Effects of Exercise and Pregnancy On Amino Acid Absorption by the Rat Small Intestine

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A Thesis

Presented To

The Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

By

Marcus Charles Dugas

August 1968

I wish to express my sincere appreciation for the guidance given to me by Dr. Robert L. Hazelwood and Dr. Addison L. Lawrence throughout this problem. I would also like to thank Charles A. Blake, Gilbert Livanec, and Ronald Jones for their excellent technical assistance. I would like to thank Dr. Darrell J. Weber for allowing me to use his Liquid Scintillation Spectrometer. My deepest appreciation goes to my wife, Pat, for typing this paper and for constantly encouraging me in my work.

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Active transport rates have been shown to be subject to change. They are likely to change in the face of different environmental conditions, pregnancy, and the additive condition of exercise and pregnancy.

Studies were undertaken to elucidate the effects of pregnancy, exercise, and a combination of both on active transport in terms of net serosal accumulation of amino acids by the gut. In addition, the effects of exercise on pregnancy were evaluated by comparing conceptus, body, and adrenal gland weight changes of exercised pregnant rats with non-exercised pregnant rats.

Groups consisted of 7, 14, and 21-day pregnant rats; 7, 14, and 21-day pregnant rats exercised on days 0-6, 7-13, 14-20, and 0-20 of gestation respectively, 7 and 21-day exercised rats, and control rats for all durations.

Net serosal accumulation studies were performed using the gut sac method of Wilson and Wiseman as modified by Lawrence and Lawrence. Amino acids employed in these studies were alanine, proline, lysine, and glutamic acid, and the amino acid analogue alpha-aminoisobutyric acid (AIB).

In general, glutamic acid and AIB were found unsuitable for net serosal accumulation studies of rat small intestine. Lysine net serosal accumulation increased in the 7-day pregnant rat and this increase was found to be due to an increased active transport rate of this substrate by the gut. Proline net serosal accumulation decreases in the 21-day pregnant group and this decrease was found to be due to a decreased intestinal

active transport rate by this group.

The accumulative effect of pregnancy and exercise increases net serosal accumulation of alanine in the second trimester and decreases it in the third trimester. This was believed to be due to an increase and decrease in active transport, respectively. Exercise during the entire period of pregnancy appears to allow transport mechanisms to adapt, or at least to remain normal.

Exercise incurred during any one trimester of pregnancy, and lasting for the duration of that trimester only, was detrimental to the rat in nearly all parameters measured. However, exercise throughout the term of pregnancy was beneficial in that no detrimental effects were noticed while maternal tissue weight decreased and active transport rates remained unchanged.

Pregnancy causes large, and exercise plus pregnancy causes smaller body weight increases. The smaller increase in body weight of one trimester, exercised pregnant animals, was due to less weight gained by maternal tissues and the conceptus. The body weight gain of pregnant animals exercised for the duration of pregnancy was found to be very similar to that of pregnant non-exercised animals. This was due to a small weight gain in maternal tissues while the conceptus weight remained unchanged.

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I. Introduction

The tremendous physiological burden that pregnancy places on the metabolic mill of the affected mother is common knowledge but perhaps not too well understood. In humans, pregnancy has been described as being everything from a period of complete mental and physical discomfort to a period of total comfort. Probably no less well known is the complete change from normal rhythmic hormonal balance to the more or less steady state hormonal condition which prevails in pregnancy.

The interplay between the nutritional demands and the endocrinological aspects of pregnancy were well demonstrated in a study of such by Hazelwood and Nelson.[1] It is interesting to note that metabolic building blocks, amino acids in this case, were transferred from the maternal muscle to be used for fetal development in steroid-injected rats kept on a protein free diet. One would assume that under normal dietary restrictions enough food is made available to satisfy the metabolic demands of both maternal and fetal bodies since it is known that pregnant rats have an increased dietary intake. Along these same lines, but of a different physiological condition, is the association of a changed appetite following physical training of normal, healthy individuals. Whether the changed dietary intake during pregnancy and that following exercise is associated with a changed dietary uptake of amino acids by way of active transport processes of the gut is the point in question.

A brief review of the literature dealing with active transport of amino acids, and the hormonal effects thereon, is in order.

