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BIOCHEMICAL CHANGES IN TISSUES DURING INFECTIOUS ILLNESS: METAB--ETC(U)

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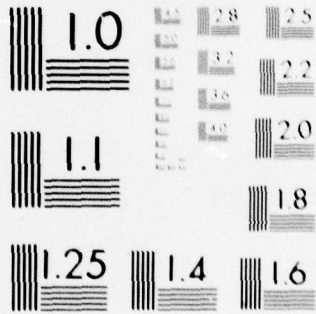
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**6** BIOCHEMICAL CHANGES IN TISSUES DURING INFECTIOUS ILLNESS: METABOLIC CONSEQUENCES OF INTERACTIONS BETWEEN INFECTIOUS ILLNESS AND EXERCISE

by

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at speeds of 10-14 rpm; the chick will accept slightly higher speeds (12-20) but time to exhaustion drops to 60 minutes.

Preliminary comparisons of forced with voluntary exercise in rats and chicks indicates that the voluntary exercise is less stressful, as measured by higher energy reserves of liver glucose and glycogen in animals under such a regimen.

We are now able to prepare S. typhimurium inocula to obtain reasonable dosage responses with regard to the survival time of rats over a 12-day period. Interactions of this disease with single sessions of 2 hours of forced exercise demonstrate conclusively the importance of time of interaction with stage of disease cycle. Highest mortalities correlated with forced exercise sessions 24 hours post inoculation. While minimal prior conditioning does help subjects when put in a forced exercise situation, there are no present indications that prior conditioning is of benefit in resisting a disease insult.

Preliminary data show that energy demands of forced exercise x infectious disease will result in significant depressions of liver glucose and glycogen; changes in levels of lipid fractions, such as mono-, di-, triglycerides, free fatty acids and the cholesterols, are of much less magnitude and more variable.

Induced spontaneous activity (wheel running) in chicks repeatedly has been shown to be significantly depressed by low levels of food toxicants, an effect similar to that resulting from an infectious disease. This phenomenon has potential not only for detecting disease insults to metabolism but also as a biological marker for further understanding infectious processes.

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## SUMMARY

A special apparatus was designed and successfully tested in which weanling rats and baby chicks can be forced to run. Standard Wahmann running wheels, equipped with pulleys, are aligned in a series of 8, all powered from a single shaft by a geared electric motor, adjustable from 0 to 100 rpms. The apparatus is such that rats or chicks or a combination of both can be forced to run simultaneously with only a single operator present.

When forced to run to exhaustion, the weanling rat will run up to 2 hours at speeds of 10-14 rpm; the chick will accept slightly higher speeds (12-20) but time to exhaustion drops to 60 minutes.

Preliminary comparisons of forced with voluntary exercise in rats and chicks indicates that the voluntary exercise is less stressful, as measured by higher energy reserves of liver glucose and glycogen in animals under such a regimen.

We are now able to prepare *S. typhimurium* inocula to obtain reasonable dosage responses with regard to the survival time of rats over a 12-day period. Interactions of this disease with single sessions of 2 hours of forced exercise demonstrate conclusively the importance of time of interaction with stage of disease cycle. Highest mortalities correlated with forced exercise sessions 24 hours post inoculation. While minimal prior conditioning does help subjects when put in a forced exercise situation, there are no present indications that prior conditioning is of benefit in resisting a disease insult.

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## INTRODUCTION

Our major effort this year has been concentrated on developing and testing a workable model of forced activity that would be superior to those reported in the literature and that could be interacted with infectious disease. The proposed improvements were that such a model should be developed from standard running wheels rather than the use of treadmills and 1) be adaptable to both rats and chicks; 2) be able to accommodate a minimum of 8 animals running simultaneously each session to make observations significant; 3) be adjustable so that various degrees of forced activity could be used, that is, the speed regulated; and 4) be easily dismantled and cleaned.

Treadmills were tried but proved unsatisfactory, principally because it was not practical to manage large numbers of animals; they were not easily dismantled for cleaning purposes; the animals, both rats and chicks, did not adjust quickly enough to the mechanism, with the result that unless each subject was carefully supervised or motivated by air blasts or electric shock it would be carried to the rear of the treadmill box, be caught in the revolving belt and suffer irreparable injury.

Swimming was tried with rats but this proved to have disadvantages, such as lack of uniformity of response and problems with attaining high enough stress loads without filling the lungs with water.

Since we had the Wahmann running wheels and a wealth of experience as to their use, we focused our efforts on working out an arrangement whereby up to 8 wheels were hooked to a common power shaft which can be operated at any speed desired with full control over the safety of the subjects. While our equipment will accept an additional 8 wheels, the procedure would require two attendants. Experiments, therefore, will run sequentially in matched groups until the required number of animals per treatment group is satisfied.

The present report covers a series of trials, with rats and chicks, which were designed to confirm the validity of our new equipment and to provide preliminary comparisons of voluntary and forced exercise regimens.

