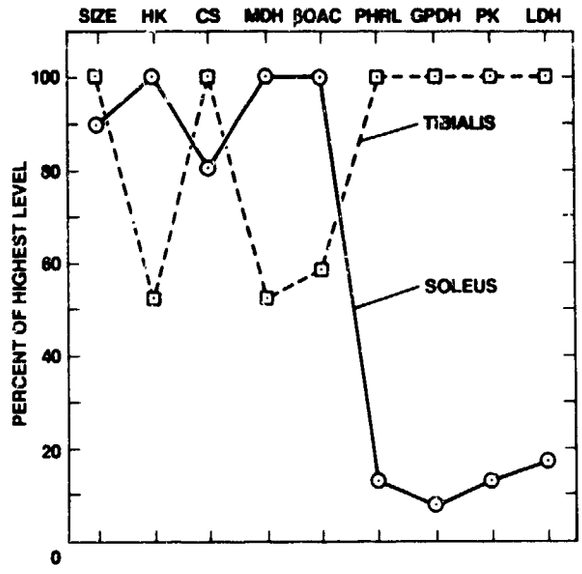

Figure 1: Comparison of average size and enzyme levels in the fibers of two synchronous soleus muscles(S7 and S9) and two synchronous TA muscles (S8 and S9). Abbreviations are HK, hexokinase; CS, citrate synthase; MDH, malate dehydrogenase; BOAC, β-hydroxyacyl CoA dehydrogenase; PHRL, glycogen phosphorylase; GPDH, glycerophosphate dehydrogenase; PK, pyruvate kinase; LDH lactate dehydrogenase.

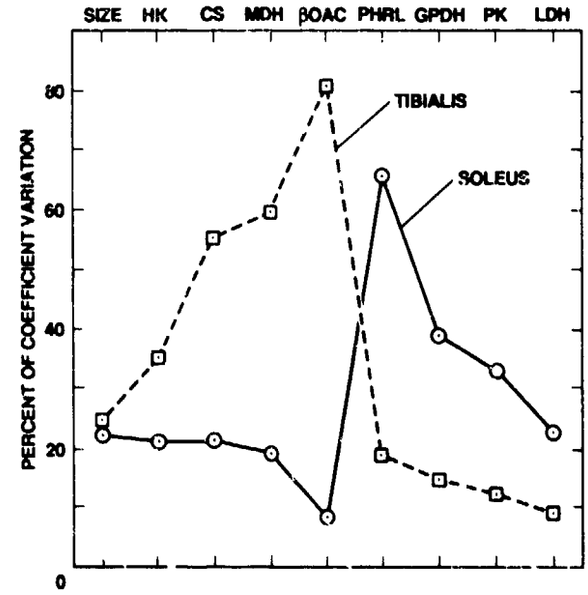

Figure 2: Average coefficient of variation for the same synchronous fibers and enzymes represented in Figure 1.

