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UNIVERSITY OF WISCONSIN-MADISON
COLLEGE OF AGRICULTURAL AND LIFE SCIENCES

POULTRY SCIENCE DEPARTMENT
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TO: Beatrice Morales
Grant Officer SLO:240A-3
NASA
Ames Research Center
Moffett Field, CA 94035-1000

FROM: Bernard Wentworth *Bernard C. Wentworth*

DATE: April 25, 1996

MAY 01 1996
CASF

RE: Grant # NAG2-1009

Please find the enclosed Progress Report on "Fecundity of Quail in Spacelab Microgravity," and budget request to rollover unencumbered Year One funds, Year Two budget and a request for budget supplement to cover the unplanned expenses associated with the STS-76 flight.

The Year One funding was not released to the University of Wisconsin until mid September of 1995 with a Year One ending date of April 30, 1996. Consequently there is unfinished laboratory work committed for Year One ($9,550) and we ask that the unencumbered funds rollover for use in Year Two. The Year Two budget ($42,504) has been adjusted to reflect the experience and shortage noted in Year One. The supplemental budget ($3,630) is requested to cover the cost of quail colony management and shipping cost associated with the fertile eggs provided for STS-76. The original plans for this flight called for obtaining fertile quail eggs from another source.

We anticipate that these budget requests will be approved and Year Two funding will be available May 1, 1996.

cc: Cheryl E. Gest, Research Admin U of W
Charles Winget, Grants Office NASA - Ames
Gary Jahns, NASA Payload Manager

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Progress Report
Fecundity of Quail in Spacelab Microgravity

B.C. Wentworth and A. L. Wentworth

Successful regeneration in space microgravity depends on successful gamete production, proliferation, differentiation and union to form a viable developing embryo. Progress in this area during 1995 using the Japanese quail, *Coturnix coturnix japonica*, is summarized below according to the following phases of Russian-American cooperative investigation: A) Embryonic development in microgravity; B) Laboratory-based tests on G-force and Vibrations (Gaveet tests at Ames, California); C. Laboratory tests conducted at the Avian Physiology laboratory U.W. Madison, Wi; D) Active participation in the coordination of Russian-American projects in Moscow and in the U.S.; E) Future Directions.

A. Development in Microgravity. Two flight experiments were performed in which fertilized Japanese quail eggs from the Moscow-based colony were allowed to develop to various ages in space, (after 7,10,14, and 16 days of incubation) at which time they were fixed in either paraformaldehyde or ethyl alcohol. After the fixed eggs were returned to earth American and Russian scientists participated in separating the specific tissues of scientific interest from the experimental flight embryos and the various controls at NASA in the Ames, California laboratories. Temperature control may have been faulty in each of these experiments so that recovery of tissues from experimental and synchronous controls was minimal. (Tables 1 and 2). Transport control fertile eggs were taken to the launch site and were returned to the Moscow laboratory and set to hatch. These eggs showed a 72.5% hatchability.

Histological Results: From the limited number of flight samples which were available for analysis, we did not observe an obvious effect of microgravity on the development and cellular differentiation of the reproductive organs of the male and female embryos. The data are recorded for the one 10-day-old embryo flight sample as well as in the few synchronous controls which were fixed at day 10 and 16. The gonads were developed normally into male or female and had an equivalent number of germ cells to that of the controls. The lungs showed that the viable 16 day bird from the synchronous control group had broken through the air sac and had commenced breathing. From these data we are encouraged that normal reproductive development of fertilized Japanese quail eggs is possible.

Progress report on Mir 21 (STS-76) Launched 3-22-96.

Forty-eight fertile Japanese quail eggs were launched on STS 76 on 3-22-96 to be incubated on Spacelab Mir and fixed after 0, 3,7,10,14 and 16 days of incubation. The eggs were from the random bred wild type Japanese quail maintained at the Avian Physiology laboratory, UW- Madison. They were placed in the Mir incubator six days from the day of lay. They will be returned in August of 1996 for subsequent tissue removal at NASA- Ames, California, and then specific analyses at the scientists' respective laboratories. Control data to date is from the transport controls and various laboratory controls. The transport controls were the fertile eggs that were shipped to Kennedy and returned to Madison for incubation to hatch @17.2 days of incubation. The eggs were held for two days at 14-21C prior to incubation). These eggs were 92% fertile, 89% viable at 16 days and 82% hatched. The laboratory controls (eggs from the same colony set as fresh eggs) showed a 94% fertility, 96% viability and 82% hatchability. (Table 3)