## I. SWIMMING AS AN EXERCISE MODEL

Our initial thoughts in studying the interactions of infectious disease with forced exercise were that swimming models, using rats, would be ideal. Such models have been employed and reported upon in the literature. While we completed a number of swimming studies, only one trial is presented here.

We found there were problems in collecting valid data. Problems ranged from the fact that: a great deal of training was required to induce rats to swim properly; water would enter the lungs; most subjects mastered the art of floating or holding their respiration while resting on the bottom of the tank. Tail weights helped but needed to be changed every day to maintain a constant ratio to body weight (2 to 8%).

The data in table 1 are from groups of 7 rats, noninfected and infected with *S. typhimurium*, and trained to swim in daily 5 min sessions over a 10-day period. At this time they were allowed to swim to exhaustion (86 min average for controls and 95 min for infected rats). They were immediately decapitated and the livers removed and frozen for biochemical analyses.

Liver weights were slightly lower in the infected group. While there was little difference in liver glucose concentrations or total quantities (concentrations x liver weight), glycogen was significantly lower in the infected rats. Due to the high variability among subjects in ability to swim to exhaustion, within group biochemical data were also highly variable. Other trials showed the same trends and demonstrated that another forced exercise model would have to be developed if we were to undertake basic studies on muscle and other tissue responses during infectious illness.

Table 1. Effect of *S. typhimurium* on liver carbohydrate reserves of trained rats forced to swim to exhaustion (11 days post inoculation)

Parameter	Control	Infected
	n = 7	n = 7
Average time swimming (min)	86	95
Liver weight (g)	5.4	5.0
Liver/body weight (%)	4.3	4.3
Glucose (mg/g liver)	2.1	1.8
Glycogen "	25.3	13.8
Glucose (mg/total liver)	11.2	8.9
Glycogen "	136.6	69.0

## II. USE OF THE CHICK AS A FORCED EXERCISE MODEL

Our initial trials have been directed toward finding out 1) whether naive or previously trained animals should be used; 2) how long a training session can be imposed and at what speeds without causing immediate death; 3) whether the equipment would accept both young weanling rats and baby chicks; 4) at what stage of an infectious disease cycle the forced exercise should be administered.

The first series of studies employed male chicks 3 days old, with the objective of determining the effects of increasingly longer training periods of forced running on the animal's physical well-being and growth. In all trials the chicks were offered a commercial chick diet ad libitum. Upon arrival from the hatchery, the day-old chicks were assigned to experimental groups so that the average group body weight was uniform between treatments. After a 3-day accommodation period, three groups were formed. Group 1, the controls, were not given any exercise; group 2 subjects were allowed constant voluntary access to running wheels; and group 3 subjects were put in a forced exercise condition at 24 rpm. Three replicate trials and one pilot trial were conducted with the following protocol for the forced exercise groups:

	# training sessions per day	Duration of Forced Running Sessions			
		Trial 1	Trial 2	Trial 3	Trial 4
Day 1	2	5 min	5 min	5 min	5 min
Day 2	2	10	6	8	10
Day 3	1	15	12	16	20
Day 4	1	20	18	24	30
Day 5	1	25	24	32	40
Day 6	1	30	30	40	50
Day 7	1	--	40	50	60

After the final session in each trial the chicks were killed and livers removed for biochemical analyses of glucose and glycogen to observe the effects of exercise on immediately utilizable and reserve sources of energy.

A comparison of the number of voluntary wheel turns produced in the voluntary group vs the forced exercise is as follows:

Table 2.	Total wheel turns, 7 days	
	Voluntary exercise	Forced exercise
Trial 1 (pilot)	--	2,880
Trial 2	8,869	3,504
Trial 3	11,826	4,512
Trial 4	15,808	5,520

At no time were there any deaths due to the forced or voluntary activity, yet at the end of each trial's final session most forced run chicks were utterly exhausted. It was observed that the chicks apparently adapted to the length of each day's training session in that exhaustion was more rapid after they had passed the previous day's time period.

The biochemical data for trials 1-4 are summarized in table 3.

Table 3. Summary of trials with chicks forced to exercise in running wheels for increasingly longer periods

Final period of forced exercise	Final body wt. g	Liver wt. g	Liver/Body %	Glucose mg/total	Glycogen liver	Glycogen/Glucose mg
<u>Trial 1, 30 min</u>						
No exercise	97.9	3.5	3.5	3.7	17.8	4.8
Voluntary <sup>1/</sup>	----	---	---	---	----	---
Forced	79.1	3.6	4.6	4.0	43.8	11.0
<u>Trial 2, 40 min</u>						
No exercise	98.0	4.1	4.2	5.8	41.0	7.1
Voluntary	92.3	4.0	4.3	4.8	40.6	8.5
Forced	80.5	3.4	4.2	1.6	10.1	6.3
<u>Trial 3, 50 min</u>						
No exercise	95.0	3.9	4.1	4.7	34.9	7.4
Voluntary	100.3	3.9	3.9	5.5	51.3	9.3
Forced	87.4	3.8	4.3	1.8	10.5	5.8
<u>Trial 4, 60 min</u>						
No exercise	90.6	3.5	3.9	2.7	37.5	13.9
Voluntary	81.3	3.5	4.3	3.1	20.0	6.5
Forced	78.0	3.5	4.5	1.9	15.7	8.3