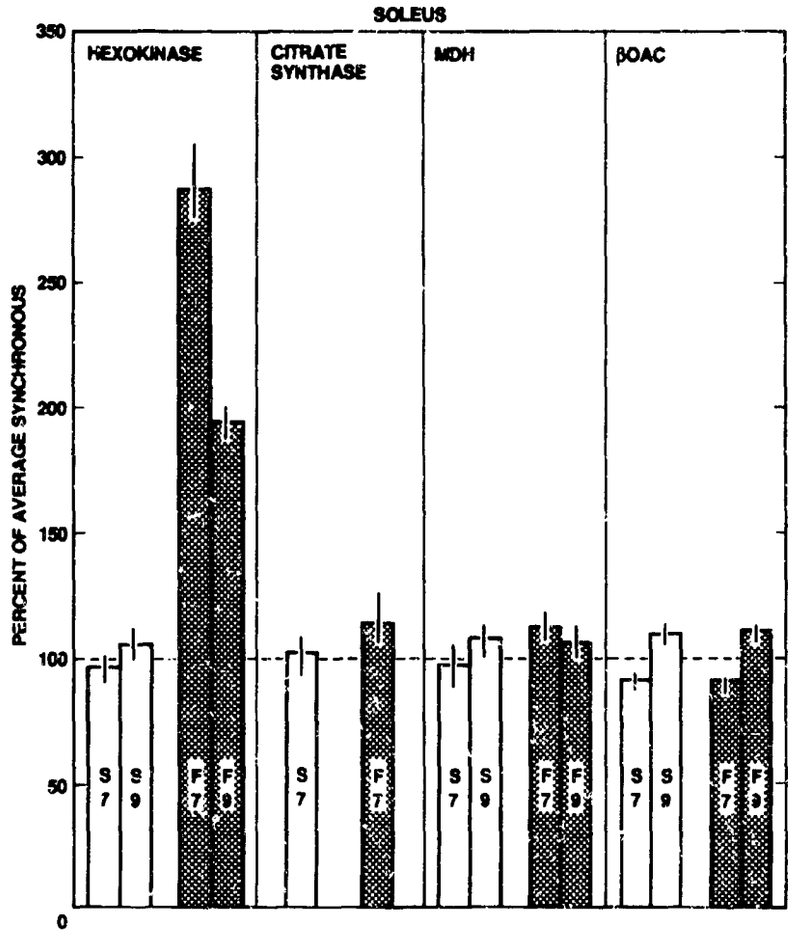

Figure 3: Comparison of synchronous and flight soleus muscle fibers in regard to levels, on a dry weight basis, of hexokinase and three enzymes of oxidative metabolism. Abbreviations as in Fig. 1.

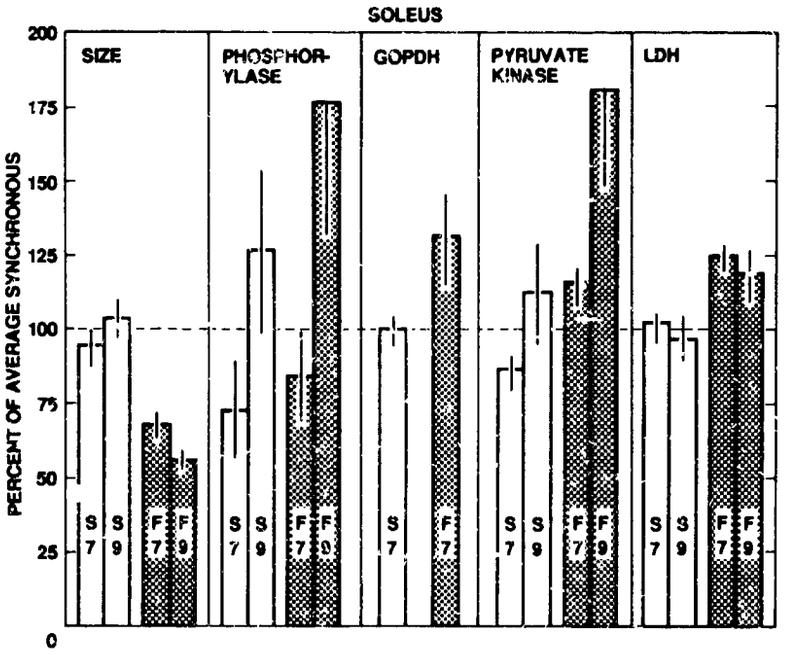

Figure 4: Comparison of synchronous and flight soleus muscle fibers in regard to size (dry weight per unit length) and the levels on a dry weight basis for four enzymes of glycolysis and glycogenolysis. Abbreviations as in Fig. 1.