A second set of laboratory controls was performed in which eggs from the same colony of Japanese quail were stored at 14C for either six (N=56) or five (N=48) days and then set to hatch. The group held for six days had a 96% fertility, 91% viability at 17.2 days and 85% hatchability of fertile eggs at 17.2 days. The group held for five days prior to incubation showed a 96% fertility, 94% viability at 17.2 days and 91% hatchability of fertile eggs at 17.2 days. (Table 4). These data are very encouraging as is the preliminary report of viable embryos on the fixation days on the Mir Spacelab.

B. Laboratory-based experiments were conducted in anticipation of problems encountered in microgravity which could hinder successful regeneration in space.

1. Embryonic development of Japanese quail in gravity using simulated vibrations and G-force. Gaveet Tests were performed in duplicate at Ames, California by the NASA staff.

Evaluations were conducted(a) by the PI's at Ames on 12-30-96 and (b) at the avian physiology laboratory at UW-Madison on 1-8-1996. No differences in development was observed in the four groups analyzed. Vibrations; Centrifuge; Centrifuge +Vibrations and Controls. (Table 5a&b labeled: " Gaveet egg data"). Additionally, the results of a sampling of gonadal tissues from the Gaveet test (B) at Ames, California, showed that neither centrifugation which simulates G-force at lift off, nor vibrations which simulated those as lift off, affected the reproductive development of the embryonic quail after 16 days of incubation.

C. Progress reports on various ground-based laboratory tests at the Avian Physiology laboratory, UW-Madison.

1.. Effects of turning and ambient temperature at various days of incubation on the development of Japanese quail, *Coturnix coturnix japonica.*

During the 1994 and 1995 telecoms among the scientists involved in this projects, two questions arose. The first was if eggs were incubated on earth for the first few days of development, a critical time of development, could they be held at ambient temperature for a day so that they could be transferred into the shuttle and resume incubation after lift off. Additionally avian eggs have to be turned a minimum of four times on earth to develop well. The question of the necessity of turning eggs in microgravity also arose. These two considerations were addressed in a 2 x2x 4 factorial design experiment which was replicated five times. The quail used in this study were from the same random bred colony that was used for the STS 76 Flight experiment and controls. Experiment 1 consisted of two different treatment groups, one turning and the other not. On different days of incubation, days 2,3,4,and 5, the eggs were placed at room temperature, 21C, for 24 hours, and were set to be either turning or not turning. Each group consisted of 100 eggs. Twenty eggs were broken out daily for five consecutive days and evaluated for development. The total number of eggs used in this experiment was 1000. Experiment 2 was a hatchability trial of the same experimental groups turning and not turning and ambient temperature for 24 hours on days 2,3,4,and 5. Hatchability was recorded at day 17, 18 and 19 to determine late hatching. At day 19, eggs that were not hatched were opened and recorded as to the stage of development. These data are currently being analyzed. Preliminary conclusions indicate that turning of quail eggs in gravity increases viability and hatchability. Many of those which did not turn did hatch. Statistical significance of these data is being calculated. Additionally, many eggs held at ambient temperature for 24 hours on the various days, 2,3,4 and 5 did develop although at a slower rate. Again, interactions and statistical significance of these data are being analyzed. The total number of eggs in this experiment was 200.

2. A method to feed and water adult and newly hatched Japanese quail, *Coturnix coturnix japonica*, in microgravity using a gelatin-based diet as a solid water supply. This experiment was designed so that adult quail and newly hatched chicks could be

maintained in space microgravity.

Japanese quail, unlike mice, depend on gravity to get their water supply from drip waterers. Therefore, a means to water adult and newly hatched quail has to be devised. We found that a 70% water based, 5% gelatin, 25% quail diet could support the body weight and egg production rate of adult female as well as male Japanese quail fertility. (Table 6a and b). This preliminary trial lasted only one week. However, quail with insufficient water(gel blocks cut up so that evaporation was rapid) stopped laying after three days. This experiment will be expanded at a later date. For the newly hatched quail, a 75% water, 2% gelatin and 23% quail starter ration could support normal growth of newly hatched chicks for 10 days. This experiment is being repeated with a modified starter ration as the feather development in these quail was slightly abnormal.