Höber and Höber provided early evidence that mechanisms other than simple diffusion are important in the dietary uptake of nutrients when they concluded from their work that there must be an "accelerating" mechanism which causes nutrients to cross the gut.[2] Further evidence was obtained by Gibson and Wiseman, who found that the L-isomers of alanine, glutamic acid, and lysine disappeared from intact rat small intestine at a faster rate than did the D-isomers.[3] The first direct evidence for an active transport mechanism of certain amino acids was obtained by Wiseman in 1953. Using an in vitro preparation of rat small intestine, he demonstrated that L-alanine was "transported" against a concentration gradient; glutamic acid, however, was not transported as such even though it disappeared from the medium on both sides of the intestine. He also showed at this time that while the L-isomer was "transported" best, the D-isomer of histidine could also be "transported" against a concentration gradient. He postulated at the time that the disappearance of glutamic acid was due to metabolism.[4] It was later shown that the disappearance of glutamic acid was due to its transamination to alanine.[5]

Along this line of evidence was the demonstration by Wilson and Wiseman that L-methionine could be transported across a concentration gradient in everted sacs of rat small

intestine under aerobic but not under anaerobic conditions.[6] Thus, oxidative phosphorylation was thought to provide the energy for transport of materials by the small intestine. With improvement of technique and experimental design have come the findings that: (1) uptake of the L-isomer of histidine by the rat intestine is inhibited by 2,4-dinitrophenol (DNP) and cyanide;[7] (2) the D-isomer of alanine is actively transported by the guinea pig intestine;[8] (3) D-methionine stereospecifically inhibits L-methionine transport in the rat small intestine;[9] and (4) that the D-isomers of serine, norvaline, and alanine are actively transported by the rat small intestine and may compete with their L-isomers for transport.[10] It, therefore, seems well established that in most instances not only the L-isomer but also the D-isomer of amino acids are transported.

Evidence regarding the specificity of transport systems was provided by Wiseman who (working with everted sacs of hamster small intestine) demonstrated that the L-isomers of the neutral amino acids methionine and histidine, but not of glutamic acid, lysine, or ornithine, slowed the rate of transport of L-proline and glycine.[11] This type of competitive inhibition indicated a separate transport system for neutral amino acids.

Hagihiri, Lin, Samiy and Wilson, using everted sacs of hamster small intestine, showed that the L-isomers of lysine, ornithine, arginine, and cystine were transported

against a concentration gradient. They concluded that there was a separate transport system for basic amino acids.[12]

In general, three intestinal amino acid transport systems have been identified. One for the neutral amino acids, one for the basic amino acids, and one for the heterocyclic amino acids.

The neutral amino acids are carried by two transport systems which show overlapping affinities among the amino acids.[13] One of the neutral systems is an alanine preferring and the other is a leucine preferring system. The leucine system shows decreasing affinities as the side chain shortens.[13] In general, there is some but not absolute optical specificity attributed to the neutral systems. The amino acid which is transported by them must have a free carboxyl group, and there must be a free alpha hydrogen. The side chain of the amino acid must be neutral with respect to charge, and as the polarity of the side chain increases the affinity of the amino acid for the carriers decreases.[14]

Basic amino acids such as L-lysine are carried by a separate transport system at approximately 5-10% the rate of transport of neutral amino acids.[14]

Heterocyclic amino acids such as proline are transported by a third system. While proline is a neutral amino acid as well as a heterocyclic one, it shows a greater affinity towards the heterocyclic carrier than it does toward the neutral carriers.[14]

Most amino acids are not extensively metabolized by the intestine; glutamic acid is an exception.[14] This amino acid has available many metabolic routes in the intestine, of which at least three have been shown to be present in the rat intestine.[15] An amino acid analogue, alpha-aminoisobutyric acid, has been shown to be transported against a concentration gradient but is not metabolized by the rat small intestine.[16] However, though this analogue could serve as a useful tool in divorcing transport from metabolism, the techniques required to demonstrate its transport are special.