<sup>1/</sup> No voluntary group in this trial

#### Comments

Forced exercise resulted in lower body weights while voluntary exercise effects on body weights were minimal. Liver/body weight ratios, which we use to evaluate the extent of stress involvement, tended to be higher in the forced exercise groups in trials 1 and 4. With the exception of trial 1, forced exercise significantly depressed glucose and glycogen reserves

compared to the groups not exercised. Values in the voluntary groups did not have a consistent pattern but tended to be the same as or slightly higher than the controls. One can speculate that the shorter exercise period of trial I (30 min) had the same effect as voluntary exercise since glucose and glycogen levels were similarly above control values.

These preliminary findings were considered biologically significant because they demonstrate 1) a definite energy reaction to exercise; 2) that the depressive reaction on energy reserves occurred in spite of prior conditioning; and 3) that the chick will be a good model for later studies on forced exercise interactions with infectious disease, especially viral infections.

In the final preliminary trial in this series with chicks, the training sessions were delayed until the chicks were 12 days old in order to observe reactions when the chicks were free of the yolk sac and less poikilothermic. They were then trained in the forced situation in 3 sessions of 5 min each with a speed of 16 rpm. The next day they also had 3 similar sessions. These brief sessions were to provide the least possible prior conditioning of the animals. On the next and final day they were forced to run for 40 min. There was no voluntary group in this trial. Immediately following the forced running session the chicks were killed and the livers obtained for analyses of glucose, glycogen and several lipid fractions.

Table 4 compares the biochemical data for the non-running control group with the forced exercise group. It should be noted that the data for the forced group were arrayed according to the grams of body weight lost in relation to the average loss during the final 40 min session of forced exercise, that is, those above the mean weight lost (4.4 g), those at the mean, and those below the mean.

Table 4. Liver weight, carbohydrate and lipid values in chicks forced to exercise.

Parameter	Control values <sup>1/</sup>	Forced runners		
		Above 4.4 g n=6	At 4.4 g n=3	Below 4.4 g n=7
Liver weight (g)	4.7	4.8	4.5	4.3
Liver/body wt. (%)	3.0	3.2	3.0	3.0
Glucose (mg/total liver)	6.8	4.3	1.3	1.6
Glycogen	69.6	34.3	2.4	6.7
Monoglycerides	7.0	7.4	8.2	5.9
Diglycerides	12.2	13.4	11.5	9.1
Triglycerides	19.9	23.0	17.9	17.0
Free fatty acids	18.9	19.7	18.1	15.8
Cholesterol	15.7	14.5	14.4	12.8
Chol. esters	10.2	11.7	10.3	9.6

<sup>1/</sup> Controls - no exercise

## Comments

It was hypothesized that the energy costs of forced exercise would equate to body weight lost during the exercise period. As shown in table 4, arraying the individual data according to loss of weight revealed that the chicks losing more than average had more total liver glucose and glycogen than the chicks with less weight loss. While differences were of less magnitude, the same trend was observed in the lipid fractions.

### III. USE OF THE WEANLING RAT AS A FORCED EXERCISE MODEL

We next evaluated our new running wheel equipment with 22-day-old male weanling rats. Here we immediately encountered the problem of the animals clinging to the wire on the wheel drum and hanging on and "looping the loop" rather than running. The solution was to install plastic baffles so that each time the wheel revolved the rat would collide with the baffle and be forced to drop off. This has been a most successful modification since the rats quickly learn to avoid colliding with the baffle. The optimum position of the baffle is still being worked out at the time of this report.

The first trial employed three groups of 6 weanling rats each: group 1, the non-running controls; group 2 was allowed to run voluntarily in running wheels only for the same length of time as group 3 which was put in a forced exercise situation. Forced running sessions began the day the rats were received and lasted 5 min/day at a speed of 7 rpm. The 5 min periods were designed to train the rats with a minimum of physical conditioning. On day 7 the rats were forced to run continuously for 2 hours at 7 rpm and were then killed and the livers immediately obtained and frozen for biochemical determinations. The rats were fed a standard rat diet on a restricted feeding schedule which was approximately 70% of ad libitum intake, an amount calculated to be equivalent to that of a disease effect on dietary intake. Clinically, at the end of the final 2 hours of forced exercise the rats, while obviously tired, were not so exhausted that they could not stand up. The data are presented in table 5.

Table 5. Liver weights and lipid fractions of noninfected weanling rats under voluntary and forced exercise regimens.