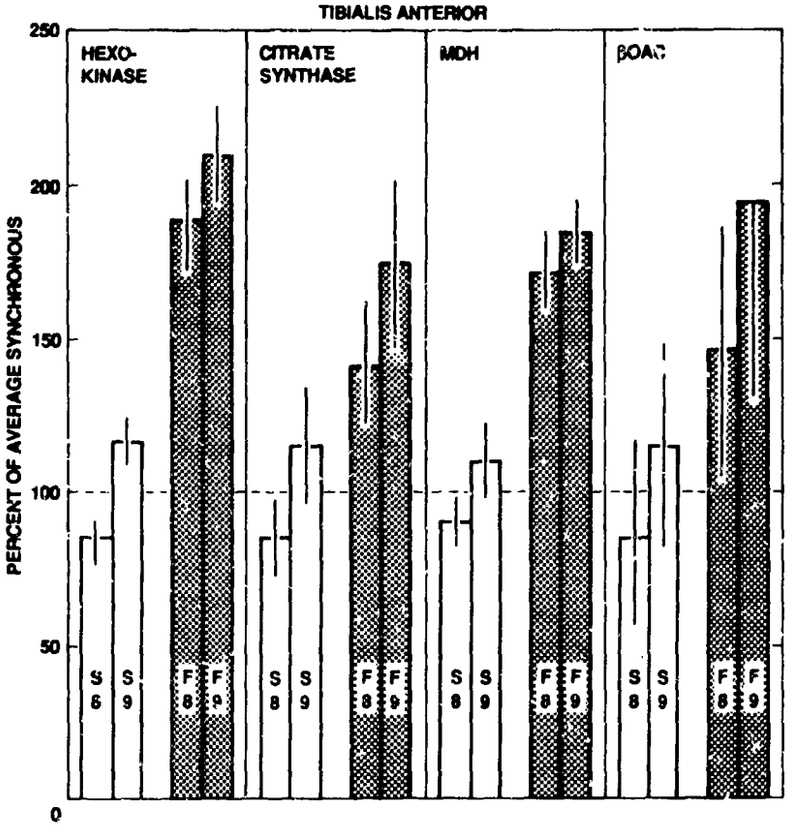

Figure 5: Comparison of synchronous and flight TA fibers in regard to levels, on a dry weight basis, of hexokinase and three enzymes of oxidative metabolism. Abbreviations as in Fig. 1.

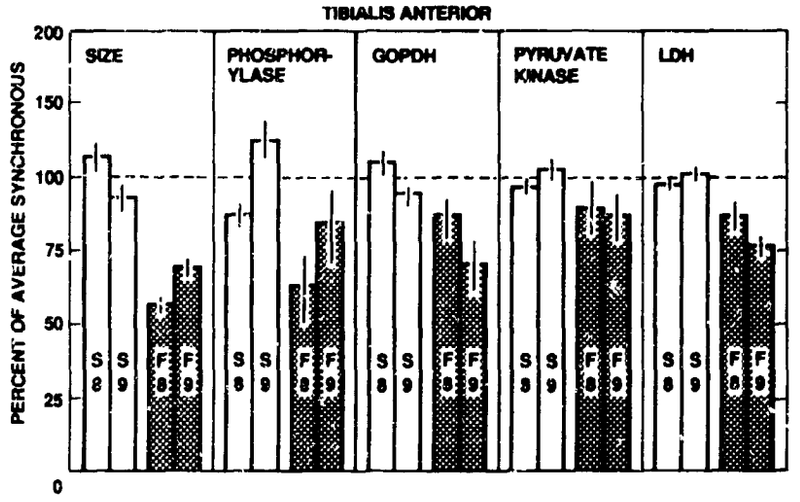

Figure 6: Comparison of synchronous and flight TA fibers in regard to size (dry weight per unit length) and the levels on a dry weight basis for four enzymes of glycolysis and glycogenolysis. Abbreviations as in Fig. 1.