Studies of the enzymatic apparatus of the intestines of various animals led Spencer and Knox to state, "When the physiological state of the animal is altered in almost any way, the activity and apparently the amount of particular enzymes in the intestinal mucosa changes. These enzyme changes are produced by substrate induction, products of the enzymes, as well as by hormones."[15] While these conclusions would tend to point to any effect of hormones on disappearance of substrate as being one of metabolism, the work of Nathans, Tapley, and Ross should be stressed. These investigators demonstrated that transport of L-(^{131}I) monoiodotyrosine, by the rat small intestine, could be shown to follow enzyme-like kinetics.[17] Earlier, Peters had proposed the concept that hormones may act on the cytoskeleton of the cell causing anatomical changes not only in the plasma membrane but in other structural elements throughout the cell so that several

enzymatic reactions are modified simultaneously.[18] It is therefore conceivable that the hormonal effects on enzymes and those on transport could be one and the same phenomenon.

Ideally, at this time one should discuss the endocrinology of pregnancy and exercise and relate specific changes in hormonal levels to documented effects of these hormones on transport. In view of the recent findings of Galton in which she points out that hormonal levels in the pregnant rat are different from what was assumed to occur by inference from human hormonal levels,[19] a general review of hormonal effects on transport processes and protein chemistry would be of more value. Noall and associates demonstrated that, in the intact rat, injection of bovine growth hormone increases the steady state distribution of the non-metabolizable amino acid analogue, AIB, in muscle with respect to extracellular fluid levels.[16] Thus, entrance of amino acids into cells was thought to be accelerated by growth hormone administration.

While these findings do not point to transport processes as the mechanism of action of growth hormone, this matter received consideration by Kostyo. He demonstrated that the accelerating action of growth hormone on entry of AIB into isolated diaphragm of hypophysectomized rats was due to an increase in transport.[20] It is interesting to note that effects of growth hormone on amino acid transport and protein synthesis are equally increased but that the free amino acid pool stays constant. This conclusion is based on the findings

of Kipnis and Reiss who demonstrated that growth hormone stimulates the transport of amino acids into rat diaphragm cells and at the same time stimulates the protein synthesizing machinery of these cells equally so that no buildup of an amino acid pool is noticed.[21] This demonstration would tend to add considerable weight to Peters' hypothesis on the actions of hormones.

Another hormone of considerable importance in protein-metabolism is insulin. Mirsky demonstrated that insulin increases removal of amino acids from the blood by muscle. This data was obtained by what he termed a "muscle preparation" which consisted of a double nephrectomized, evicerated dog which eliminated the tendancies for excretion of amino acid nitrogen and metabolism of amino acids by the viscera.[22]

Krahl's demonstration that insulin increases incorporation of L-alanine into protein of the isolated rat diaphragm[23] led to the speculation that insulin induces amino acid loss from the blood secondarily by creating a concentration gradient caused by utilization of cellular amino acids for protein synthesis.

The possibility that insulin affects amino acid transport, in a manner similar to its action on glucose transport, has been examined with the non-metabolizable amino acid AIB. Transport of AIB into skeletal muscle in presence of insulin was increased whether or not glucose was present in the incubating medium.[24] These facts were further supported by

the findings that AIB transport into skeletal muscle *in vivo* and *in vitro* was depressed by the condition of diabetes mellitus and accelerated by insulin.[24, 25, 26]

Various steroids have been shown to affect amino acid transport into cells. Estrogens have been shown to stimulate transport of AIB in the rabbit uterus. A pecularity noticed in the demonstration of this effect was that estradiol injected *in vivo* (but not *in vitro*) caused the increase. Thus, it seems that the estradiol has to be activated *in vivo* before it can exert its characteristic action.[27]

Noall and associates reported that the glucocorticoids markedly accelerated the intracellular transport of amino acids in the liver.[16] This coupled with the failure to demonstrate an increase in the intracellular amino acid pool[28] suggests that these metabolites are being shunted at increased rates into the metabolic pathways concerned with gluconeogenesis, oxidation, deamination, and protein formation. The peripheral tissues of corticosteroid-treated animals, principally skeletal muscle, supply the amino acids needed to keep up with the increased hepatic demands. This was demonstrated by Kline when he found that the rate of release of amino acids by the isolated rat diaphragm was decreased by adrenalectomy and increased by adrenal cortical extract administration.[29] It is interesting to note that in the work of Hazelwood and Nelson the shift of proteins from maternal muscle to fetal tissues was postulated to be due in part to ovarian hormone

stimulation of adrenal corticosteroid secretion with their subsequent action on protein metabolism.[1]

In exercised rats there is a highly significant hypertrophy of the adrenal glands that requires three weeks to occur. The secretion of corticoids parallels the adrenal hypertrophy for three weeks then falls off toward pretraining levels.[30]