Parameter	Control values <sup>1/</sup>	Voluntary % of control values	Forced
Liver weight	1.91 g	100	92
Liver/body weight	3.3%	96	98
Monoglycerides	3.6 mg/total liver	151	142
Diglycerides	2.5 "	139	118
Triglycerides	2.2 "	171	140
Free fatty acids	7.8 "	120	124
Cholesterol	5.4 "	115	104
Chol. esters	3.7 "	116	107
Body wt. lost during final 2 hr forced exercise session		0.7 g	1.5 g
Wheel turns final 2 hr session		1076	840

<sup>1/</sup> Controls - no exercise

### Comment

Liver/body weight ratios were not affected by exercise. Loss in body weight during the exercise period the final day was twice as much in the forced group. Wheel turns for the voluntary animals during the 2 hours allotted them were 1076 or 28% more than the rats on the forced regimen. Total quantities of liver lipid fractions were increased by exercise, with values slightly higher in rats running voluntarily.

These data for the rat are in line with the chick data shown in table 3 where liver glucose and glycogen values tended to be higher in chicks running on a voluntary basis than in control and forced groups. Present data do not explain this apparent conservation of energy resources.

The second trial was similar to the first but each activity condition contained a group of noninfected rats and a group inoculated with S. typhimurium. Again, rats were trained by being forced to run in the wheels 5 min/day and at 6 days post inoculation were forced to exercise 2 hours at 12 rpm (an increase of 5 rpm) and then killed by decapitation. Livers and spleens were removed to determine the extent of disease involvement. Noninfected rats running voluntarily produced 786 wheel turns and the infected rats 849, vs 1440 wheel turns for those forced to run. As in the previous trial, some of the forced rats, exhausted at the end of the 2 hour session, appeared to recover rapidly when returned to the cage. At 6 days post inoculation the S. typhimurium infection, however, was not advanced enough to provide valid comparisons between noninfected and infected animals.

A third trial repeated the conditions of trial 2 except that the termination was set for 7 days post inoculation. To test the effect of the prior 5 min daily training sessions, an extra noninfected "naive" group was included which was not forced to exercise until the final day at which time it was forced to run in the wheels for 2 hours. The spleen/body weight ratio of the noninfected, nonactive group was 0.52% compared to 2.03% for the infected non-runners, indicating a satisfactory degree of disease involvement. Again, the 2 hour session did not result in deaths due to the exercise even though, as before, some rats were completely exhausted. Results of this trial are summarized in table 6.

Table 6. Effect of voluntary and forced exercise on liver lipids of weanling rats infected with *S. typhimurium* (7 days post inoculation)

Parameter	Control values <sup>1/</sup>	Noninfected			Infected		
		V <sup>2/</sup>	F <sub>1</sub> <sup>2/</sup>	F <sub>2</sub> <sup>2/</sup>	No exer.	V	F <sub>2</sub>
		Percent of control values					
Liver/body wt. (%)	3.9	100	95	100	146	138	123
Per total liver (mg)							
Monoglycerides	2.25	97	97	145	193	165	157
Diglycerides	2.51	110	104	129	161	146	143
Triglycerides	6.04	121	104	144	137	119	139
Free fatty acids	6.13	114	105	127	160	135	135
Cholesterol	6.95	95	88	104	164	137	120
Chol. esters	4.42	92	86	117	131	136	117
Wheel turns	----	1651	1440	1440	---	1658	1440
Weight lost due to running (g)	----	0.8	0.9	2.8	---	2.0	3.1

<sup>1/</sup> Controls: no exercise, noninfected

<sup>2/</sup> V = voluntary access to wheels same length of time as forced group

F<sub>1</sub> = rats not allowed exercise until final 2 hour session

F<sub>2</sub> = rats given daily 5 min sessions of forced exercise and 2 hours on last day

#### Comments

Liver/body weight ratios were significantly higher in all infected groups, but the type of exercise interacted with the *S. typhimurium* infection with the result that the forced group had the least disease-related increase.

In the noninfected rats, values for the lipids were highest in the forced group which had prior conditioning. Untrained rats forced to run for 2 hours had values close to the non-running controls except for liver cholesterol and esters. Lipids in the non-exercising, infected rats were significantly elevated. Voluntary exercise, even such a slight amount as 1658 wheel turns in 7 days, reduced these lipid quantities. Forced exercise caused an even further reduction so that both the noninfected and infected rats forced to run had comparable levels of liver lipids.

Since the data from trial 3 with rats showed that young, naive rats were able to sustain a 2 hour forced exercise situation, in the fourth trial the same overall design as the previous trial was used but with none of the rats given prior training. On the final day they were forced to run for 2 hours; this was 9 days post *S. typhimurium* inoculation in order to allow a more advanced state of infection. Two additional groups, noninfected and infected, were allowed continuous voluntary access to running wheels and on the last day of conditioning were forced to run for 2 hours the same as the other groups. Speed of the wheels was increased from 12 to 14 rpm. Spleen/body weight ratio of nonactive controls was 0.60% and for the infected non-exercised group 1.52%, indicating a reasonable degree of infection. Results are summarized in table 7.