D. Participation in interactive communication between Russian and American scientists at the Moscow and the American-based laboratories

Trip to Russia. A major part of the information exchange involved future missions and technical details of logistics and required hardware. It was apparent from the meeting that the following things must be accomplished.

Complete success in the incubator project STS76 - MIR21

Make preparation for MIR 21.

Make preparation for NASA 4 & 5.

Determine who will supply eggs for MIR21.

What vehicle will be used to transport hardware and who's payload manifest (U.S. or Russian) will provide space for hardware.

My major contribution in flight programing to meet this accomplishment was associated with the incubator hardware requirements, general and specific knowledge concerning avian physiology, and a continuous source of quail eggs. It was agreed that Dr. Hester would provide the eggs for MIR 21. We agreed that 150 sexually mature birds in a ratio of three females to one male would be adequate to meet the March flight. We maintained a colony of random bred wild-type quail at the UW - Madison Avian Physiology laboratory as a back up egg supply to the planned egg supply.

E. Future Directions

1. Adult quail maintenance in space microgravity.

2. Hatching fertile quail eggs in space microgravity and maintaining quail chicks in space microgravity.

The ground research which was performed on this project to prepare for future flights includes:

- Analysis of the Russian quail feed.
- Analysis of UW Madison quail adult and quail chick starter rations.
- Effect of turning during incubation
- Effect of chilling during incubation
- Gel feed containing adequate water for day-old chicks, and adults
- Artificial insemination of quail
- Freezing of semen

3. Cell-cell and cell-matrix interactions in migration, morphogenesis, and differentiation. Gravity is postulated to play a critical role in embryogenesis. In the case of *Xenopus* oocytes, gravity appears to be essential for the determination of the dorso-ventral axis. Turning of avian eggs is essential for the normal development of embryo in gravity. However, the requirement for turning of embryonated eggs for proper development of the avian embryo in space microgravity has not been resolved. One could postulate that gravity may not be the determining factor in embryogenesis. Rather cell-cell adhesion and cell-substrate interactions involving a variety of integrins, growth factors and extracellular matrix components may be the predominant contributing factors toward morphogenesis and tissue differentiation.

Space microgravity provides the ideal environment for studies involved in cell-matrix interactions. The major focus of the avian physiology laboratory is the primordial germ cell. Because of its unique extraembryonic segragation, migration and differentiation processes the primordial germ cell is the ideal cell to explore mechanisms which regulate cell adhesion, migration, chemotaxis and differentiation. The primordial germ cell possesses integrins which are presumed to react with specific extracellular matrix proteins and specific growth factors along its migratory route. Insights in this system of cell-ECM interactions could provide insights into other systems of cell-cell, cell-matrix interactions involved in development and in several physiological and pathological processes.

Table 1: Results from MIR 18 (STS 74);launched eggs ; synchronous and laboratory controls

Age at Fixation/ Number fixed on Fixation Day ( )	Flight/ Number Set Results				Synch Control/ Number Set Results				Lab. Control/ Number Set Results			
	48				48				48			
	Results				Results				Results			
	N	I	ED	#LD	N	I	ED	#LD	N	I	ED	#LD
Day 7 (8)	0	1	7		5		3		6	1	1	
Day 10 (8)	1	1	6		3		5		7		1	
Day 14 (8)	0	1	7		3		5		7	1		
Day 16 (8)	0		8		1		4	3	8			
(ETOH) (16) Day 16	0		16		0		13	3	11	2	3	

Legend: N = number fixed alive at designated age;I = infertile; ED = early dead; LD = late dead; ETOH: 16 eggs fixed at day 16 in ethyl alcohol. Flight = launched eggs;Synch. = synchronous controls= simulated temperature aboard MIR; Lab controls = Controls set in Moscow laboratory standard conditions.