The interrelationships of exercise, hormonal levels, and amino acid absorption have been hinted at by Sukkar and his associates.[31] They found that fasting decreased insulin and plasma amino nitrogen levels in humans. After ingestion of a meal of protein, plasma insulin, growth hormone, and plasma amino nitrogen were elevated; the rise in insulin preceded that of growth hormone. Moderate exercise in the absorptive stage of the meal abolished the insulin response to the meal and enhanced the growth hormone response while the plasma amino nitrogen remained elevated. These workers were led to postulate that "The value of increased insulin secretion after a protein meal may lie in its ability to enhance the tissue uptake of amino acids."[31] Then the failure of exercise in preventing an increased plasma amino nitrogen while causing a decreased plasma insulin level could be due to diminished intestinal absorption of amino acids together with the decreased insulin induced tissue uptake of amino acids. Growth hormone action during exercise could be exerted as one means of drawing on the fuel stores of adipose tissue. Other workers report no effect of exercise

on intestinal absorption of a sugar by humans.[32]

The endocrinology of the pregnant rat is marked by increased plasma levels of a substance with growth-promoting activity.[33] This growth-promoting substance was thought to be a contribution of the placenta and was found to possess prolactin-like activity.[34] There is also a high concentration of estrogen during pregnancy in the rat.[35] The condition of pregnancy can be considered as a stressful stimulus to the pregnant rat since the adrenal gland hypertrophies.[36] A demonstration of the interrelation between pregnancy and intestinal transport was provided by the findings of Larralde, Fernandez-Otero, and Gonzalez who found an increase in D-glucose and glycine intestinal absorption rate in pregnant rats over non-pregnant rats.[37]

There is, therefore, considerable evidence which would warrant investigation of the effects of exercise and pregnancy on intestinal absorption of amino acids and the effects of exercise on pregnancy.

II. Statement of Problem

The purpose of this investigation was to determine whether the normal physiological changes associated with pregnancy and/or exercise will induce changes in the intestinal absorption rate of several amino acids. This was studied in terms of active transport of basic, neutral, and heterocyclic neutral amino acids. Finally, the effects of exercise on the condition of pregnancy was determined.

III. Methods and Materials

HOUSING AND CONDITIONING OF ANIMALS:

Female rats derived from the Holtzman strain were used in this study. All rats were kept in wire-bottom cages housed in one of three animal quarters. For the purpose of this study, two of the quarters were sufficiently similar in housing conditions to be considered as one.

All animals used in the pregnancy studies were housed in Animal Quarters I which had a room temperature range of 65°-75°F. All animals used in exercise studies were housed in Animal Quarters II which had a temperature range of 70°-76°F. All rats were fed Purina laboratory chow and water ad libitum.

Breeding was accomplished by following daily vaginal smears and placing two females (190-210 grams) which were about to enter estrus in a large wire-bottom cage containing a male. The presence of sperm eight to twelve hours after placing the females in with the male was taken as an indication of pregnancy and noted as day "zero" of gestation.

Virgin rats were exercised on a Collins Rodent Treadmill (Model 297) for thirty minutes each day at a pace of either 15, 20, 25, or 30 meters a minute for seven days in an initial study to observe the effects of running on body weight. The effects of several intensities of running on net serosal accumulation of lysine was also studied. The intensities of running were 7, 12, and 15 M/min. for 30 minutes a day for seven days. The most desirable intensity was determined as being 15 meters per minute, because this intensity of

running caused a significant change in net serosal accumulation of lysine. In all further studies, rats were run at 15 meters per minute for thirty minutes a day for seven days except for one group which ran every day for 21 days. All exercised rats were sacrificed 24 hours after the last exercise period.

Groups consisted of 7, 14, and 21 day pregnant rats; 0-6, 7-13, 14-20, and 0-20 day exercised pregnant rats. These groups are referred to as 7 D-P, 14 D-P, 21 D-P, 0-6 D-P-E, 7-13 D-P-E, 14-20 D-P-E, and 0-20 D-P-E, respectively. At all times at least one control animal was exercised with each exercised group; at least one non-exercised control used during each transport study.