Table 7. Effect of voluntary and forced exercise on liver lipid fractions in weanling rats infected with *S. typhimurium* (9 days post inoculation)

Parameter	Control values <sup>1/</sup>	Noninfected			Infected			
		V <sup>2/</sup>	F <sup>2/</sup>	V/F <sup>2/</sup>	No exer.	V	F	V/F
		Percent of control values						
Liver/body wt. (%)	3.9	108	100	105	133	123	121	128
Per total liver (mg)								
Monoglycerides	3.78	120	145	101	117	109	124	128
Diglycerides	4.08	91	97	104	134	109	112	107
Triglycerides	5.94	120	156	123	148	127	134	128
Free fatty acids	13.39	118	137	106	115	112	120	122
Cholesterol	9.02	101	106	95	124	113	111	107
Chol. esters	6.74	107	95	88	110	113	107	118
Wheel turns	----	620	1680	35,304	---	695	1680	14,370
Weight lost due to running (g)	----	1.9	1.7	3.2	---	1.8	1.2	2.9

<sup>1/</sup> Controls: no exercise, noninfected

<sup>2/</sup> V = ran voluntarily at same time as forced group, no previous training

F = forced to run 2 hours on final day only; no previous training

V/F = ran voluntarily from day 0; forced to run 2 hours on final day;

Wheel turns are total of 9 days plus 1680 wheel turns final day.

#### Comments

As in the previous study, infection significantly increased liver/body weight ratios irrespective of exercise regimen, with those of the exercising

groups being slightly below the non-runners. As indicated by the spleen/body weight ratios at 9 days post inoculation, the infection was not severe at that time (there were no deaths). Nevertheless, lipid values in the infected non-exercising rats were increased above their noninfected counterparts. Regardless of type of exercise, values were no higher in these groups and in certain instances lower. These data follow the same trend as the data in table 6 with rats sacrificed 7 days post inoculation.

#### IV. EFFECT OF FORCED EXERCISE ON SURVIVAL OF RATS INOCULATED WITH VARYING LEVELS OF S. TYPHIMURIUM

Prior to undertaking these final experiments of the 1979 year, we consulted with our colleagues at the Army Institute of Infectious Diseases. We reported that we had developed and defined a forced exercise model that would be equally applicable to rats and chicks. The use of more than one species is believed to offer closer simulation of human responses and the potential of covering a greater variety of diseases, especially the avian viruses.

In the first trial of the new series, two groups of weanling rats, 16/group, were fed our standard energy diet ad libitum. Two days after arrival (23 days old) both groups were inoculated with 0.1 ml of a S. typhimurium culture calculated to yield 50% mortality. Twenty-four hours post inoculation one group was forced to run at 14 rpm until 25% of the group reached complete exhaustion. This criterion was met with 75 min of continuous running in the wheels. The rats were then returned to their home cages and given no further opportunity to exercise for the duration of the trial which was designed to last 12 days in order to fully cover the period of overt illness and mortality.

In the second trial the design was the same but the strength of the inoculum was increased by injecting 0.2 ml of the S. typhimurium in order to compare mortality rates following very high levels of overt illness.

Table 8 shows the cumulative mortality for the two trials. In trial 1, first death occurred on day 7 in the forced group and on day 10 in the non-exercised group. At the end of the 12-day trial, 17% of the non-runners had died and 38% of the rats forced to run for only 75 min 24 hours post inoculation.

With the severe infection used in trial 2, there was no difference in rate of death or end point mortality; this trial necessarily concluded at the end of 6 days and demonstrated that the high level of disease insult masked separation of exercise x disease interactions.

Fig. 1 shows the daily growth rate of rats that lived and those that died in trial 1. The forced-run rats that survived the trial continued growing at the same rate as the nonactives until day 4 when their rate of

gain became somewhat less than the nonactives. In the forced run rats that died, growth rate was affected immediately by the session of forced running and the curve continued to be well below the nonactives that eventually died.