Table 2: Results from MIR 19 (STS 76) launched eggs ; synchronous and laboratory controls

Age at Fixation/ Number fixed on Fixation Day ( )	Flight/ Number Set				Synch Control/ Number Set				Lab. Control/ Number Set			
	48				48				48			
	Results				Results				Results			
	N	I	ED	#LD	N	I	ED	#LD	N	I	ED	#LD
Day 7 (8)	2		6				8		6		1	
Day 10 (8)		1	7		?		6		5		3	
Day 14 (8)	0		8		0		8		7	1		
Day 16 (8)	0		8		0		7	1	8			
(ETOH) (16) Day 16	0		12	1	0	1	9		13			

Legend: N = number fixed alive at designated age;I = infertile; ED = early dead; LD = late dead; ETOH: 16 eggs fixed at day 16 in ethyl alcohol. Flight = launched eggs;Synch. = synchronous controls= simulated temperature aboard MIR; Lab controls = Controls set in Moscow laboratory standard conditions

Table 3. Results of transport controls and laboratory controls taken to hatch at Madison laboratory.

# Set	#F	#I	#C	#ED	#LD	#H	#P	%F	%V
LBG2 48	44	4	4	1	1	36	3	92%	89%
Lab 48	45	3	0	2	1	37	6	94%	96%

Legend: F=Fertile;I=Infertile; C=cracked;ED=early dead; LD=late dead; H=hatched; P=pipped or alive and not pipped. LBG2 = launch back up group 2; Laboratory = fresh laboratory controls.

Table 4. Laboratory test on hatchability of fertile Japanese quail egg storage at 40F for 6 and 5 days prior to incubation.

# Set (DSt)	#F	#I	#ED	#LD	#H	#P	%F	%H of F	%V
56 (6)	54	2	1	2	46	5	96.%	85%	91%
48 (5)	46	2	3	0	42	1	96%	91	94%

Legend: F=Fertile;I=Infertile; ED=early dead; LD=late dead; H=hatched; P=pipped or alive and not pipped. DST=days in storage before incubation; %V=%viable but not hatched by 17.2 days.

Table 5A GAVEET egg data

GAVEET Egg Data										
EGG ID	Date of Egg opening	Initial Egg Wt (g)	Final Egg Wt (g)	Embryo Age (d)*		EGG ID	Fix Date	Initial Egg Wt (g)	Final Egg Wt (g)	Embryo Age (d)*
E6						**E16**				
CENTRIFUGE (Group I)										
23	12/20/95	9.66	9.26	6		24	12/30/95	9.98	9.3	16
5	12/20/95	9.77	9.31	6		9	12/30/95	11.41	8.48	Infertile
14	12/20/95	11.21	10.89	6		13	12/30/95	11.57	10.73	
15	12/20/95	10.37	10.03	6		20	12/30/95	9.34	8.45	16
17	12/20/95	10.6	10.29	6		2	12/30/95	1/12/04	10.34	16
1	12/20/95	10.5	10.26	6		18	12/30/95	1/12/04	9.89	16
21	12/20/95	10.54	9.87	6		3	12/30/95	1/11/04	9.68	16
6	12/20/95	9.06	8.78	6		7	12/30/95	1/12/04	10.79	16
10	12/20/95	10.23	9.92	6		12	12/30/95	1/12/04	10.95	16
16	12/20/95	10.7	10.36	6						
8	12/20/95	11.02	11.3	6						
22	12/20/95	9.77	9.56	6						
CENTRIFUGE AND VIBE (Group II)										
43	12/20/95	10.35	10.08	6		45	12/30/95	10.46	9.75	16
34	12/20/95	11.27	10.89	6		28	12/30/95	10.67	9.82	16
47	12/20/95	10.27	9.87	Infertile		48	12/30/95	9.5	8.68	16
26	12/20/95	9.86	9.49	Infertile		36	12/30/95	10.76	10.05	16
30	12/20/95	10.58	10.2	6		41	12/30/95	10.22	9.55	15.5
33	12/20/95	9.76	9.43	6		31	12/30/95	10	9.38	1
27	12/20/95	10.48	10.21	6		42	12/30/95	9.22	8.38	1
29	12/20/95	10.59	6.83	2		25	12/30/95	9.46	8.63	16
44	12/20/95	9.62	9.27	6		37	12/30/95	10.49	9.8	16
38	12/20/95	10.13	9.77	6		46	12/30/95	10.11	8.94	Infertile
35	12/20/95	10.49	10.21	Infertile		39	12/30/95	9.69	8.77	15.5
40	12/20/95	9.37	8.96	6			12/30/95			