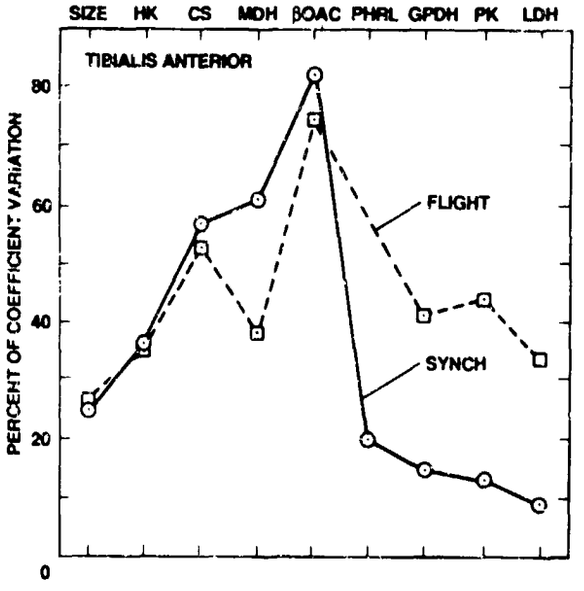

Figure 7: Average coefficients of variation for size, and the levels of eight enzymes of individual fibers from the two flight and two synchronous TA muscles of Figures 5 and 6.

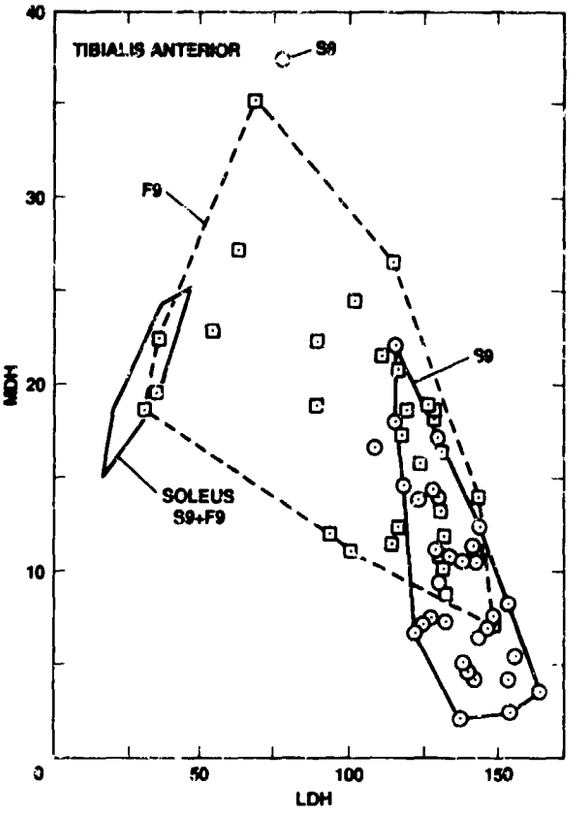

Figure 8: Malate dehydrogenase (MDH) and lactate dehydrogenase (LDH), mol h^{-1} kg^{-1} (dry wt.), plotted against each other for individual fibers from TA muscles S9 and F9. The domain occupied by 6 soleus S9 and 6 soleus F9 fibers is also indicated but without showing the individual values. Activities are mol h^{-1} kg^{-1} (dry wt.).

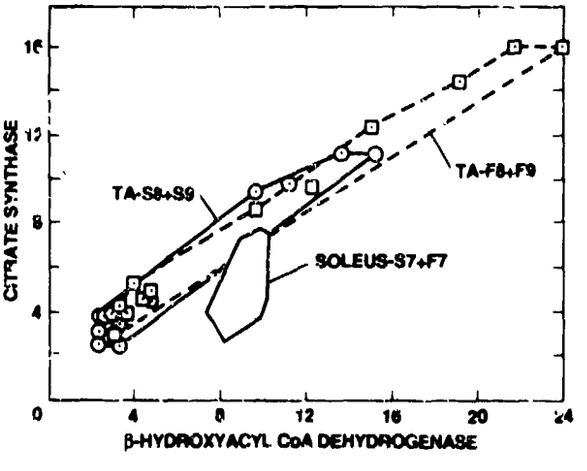

Figure 9: Citrate synthase and ß-hydroxyacyl CoA dehydrogeanse, mol h^{-1} kg^{-1} (dry wt.), plotted against each other for individual fibers from TA muscles S8, S9, F8 and F9. Six fibers were analyzed from each muscle; the flight fibers are indicated by x, the synchronous fibers by 0. Also indicated is the domain without showing individual values for 16 S7 and 16 F7 soleus fibers.