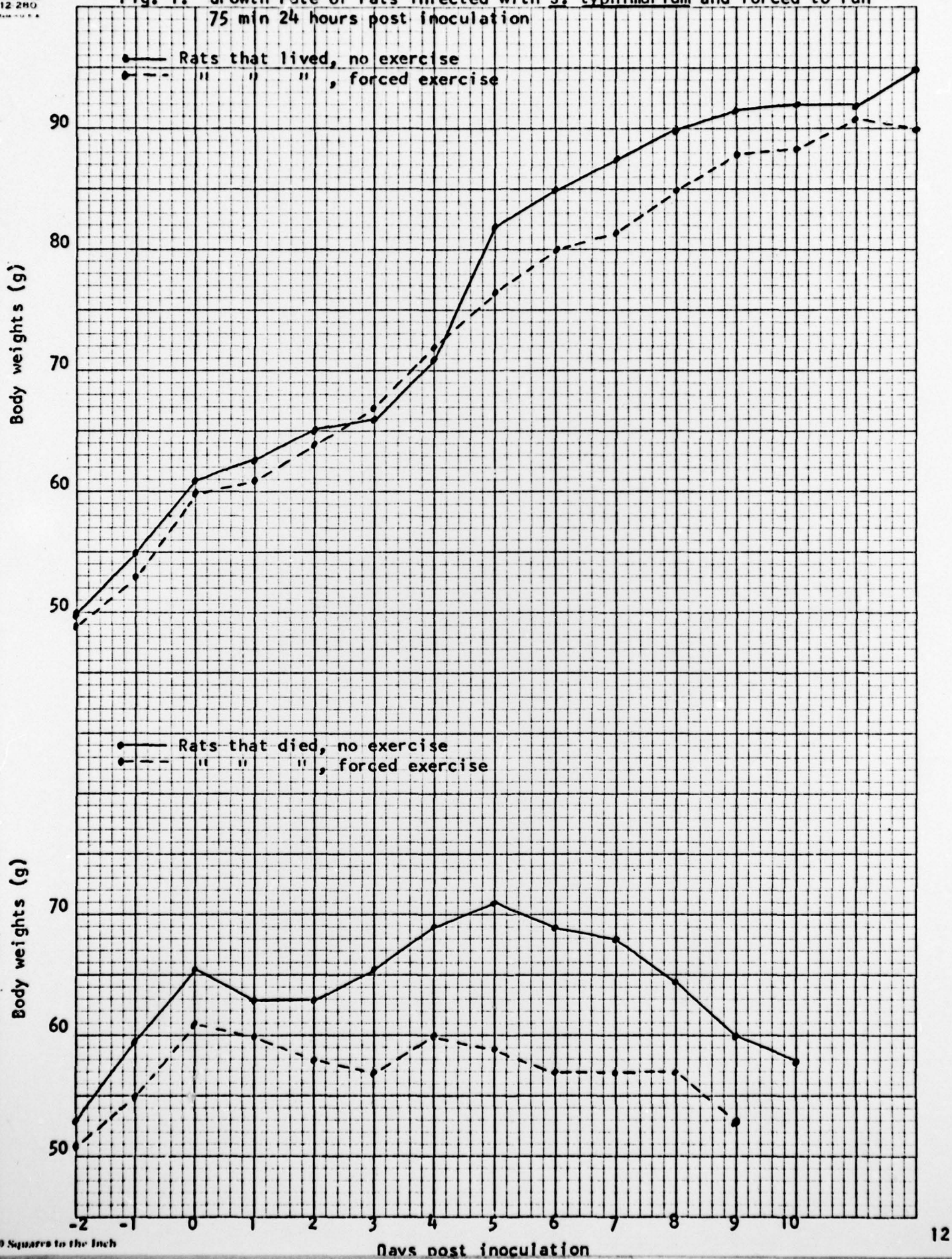
Table 8. Rate of mortality of weanling rats infected with *S. typhimurium* and forced to run for 2 hours 24 hours post inoculation.

Days post inoculation	Trial 1		Trial 2	
	No exercise	Forced exercise	No exercise	Forced exercise
Daily mortality, n = 16/group				
1	0	0	0	0
2	0	0	2	1
3	0	0	3	6
4	0	0	7	4
5	0	0	0	2
6	0	0	0	0
7	0	1	trial ended	
8	0	1		
9	0	1		
10	0	3		
11	2	0		
12	0	0		
Total	2	6	12	13

#### Comments

These trials raise questions as to effects on survival time of forced exercise in relation to the disease cycle. More importantly, they establish the fact that we now have a working model that will permit basic studies on energy demand and biochemical changes in tissues, especially heart and skeletal muscle, liver, spleen and brain.

Fig. 1. Growth rate of rats infected with *S. typhimurium* and forced to run 75 min 24 hours post inoculation



## V. BIOCHEMICAL BASES OF VOLUNTARY WHEEL RUNNING IN CHICKS

Parallel studies in our laboratories which are tied to our Army research are concerned with searching for biochemical bases of voluntary wheel running in rats and chicks. Ad libitum fed animals (rats and chicks) will seldom run over 1,000 wheel turns in a 24-hour period. This level of running is not sufficient for comparing effects of treatment on voluntary activity. The desire to run, however, can be induced by restricting food intake. Food restriction of 70 to 90% of ad libitum induces spontaneous activity and results in a high number of wheel turns (6,000 to 10,000).