TECHNIQUE USED FOR TRANSPORT STUDIES:

Sacrifice was accomplished by cervical dislocation. The abdomen was immediately opened and the small intestine excised and placed in a linear measuring well containing cold Krebs-Ringer buffer (phosphate or bicarbonate buffer uas used, depending on amino acid being studied).[38] No glucose was added to either buffer. Intestine from 21 and 46 centimeters proximal to the ileo-cecal junction was removed and washed out by gently flushing 10 cc of the buffer through its lumen. This rinsed section of intestine was then everted on a glass rod and devided into three equal segments. The everted segments were mounted for transport studies using a

transport apparatus and procedure identical with that of Wilson and Wiseman[6] as modified by Lawrence and Lawrence.[39] The entire transport apparatus was then placed in a 37°C water bath. The incubating media in the mucosal compartment was gassed with 95% O_2-5%CO_2 when the bicarbonate buffer uas used, and 100% O_2 when the phosphate buffer was used.

The time of incubation was 30 minutes with the initiation of gassing serving as the starting time. All initial and final serosal volumes were recorded. A recent survey of the literature, however, indicated that little or no water movement occurs with this preparation. Little water movement occurred in our observations, with difference in final and initial serosal volumes indicating a loss of fluid. Practically all fluid loss could be accounted for by adding to the final serosal volume the volume of fluid adhering to the pipetting apparatus. The amount of fluid loss in most instances was less than 0.09 ml. For these reasons the initial serosal volume was used in all calculations. The time from sacrifice to initiation of gassing of the last segment was between 12 and 15 minutes with the first segment taking 3-6 minutes and subsequent segments 2-4 minutes each. Usually, four animals were used per transport study (run).

After the intestine was excised and during the time it was being made ready for transport studies, data were recorded pertaining to the condition of pregnancy. These data included total conceptus weight, number of fetuses,

number of placentae, and adrenal gland weights in all cases and the above plus total fetal weight and total placental weight in all cases except the exercised and non-exercised 7-day pregnant group. Weight of the adrenal glands of all groups and conceptus of the non-exercised and exercised 7-day pregnant groups were obtained on a Roller-Smith Torsion Balance. All other tissues were weighed on an Ohaus Triple Beam Balance.

In all studies the Krebs-Ringer buffer contained one micromole of unlabeled L-isomer and 0.0024 μ curies of the labeled racemic amino acid per milliliter. This gave a final concentration of 1.000 micromoles amino acid per milliliter buffer. The amino acids used for transport studies were alanine, lysine, proline, alpha-aminoisobutyric acid, and glutamic acid. The Krebs-Ringer bicarbonate buffer was used for all amino acids studied except alpha-aminoisobutyric acid in which case the Krebs-Ringer phosphate buffer was used.

At the end of the incubation period the final serosal fluid was obtained and either 100 lambda or 200 lambda placed in 15 milliliters of a Dioxane cocktail containing 100 grams napthalene/liter, 6 grams 2,5-diphenyloxazole (PPO)/liter, and 100 ml water/liter of dioxane for counting on a liquid scintillation spectrometer. A similar aliquot of mucosal fluid was treated identically. Several standards were prepared by pipetting either 100 or 200 lambda of buffer, containing the labeled and unlabeled amino acid, into 15 milliliters of Dioxane

cocktail. Serosal and mucosal samples (5 lambda each), along with standards, were prepared for paper chromatography.

All counting was done on a Packard Tricarb Liquid Scintillation Spectrometer (Model3003) with an automatic control (Model 574). The gain was set at 12.7 and the windows were set from 50 to 1000. An automatic external standard was counted with each sample for one minute while each sample was counted for five minutes. Each sample contained at least 1000 counts per minute. At the 96% confidence level the percent error range of the serosal counts was 1.41-2.00, and 2.00-2.98 for the mucosal counts. No correction was made for quenching which was negligible for the system used as shown by the counts of the external standard. The standards prepared from the radioactive buffer were counted under identical conditions.

Calculations of final serosal and mucosal concentrations were performed as follows:

A. $\frac{\text{Counts of unknown per 5 min.}}{\text{Counts of standard per 5 min.}}$ times the concentration of the standard (μ M/ml) = μ mole/ml of unknown

Total amount transported was calculated as follows:

A. Concentration of final serosal solution X initial volume of gut segment = total μ moles in serosal compartment at end of study

B. Concentration of initial serosal solution X initial volume of gut segment = total μ moles in serosal compartment at beginning of study.