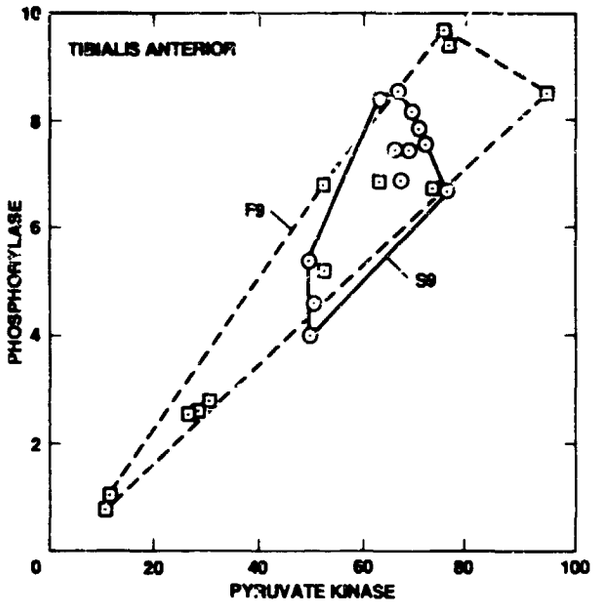

Figure 10: Phosphorylase and pyruvate kinase, mol h^{-1} kg^{-1} (dry wt.), plotted against each other for TA fibers.

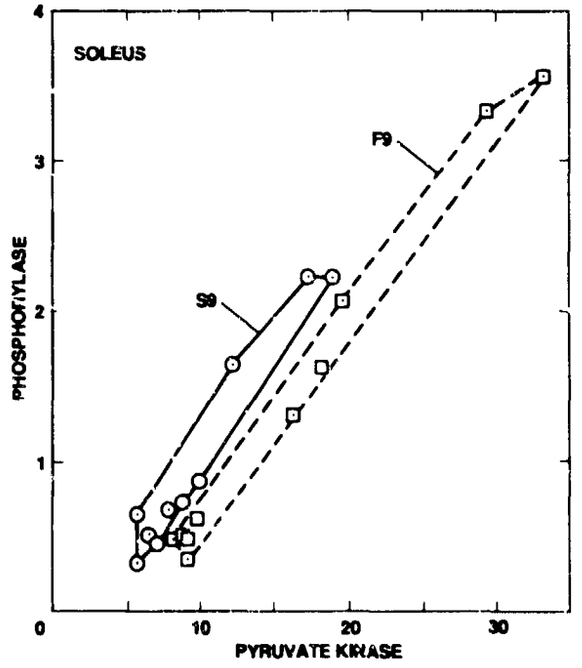

Figure 11: A plot similar to Figure 10 for 10 S9 and 10 F9 soleus fibers.

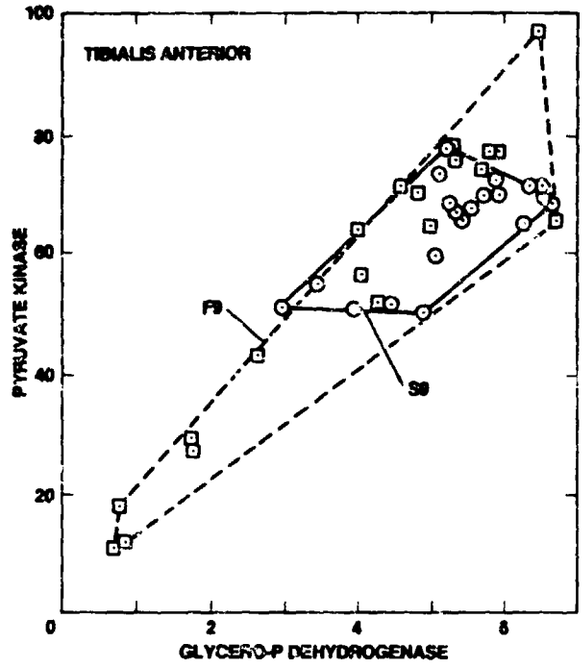

Figure 12: Pyruvate kinase and glycerophosphate dehydrogenase (GOPDH), mol h^{-1} kg^{-1} (dry wt.), plotted against each other for 20 S9 and 19 F9 TA fibers.