We have used this phenomenon in our voluntary exercise x infectious disease studies because animals suffering with an infectious disease seldom have normal dietary intakes. Our technique is to project a desired level of disease involvement and then estimate the amount of diet which will be totally consumed so that intake of dietary nutrients can be calculated. While they are not studying exercise interactions, our colleagues at the U. S. Army Institute of Infectious Diseases also use feed restriction techniques during infectious illness (Dr. Neufeld, personal communication).

The studies reported below were carried out by one of our graduate students to determine whether normal breakfast cereals would contain a factor(s) which would influence rate of voluntary wheel running. Baby chicks were selected for the study because their nutritional requirements are well known.

A popular breakfast cereal, "Life", was purchased in a local supermarket and fed on a restricted basis to 9-day-old chicks given continuous voluntary access to running wheels. A companion group was fed an isocaloric, isonitrogenous diet.

The effect of the Life cereal was immediate in that this group ran significantly less than the controls and continued to do so until the end of the 12-day trial. A second trial was conducted, replicating all conditions of the first, and again the Life group ran less than the chicks on the control diet. There was no diet effect on body weights of the nonactive chicks in either trial, but because running was so much less in the Life chicks their body weights were above those of the active controls. Fig. 2 shows the typical activity curves for the two diet treatments.

At the end of the second trial livers were obtained for biochemical analyses. These results are summarized in table 9.

FIGURE 2  
DAILY WHEEL TURNS OF CHICKS FED LIFE CEREAL

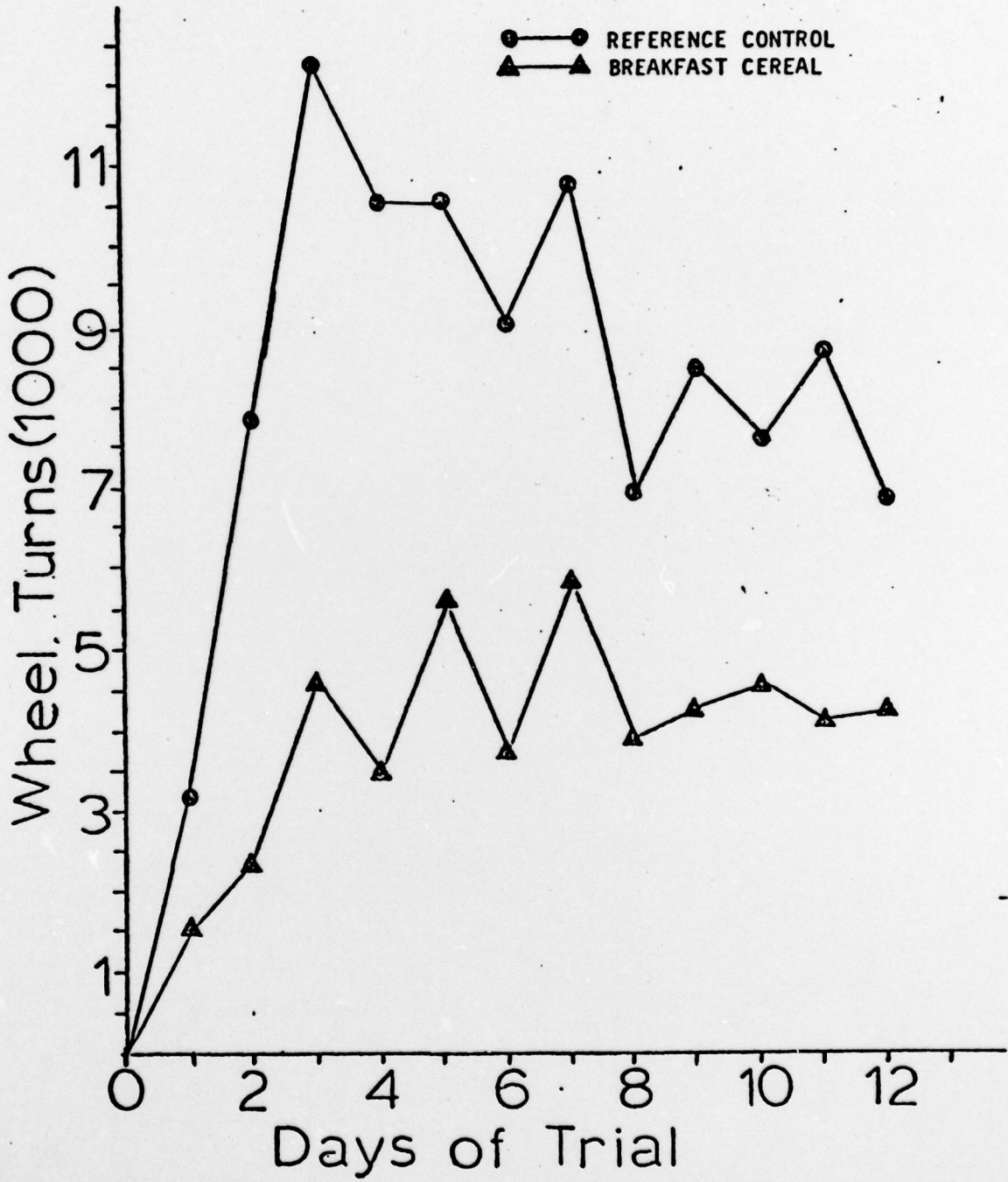


Table 9. Effect of Life breakfast cereal on carbohydrate and lipid fractions in chicks exercising voluntarily.