C. A. - B. = total μ moles accumulated by serosal compartment of gut segment

$$D. \quad \frac{C}{\text{Dry wt. of gut (gm.)}} = \mu \text{ moles accumulated per gm. dry gut wt.}$$

Chromatographs were developed using descending chromatography techniques with a solvent consisting of n-butyl alcohol, acetic acid, and water in the proportions of 4:1:1.[40] The solvent front was marked and radiographs prepared using Kodak No-Screen Medical X-ray Film and Kodak x-ray film developers. Similar Rf values for developed serosal and mucosal spots were taken as an indication that no appreciable metabolism had occurred. In those instances where developed paper chromatograms showed only one spot each for serosal and mucosal samples, and if similar Rf values were obtained, then this was taken as an indication that no appreciable metabolism had occurred.

MATERIALS:

The D-L-lysine-1-C^{14}, D-L-proline-5-C^{14}, and alpha-aminoisobutyric acid-1-C^{14} were purchased from New England Nuclear Corporation and came from lot numbers 134-220-20, 134-297-14, and 305-73-4 respectively. The D-L-alanine-1-C^{14}, lot number EB-5724, was purchased from Volk Chemicals. The D-L-glutamic acid-1-C^{14}, lot number 880220, was purchased from CalBioChem.

The L-proline lot #95B-1880, L-alanine lot#55B-1230, and L-lysine lot #54B-1180 were of sigma grade and purchased from the Sigma Chemical Company. The L-glutamic acid lot #43340 and the alpha-aminoisobutyric acid lot # 45854 were

A-Grade and were purchased from CalBioChem.

IV. Results

The results are clearly indicative of the complex nature of the problem. In some instances assessment of group relations and interrelations were made only after comparison of four groups. In all instances Students t-tests were performed according to the method described by Bailey in "Statistical Methods in Biology".[41]

EFFECT OF EXERCISE ON BODY WEIGHT AND NET SEROSAL ACCUMULATION OF LYSINE:

Preliminary studies on the effect of intensity of treadmill running on body weight are shown in Table I (Preliminary Study I). Starting weights of rats used in this study varied from 180-200 grams. All rats were exercised for 30 minutes a day for seven days. At 15 M/min the gain in body weight was similar to that of control rats. As the rate of running was increased the average gain in body weight decreased with respect to control rats which had gained an average of 22 grams over the same period of seven days. It was concluded that if a change could be induced in net serosal accumulation by the gut at the lower intensity of 15 M/min, or at an intensity lower than this, then the lower intensity would be an acceptable one for the study. This conclusion is strengthened when one considers the fact that third trimester pregnant rats would be carrying the additional weight of the conceptus when running on the treadmill. By following this line of investigation, the threshold for significant change in net

serosal accumulation could be exceeded.

Accordingly, a study was made on the effect of intensity of exercise on net serosal accumulation of lysine (Preliminary Study II). As can be seen from Table I, as the intensity of running increased, net serosal accumulation decreased. At 15 M/min there was significantly less serosal accumulation of lysine than the control value of 2.15 μ moles per gram dry gut weight (Table I). There was also identical gain in average body weight in this group as in the control group. The reason the body weight changes of the control rats of Preliminary Study II are tabulated separately from those of Preliminary Study I in Table I is because the starting weight of rats in these two groups differed. This difference in starting weight (32 grams) was believed to be large enough to affect weight gained in a seven day period. Also, since accumulation studies were not performed on rats of Preliminary Study I, body weight values of rats from the two groups are tabulated separately in Table I. The fact that exercise at a rate of 15 M/min produces a significant decrease in body weight gain, which is indicative of stress, can be further ascertained by referring to Table VI. Seven day exercised control virgin rats gained significantly less weight than did control virgin rats over the same time period ($P<0.01$).