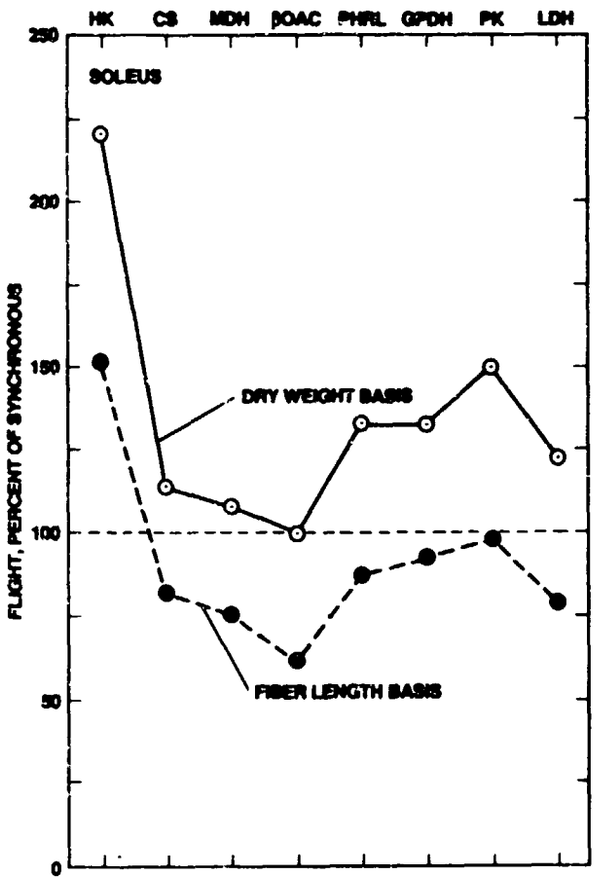

Figure 13: Average enzyme levels for soleus fibers from the two synchronous and two flight animals compared on the basis of dry weight and on the basis of fiber length.

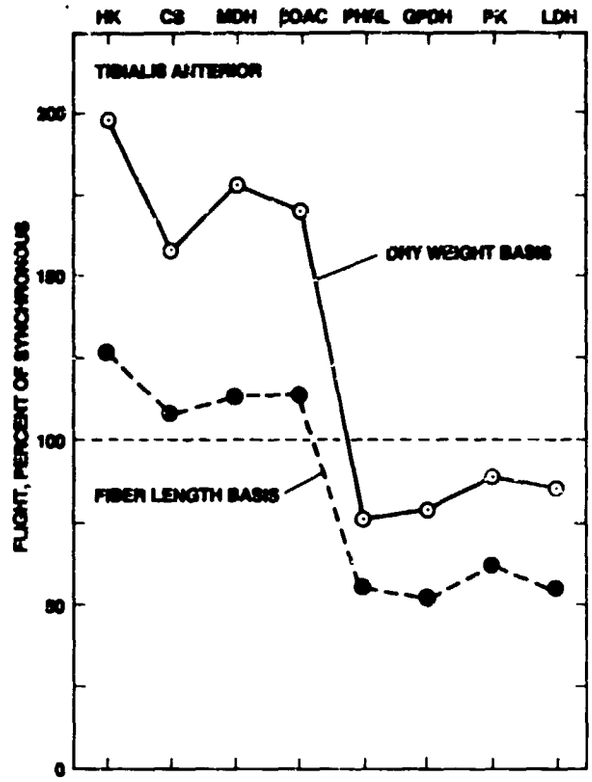

Figure 14: Average enzyme levels for TA muscles from the two synchronous and two flight animals compared on the basis of dry weight and on the basis of fiber length.