Table 5B GAVEET egg data

EGGID	Date of Egg opening	Initial Egg Wt (g)	Final Egg Wt (g)	Embryo Age (d)*		EGGID	Fix Date	Initial Egg Wt (g)	Final Egg Wt (g)	Embryo Age (d)*
VIBE (Group III)										
49	12/20/95	9.14	8.83	6		50	12/30/95	9.77	8.91	16
54	12/20/95	9.34	8.98	6		58	12/30/95	10.14	9.4	Infertile
51	12/20/95	10.48	10.03	6		71	12/30/95	10.48	9.66	16
70	12/20/95	10.57	10.26	6		69	12/30/95	10.77	9.94	16
65	12/20/95	9.93	9.61	6		72	12/30/95	9.64	8.98	16
57	12/20/95	9.9	9.53	Infertile		53	12/30/95	9.28	8.43	16
63	12/20/95	10.1	9.75	6		62	12/30/95	11.5	10.63	16
61	12/20/95	9.63	9.37	6		56	12/30/95	8.65	7.87	Infertile
55	12/20/95	9.61	9.27	6		60	12/30/95	9.87	9.03	16
64	12/20/95	10.29	9.92	6		66	12/30/95	10.78	9.72	Infertile
68	12/20/95	10.34	8.56	6						
67	12/20/95	10.11	9.8	Infertile						
CONTROL (neither vibe or centrifuge - Group IV)										
83	12/20/95	9.38	9.08	6		73	12/30/95	11.09	8.68	16
86	12/20/95	9.6	9.3	6		89	12/30/95	10.74	8.49	16
92	12/20/95	11.05	10.71	6		74	12/30/95	9.82	8.9	16
80	12/20/95	11.87	10.57	6		96	12/30/95	9.03	8.27	16
88	12/20/95	9.88	9.52	6		94	12/30/95	9.45	8.65	2
75	12/20/95	9.59	9.29	6		93	12/30/95	11.9	10.95	16
84	12/20/95	9.2	7.75	6		97	12/30/95	9.85	9.1	Infertile
90	12/20/95	9.6	9.26	6		76	12/30/95	9.35	8.62	Infertile
87	12/20/95	10.11	9.78	6		85	12/30/95	9.5	8.64	Infertile
82	12/20/95	9.53	9.23	6		91	12/30/95	10.06	9.28	16
81	12/20/95	11.14	10.8	6		77	12/30/95	11.62	10.4	16
78	12/20/95	9.25	8.7	6		79	12/30/95	10.05	9.24	16

*Age of embryo as determined by PI team at time of dissection

All eggs placed in incubator 12/14/95

Egg marked with sharpie was 16 days embryo

Eggs #11,19,32,52 broken at start of incubator, egg #4 cracked during centrifugation/vibration

Table 6a. Body Weights of male and female adult Japanese quail maintained on a gelatin based diet for one week. Preliminary data.

	Ave. Weight @ Start	Ave. Weight After 7 days
Control Females	149.5	156.5
Experimental Females	150.8	152.5
Control Males	119.8	114.9
Experimental males	119.0	115.0

Table 6b. Egg production and egg weights from Japanese quail females for one week prior to being placed on experimental gelatin ration and for one week after being on the gelatin based diet. (Three birds)

	Control (prior to exp't)	Experimental
Number of Eggs	17	17
Average Egg weight	9.87	9.5

* Birds fed limited water (i.e. gelatin diet cut up so that it evaportated stopped laying at day three of the experiment.

Request roll over of 1995-1996 NASA grant (October, 1995 - April 30, 1996) as it was an abbreviated year with unfinished laboratory work as a result of the two flights during the grant period. NASA Grant # NAG 2-1009