EFFECT OF PREGNANCY ON NET SEROSAL ACCUMULATION OF AMINO ACIDS:

When considering the effect of any one parameter on

TABLE I

EFFECT OF EXERCISE ON BODY WEIGHT

AND NET SEROSAL ACCUMULATION OF LYSINE

Intensity of Treadmill Running*	Mean Body Weight In Grams		Net Serosal[e] Accumulation As Mean ± S.E.M.
	Initial	Change	
Nonexercised Controls	180[a] (4)[c]	+22 (4)	———
	212[b] (3)	+ 8 (3)	2.15 ± 0.25 (9)
7 Meters/min.	206 (3)	+ 7 (3)	2.60 ± 0.38 (9)
12 Meters/min.	203 (3)	+ 9 (3)	1.99 ± 0.32 (9)
15 Meters/min.	198[b] (3)	+ 8 (3)	1.43[d] ± 0.15 (9)
	196[a] (1)	+22 (1)	———
20 Meters/min.	202 (1)	+18 (1)	———
25 Meters/min.	182 (1)	+12 (1)	———
30 Meters/min.	188 (1)	+ 10 (1)	———

*All rats were exercised at the stated intensity for 30 minutes per day for 7 days

a=Preliminary Study I

b=Preliminary Study II

c=Number of Observations

d=$P < 0.05$ as compared with control rats

e=μ Moles/gm dry gut weight

net accumulation it should be remembered that net accumulation is the result of the balance between efflux and influx due to simple and facilitated diffusion, active transport, and tissue metabolism of the substrate under consideration. Pinpointing which one of these processes is affected the most by an experimental condition is very difficult in studies of this type.

It should be indicated at this time that the factors affecting net accumulation are indeed susceptible to changes. By referring to Table III, it can be seen that in two instances out of three net serosal accumulation differed significantly between control rats housed in Animal Quarters I and II. In both instances, alanine and lysine, control rats for exercise studies which were housed in Animal Quarters II showed less net serosal accumulation than did control rats for pregnancy studies which were housed in Animal Quarters I ($P<0.05$ for alanine and $P<0.02$ for lysine). There was no noticable effect of the estrus cycle on net serosal accumulation.

Table II indicates that no significant difference in net serosal accumulation of alanine occurred between pregnant and control rats during any of the trimesters studied. It is interesting to note that values for all groups were the same or consistently close to control values. Accumulation studies using lysine revealed that the 7 D-P group showed a very significant increase in net serosal accumulation when compared with control rats for pregnancy experiments ($P<0.001$).

Similar studies with proline indicate a significant decrease in net serosal accumulation by the 21 D-P group when compared with control rats for pregnancy experiments ($P<0.05$).

Accumulation studies on AIB yielded interesting results. In no instance was there a mean positive net serosal accumulation. If this had been observed it would presumably have resulted strictly from active transport processes since there is supposedly no appreciable metabolism of this amino acid analogue.[16] The negative net serosal accumulation of this analogue could have resulted from AIB being actively transported out of the serosal compartment (ie. secreted), but it is doubtful that such took place. If one assumes that the negative net serosal accumulation of AIB was due to the balance between tissue uptake and/or metabolism of this substrate, then the following can be concluded:

The 7 D-P group had less AIB taken up and/or metabolized by the tissue than did the control rats for pregnancy experiments ($P<0.001$). The 14 D-P group showed more and the 21 D-P group showed less tissue uptake and/or metabolism of AIB than did the control rats for pregnancy experiments ($P<0.02$ and $P<0.05$, respectively). Assessing the effect of any one experimental condition on net serosal accumulation (or active transport) of AIB (and glutamic acid) was not attempted because of the apparently low transport rate of these substrates. It is worthwhile noting that there was little absolute change in the serosal concentration of AIB of these segments. Inferences

TABLE II

EFFECTS OF PREGNANCY

ON NET SEROSAL ACCUMULATION OF AMINO ACIDS

Group	Alanine	Lysine	Proline	AIB	Glutamic Acid
Virgin Controls For Pregnancy Experiments	5.89 ± 0.61* (12)e	2.63 ± 0.29 (24)	3.37 ± 0.67 (12)	0.64 ± 0.06 (14)	0.54 ± 0.08 (20)
7 Day Pregnant Rats	6.83 ± 0.92 (9)	5.60 ± 0.77[a] (9)	1.95 ± 0.47 (9)	0.08 ± 0.14[a] (9)	0.79 ± 0.08[b] (9)
14 Day Pregnant Rats	5.89 ± 0.88 (8)	2.39 ± 0.32 (15)	3.03 ± 0.49 (8)	1.08 ± 0.19[c] (8)	0.01 ± 0.09[d] (9)
21 Day Pregnant Rats	6.52 ± 0.72 (12)	1.90 ± 0.26 (9)	1.62 ± 0.62[b] (12)	0.46 ± 0.21[b] (9)	0.37 ± 0.08 (9)