EXPERIMENT K-6-21

PART II: METABOLIC ENZYMES OF HIPPOCAMPUS AND SPINAL CORD

INTRODUCTION

The question of possible enzyme changes due to exposure to microgravity is much more complicated in the case of the central nervous system than it is for skeletal muscle. The brain is enormously complex. Valid comparisons must be made between exactly the same regions of control and flight brains, otherwise natural differences will confuse the issue.

METHODS

We have measured 9 different enzymes in 6 regions of the hippocampus and 4 and 5 enzymes in 5 regions of the spinal cord (a total of almost 500 quantitative measurements) and found differences that may be meaningful in three enzymes in a few areas of flight brains. However, statistical proof (either way) will require substantially more data. Probably, the present results should be regarded as a guide to future and more definitive studies.

Wherever possible, the assays for a number of enzymes were made in duplicate with aliquots from an extract of a single, relatively large, tissue sample, made with a medium similar to that described for muscle (Part I). For Table 1, the dry tissue samples weighed about 0.2 µg and were dispersed in 5 µl of extraction medium. The assays were made with 0.2 µgl aliquots of these extracts, each equivalent to about 8 ng of dry tissue. This methodology was not possible or practical in all cases. Glutaminase, for example, is difficult to stabilize in extracts, and enzymes of low activity are easier to measure accurately with samples added directly into the assay reagent.

RESULTS

The six enzymes of the hippocampus shown in Figure 4 of Part I were in most cases remarkably similar in flight and control (vivarium) brains. ß-hydroxyacyl CoA dehydrogenase was 35% lower in the molecular layer of CA1 of the flight brain, and should be reinvestigated, however, the other two enzymes of oxidative metabolism in this region were within 10% of the control.

GABA and two enzymes of GABA metabolism were measured in the hippocampus of two vivarium and two flight animals (Tables 2 and 3). The GABA levels are probably not definitive, since they are known to be sensitive to post mortem increase. The glutamate decarboxylase activities were quite variable, nevertheless it probably should not be ignored that the levels for the flight samples from all of the hippocampal regions were on average 30% higher than for the controls. This result is strengthened by the data for GABA transaminase: in each of the six hippocampal areas assayed the average activity for the two flight animals was higher (by 8% to 35%) than the average of the two vivarium controls. The overall average difference was plus 16%.

Another positive result for hippocampus concerns glutaminase. In CA1, average values for the three regions assayed were 25% to 59% higher in the flight than the control tissues (Table 2). In fascia dentata (Table 3), the differences were much smaller (+2% to +10%) and probably have little meaning.

The spinal cord data are limited to one (synchronous) control and one flight animal. The higher aspartate aminotransterase values for the pyramidal tract and outer dorsal horn for the flight specimens would be worth further investigation. The same is true for the lower glutaminase levels in the dorsal column and pyramidal tract of the flight animal and the higher level in the outer dorsal horn. All three differences are statistically significant ($P<0.01$), but they only concern single animals.

CONCLUSION

To reiterate, these somewhat fragmentary results suggest that future, more extensive, studies of this type with selected and carefully matched brain areas, should be quite rewarding.

TABLE 1

SIX ENZYMES IN SIX REGIONS OF THE HIPPOCAMPUS

	Hexokinase		G6PDH		Citrate Synthase		MDH		BOAC	
CA1	V10	F8	V10	F8	V10	F8	V10	F8	V10	F8
Pyramidalis	3.4	4.1	0.051	0.052	6.9	6.0	8.4	10.0	0.51	
0.59	8.8	8.4								
Radiata	6.6	6.3	0.056	0.058	9.4	7.7	9.9	8.1	0.52	
0.49	9.7	9.2								
Molecularis	5.7	5.7	0.057	0.061	10.1	9.4	10.5	10.3	1.05	
0.68	10.4	8.2								
Area dentata										
Molecularis	6.2	6.0	0.060	0.055	10.9	9.8	13.3	11.3	1.00	
.095	12.1	11.1								
Granularis	3.9	4.4	0.057	0.055		8.0	9.3	9.9	0.76	
0.74	10.4	11.3								
Hilus	6.0	5.7	0.053	0.047	8.1	7.1	8.3	9.1	0.62	
0.62	9.2	8.2								

Activities are moi h^{-1} kg^{-1} (dry wt.) at 20°. Abbreviations are: G6PDH, glucose 6-phosphate dehydrogenase; MDH, malate dehydrogenase; ßOAC, ß-hydroxyacyl CoA dehydrogenase; amino-T, aminotransferase.