Parameter	Reference control diet		Life cereal	
	-Exercise	+Exercise	-Exercise	+Exercise
Liver wt. (g)	3.4	3.3	4.1	3.8
Liver/body wt. (%)	3.4	3.9	3.9	3.9
Glucose (mg/total liver)	10.1	8.8	21.1	19.1
Glycogen "	90.5	79.0	275.3	216.0
Monoglycerides "	5.3	6.0	7.5	5.5
Diglycerides "	7.1	6.8	7.8	7.1
Triglycerides "	16.0	14.8	18.9	17.6
Free fatty acids "	12.3	11.3	13.5	12.2
Cholesterol "	11.5	11.6	13.0	12.3
Chol. esters "	10.2	9.9	11.6	10.3

As shown in the table, voluntary exercise reduced carbohydrate liver reserves, with no apparent effects on lipid fractions. These data are similar to those seen in *S. typhimurium* infected rats. Animals fed the Life cereal had excessively high levels of liver glucose (two fold) and glycogen (5 fold) but levels of the lipid fractions were similar to those of controls. While these high carbohydrate levels correlate with a depression of running, they do not explain the depressive effect on voluntary exercise.

In view of the above findings, Life cereal was tested in a different experimental model. In this model, a high level of running was induced in control chicks by feeding a constant but reduced amount of food per day. From previous trials, we have observed that under such a regimen wheel turns in control chicks will peak at 7,000 to 8,000 on day 3 and then decrease, with first mortality occurring on day 5. Would Life cereal have a double jeopardy effect on activity under such a model and would the mortality rate be altered?

Under this design a constant intake of 5 g/day was barely sufficient to enable the chick to survive over a 10-day period. It was hypothesized that a residual toxicant would create a state of double jeopardy which would result in greater mortality and significant biochemical changes in tissues.

As shown in table 10, the higher trend of mortality in the Life group confirmed the double jeopardy hypothesis. To gain further understanding of the relationship of voluntary exercise to mortality, the data were arrayed in table 11 according to the number of days the chicks lived in the 10-day test period.

Table 10. Daily wheel turns and mortality rate of chicks fed Life cereal and given constant access to running wheels.

Day of trial	Wheel turns		No. dead chicks	
	Reference control n = 12	Life cereal n = 12	Reference control n = 12	Life cereal n = 12
1	435	2,425	0	0
2	2,535	5,457	0	0
3	6,695	5,954	0	0
4	7,561	7,151	0	0
5	6,220	7,192	0	0
6	2,903	6,939	2	1
7	2,658	3,705	2	4
8	2,837	4,645	0	1
9	575	3,185	2	3
10	366	2,454	3	2
Total	32,785	49,107	9	11

In table 11 it can be seen that there was a high correlation between survival and voluntary exercise levels. The highest runners died on days 5, 6 and 7. Those that lived longer ran consistently less each day than the chicks that failed to survive.

These data are believed to be highly applicable to our continuing studies on exercise x infectious disease. They portend that animal subjects, like humans, are unable to detect and defend against serious stressors. In this case, mere reduction of their voluntary exercise would have reduced or prevented mortality.

Table 11. Relation of survival time to voluntary exercise in chicks.

Day of trial	W h e e l t u r n s o f c h i c k s s u r v i v i n g											
	5 days		6 days		7 days		8 days		9 days		10 days	
	Control diet	Life	Control diet	Life	Control diet	Life	Control diet	Life	Control diet	Life	Control diet	Life
1	286	3,614	1,334	3,701	1/	7,652	30	462	449	399	191	844
2	3,695	13,376	6,100	7,320	--	11,771	634	3,037	458	665	2,732	622
3	13,373	5,698	13,030	10,726	--	12,206	1,314	2,208	469	1,224	7,833	1,565
4	15,803	7,966	16,309	12,390	--	7,223	2,245	4,865	745	1,145	6,595	1,032
5	14,195	8,449	13,161	10,739	--	15,206	2,379	4,920	800	1,955	4,255	1,018
6	--	--	3,358	9,071	--	12,907	2,876	6,210	1,034	3,320	4,485	1,862
7	--	--	--	--	--	6,542	2,633	4,000	1,832	2,102	3,500	3,192
8	--	--	--	--	--	--	1,681	4,760	2,603	5,207	3,840	3,206
9	--	--	--	--	--	--	--	--	1,680	7,710	1,051	1,971
10	--	--	--	--	--	--	--	--	--	--	366	2,454

1/ No control chicks died on this day

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