FORM B

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: B. Wentworth

DETAILED BUDGET FOR 12-MONTH BUDGET PERIOD DIRECT COSTS ONLY		**FROM**		**THROUGH**	
Duplicate this form for eacy year of grant support requested		**DOLLAR AMOUNT REQUESTS** *(Omit cents)*			
PERSONNEL *(Applicant Organization Only)*		**EFFORT ON PROJECT**	**SALARY**	**FRINGE BENEFITS**	**TOTALS**
NAME	**ROLE IN PROJECT**				
B. Wentworth	Principal Investigator	10%	0	0	0
	Student labor	35 hrs.	$210	$5	$215
	SUBTOTALS →				
CONSULTANT COSTS	Slide sectioning (Sch. of Vet. Med.)				1,500
EQUIPMENT *(Itemize, use additional sheet if needed)*	transilluminator (white light)				375
SUPPLIES *(Itemize by category, use additional sheet if needed)*	Expendable supplies				1,204
TRAVEL	**DOMESTIC**				
	FOREIGN Russia				4,700
OTHER EXPENSES *(Itemize by category, use additional sheet if needed)*	current protocols				250
	maintain quail colony				1,000
	air and fed ex charges				306
TOTAL DIRECT COSTS FOR FIRST 12-MONTH BUDGET PERIOD *(Item 8a, Form A)*				$	9,550
INDIRECT COSTS FOR FIRST 12-MONTH BUDGET PERIOD				$	
TOTAL COSTS FOR FIRST 12-MONTH BUDGET PERIOD *(Item 8b, Form A)*				$	9,550

G0018RM

Continuation of NASA Grant # NAG 2-1009

FORM B

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: B. Wentworth

DETAILED BUDGET FOR 12-MONTH BUDGET PERIOD DIRECT COSTS ONLY		FROM		THROUGH	
Duplicate this form for eacy year of grant support requested		DOLLAR AMOUNT REQUESTS *(Omit cents)*			
PERSONNEL *(Applicant Organization Only)*					
NAME	ROLE IN PROJECT	EFFORT ON PROJECT	SALARY	FRINGE BENEFITS	TOTALS
B. Wentworth	Principal Investigator	10%	0	0	0
	Research Associate	20%	$6,388	$2,537	$8,925
	Student labor	165 hrs.	990	19	1,009
		SUBTOTALS →			
CONSULTANT COSTS	slide sectioning (Sch. Vet. Med.)				3,500
EQUIPMENT *(Itemize, use additional sheet if needed)*	bar code scanner				2,200
SUPPLIES *(Itemize by category, use additional sheet if needed)*	expendable supplies				4,860
TRAVEL — DOMESTIC	Ames (4), Kennedy (1), Houston (1)				7,100
TRAVEL — FOREIGN					
OTHER EXPENSES *(Itemize by category, use additional sheet if needed)*	current protocols				465
	maintain quail colony				1,800
	publication costs				1,200
TOTAL DIRECT COSTS FOR FIRST 12-MONTH BUDGET PERIOD *(Item 8a, Form A)*				$	31,059
INDIRECT COSTS FOR FIRST 12-MONTH BUDGET PERIOD				$	11,445
TOTAL COSTS FOR FIRST 12-MONTH BUDGET PERIOD *(Item 8b, Form A)*				$	42,504

G0018RM

Supplemental budget to cover an unplanned expense of providing eggs for the STS-76 flight. NASA Grant # NAG 2-1009

FORM B

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: B. Wentworth

DETAILED BUDGET FOR 12-MONTH BUDGET PERIOD DIRECT COSTS ONLY		FROM		THROUGH
Duplicate this form for eacy year of grant support requested	**DOLLAR AMOUNT REQUESTS** *(Omit cents)*			

PERSONNEL *(Applicant Organization Only)*					
NAME	**ROLE IN PROJECT**	**EFFORT ON PROJECT**	**SALARY**	**FRINGE BENEFITS**	**TOTALS**
B. Wentworth	Principal Investigator	10%	0	0	0
	Student labor	50 hrs.	$300	$6	$ 306
	SUBTOTALS →				
CONSULTANT COSTS					
EQUIPMENT *(Itemize, use additional sheet if needed)*					
SUPPLIES *(Itemize by category, use additional sheet if needed)*					
TRAVEL	DOMESTIC				
	FOREIGN				
OTHER EXPENSES *(Itemize by category, use additional sheet if needed)*	maintain quail colony				1,800
	air and fed ex charges				894
TOTAL DIRECT COSTS FOR FIRST 12-MONTH BUDGET PERIOD *(Item 8a, Form A)*				$	3,000
INDIRECT COSTS FOR FIRST 12-MONTH BUDGET PERIOD				$	630
TOTAL COSTS FOR FIRST 12-MONTH BUDGET PERIOD *(Item 8b, Form A)*				$	3,630

G0018RM