TABLE 2

GLUTAMINASE, GABA AND TWO ENZYMES OF GABA METABOLISM IN FOUR LAYERS OF CA1 OF THE HIPPOCAMPUS

		GABA	GAD	GABA trans	Glutaminase
		mmol kg^{-1}	mmol h^{-1} kg^{-1} (dry)		
Pyramidalis	V9	11±3	59±2	471±5	2390±200
	V10	17±3	53±1	441±34	1670±60
	F8	39±7	63±8	529±36	3230±290
	F9	24±2	56±4	483±12	3240±220
Radiatum	V9	30±2	12±2	265±16	4810±120
	V10	12±1	27±0.3	259±15	4020±260
	F8	21±3	38±1	290±18	5600±80
	F9	22±1	28±1	277±13	5620±240
Lacunosum	V9	27±5	16±1		
	V10	13±1	28±2		
	F8	39±6	49±5		
	F9	19±2	29±2		
Molecularis	V9	27±5	34±2	394±20	3700±100
	V10	26±2	48±4	210±10	3960±230
	F8	25±8	52±4	269±6	4580±180
	F9	20±2	49±5	421±21	5270±240

Abbreviations: V, vivarium; F, flight; GAD, glutamate decarboxylase; trans; transaminase. GAD activity was measured at 38°C, the activities for the other two enzymes were measured at 20°C. Standard errors are shown for usualy 3 samples.

TABLE 3

GLUTAMINASE, GABA AND TWO ENZYMES OF GABA METABOLISM IN THREE REGIONS BY FASCIA DENTATA

		GABA	GAD	GABA trans	Glutaminase
		$mmol^{-1}$	$mmol\ h^{-1}\ kg^{-1}$ (dry)		
Granular	V9	27±4	59±1	436±31	2500±80
	V10	36±2	42±1	384±16	1820±150
	F8	22±1	41±4	442±5	2210±100
	F9	28±2	55±13	491±8	2540±330
Molecular	V9	19±1	50±6	289±43	5240±330
	V10	23±2	30±2	320±7	4350±330
	F8	25±1	81±8	386±18	4880±410
	F9	29±1	48±2	437±19	5250±550
Hylus	V9	21±2	30±3	364±38	4980±210
	V10	26±1	29±1	284±11	3070±310
	F8	19±1	30±2	356±12	4140±210
	F9	23±2	22±2	398±13	4050±270

TABLE 4

FIVE ENZYMES IN FIVE REGIONS OF THE SPINAL CORD AT ABOUT T11

		Hexo-kinase	G6PDH	Citrate synthase	Aspart amino-T	Glutam-inase
		mol h^{-1} kg^{-1} (dry), 20°C				
Dorsal Col.	S4	0.10	0.262	0.94	3.28	0.366±0.023 (8)
	F3	0.19	0.256	0.88	3.36	0.276±0.018 (11)
Pyram tract	S4	1.34	0.237	4.45	8.5	0.819±0.066 (4)
	F3	1.37	0.261	4.61	11.3	0.612±0.037 (4)
Rexed I-III	S4	3.52	0.263	6.66	12.7	2.06±0.11 (14)
	F4	3.28	0.285	6.29	16.6	2.57±0.13 (12)
Rexed IV-V	S4	3.54	0.228	6.61	16.4	
	F3	3.40	0.276	7.31	16.8	
Rexed VII	S4	3.32	0.254	6.21	15.1	
	F3	3.92	0.253	9.52	15.4	

Data for the first four enzymes are from duplicate assays made with 0.05 or 0.1 µl aliquots from an extract of about 0.5 µg of dry tissue made with 2 µl glycerol-detergent medium. The glutaminase assays were made with individual samples of about 25 ng dry weight.