*Mean ± S.E.M. μ moles serosal accumulation per gram dry gut weight

When compared with respective control group, a=P<0.001; b=P<0. 05; c=P<0.02; and d=P<0.002

e=Number of segments

as to the tissue uptake and/or metabolism of all amino acids studied should not be made from the findings on AIB since all amino acids are not metabolized equally or by completely identical metabolic pathways by the same tissue. Using the same reasoning, reference to Table II indicates more tissue uptake and/or metabolism of glutamic acid by the 7 D-P group and less by the 14 D-P group when compared to control animals ($P<0.05$ and $P<0.002$, respectively). The 21 D-P group did not differ significantly from control animals.

EFFECT OF EXERCISE AND PREGNANCY ON NET SEROSAL ACCUMULATION OF AMINO ACIDS:

Because studies on net serosal accumulation of AIB and glutamic acid yielded negative results, these two substrates were not used in further accumulation studies. Furthermore, except for accumulation studies of alanine, only animals in the last trimester of pregnancy were used in exercise studies. All animals used in this study were housed in Animal Quarters II.

As mentioned above, alanine and lysine control rats for the exercise studies showed significantly less net serosal accumulation than did control animals for pregnancy studies (Table III). For this reason, pregnant exercised rats were compared with control rats for the exercise studies for these two amino acids and compared with all control animals for proline studies.

TABLE III

EFFECTS OF EXERCISE AND PREGNANCY

ON NET SEROSAL ACCUMULATION OF AMINO ACIDS

Group	Alanine	Lysine	Proline
Virgin Control Rats for Pregnancy Experiments	5.89 ± 0.61[a] (12)[b]	2.63 ± 0.28 (6)	3.37 ± 0.67 (12)
Control Rats For Exercise Experiments	4.36 ± 0.43[c] (24)	1.09 ± 0.28[d] (6)	3.01 ± 0.34 (6)
Control Rats For Both Pregnancy and Exercise Experiments	——	——	3.25 ± 0.44 (18)
Pregnant Rats* Exercised on Days 0-6	5.30 ± 0.50 (9)	——	——
Pregnant Rats Exercised on Days 7-13	6.84 ± 0.69[e] (9)	——	——
Pregnant Rats Exercised on Days 14-20	3.05 ± 0.50 (12)	1.06 ± 0.25 (9)	2.19 ± 0.23 (12)
Pregnant Rats Exercised on Days 0-20	4.95 ± 0.51 (9)	——	——

*All exercised rats ran on a treadmill at 15 M/min for 30 min/day for the stated number of days

a=Mean ± S.E.M. μ moles serosal accumulation per gram dry gut weight

b=Number of segments

When compared with control rats for pregnancy experiments, c=$P<0.05$, d=$P<0.02$

e=$P<0.01$ when compared with control rats for exercised experiments

The 0-6 D-P-E, 14-20 D-P-E, and 0-20 D-P-E groups did not show any significant changes in net serosal accumulation of alanine when compared with the control rats for exercise experiments. The mean value for the 0-6 D-P-E group was nearly 1 μ mole per gram dry gut weight higher than the mean value of the control rats for exercise experiments. The 14-20 D-P-E group mean value was one μ mole per gram dry gut weight less and the 0-20 D-P-E group mean nearly the same as the mean value of the control rats for exercise experiments. The 7-13 D-P-E group showed significantly more net serosal accumulation than did the control rats for exercise experiments ($P<0.01$).

The 14-20 D-P-E group for lysine and proline studies showed no significant change from their respective control values.

EFFECT OF PREGNANCY ON EXERCISED RATS AS SEEN IN NET SEROSAL ACCUMULATION OF AMINO ACIDS:

The effect of exercise on pregnancy was to be a major point of comparison in this study. A brief look at all of the control values indicates that while such a comparison is desirable, it was not possible to accomplish in a direct manner. In other words, if exercised pregnant animals are compared with control animals then the accumulative effect of exercise and pregnancy can be evaluated. It the exercised pregnant group is compared with the exercised non-pregnant

group, the effect of pregnancy above that of exercise can be determined. If the same exercised pregnant group is compared with the pregnant group of like duration, then the effect of exercise above that of pregnancy can be determined for any one parameter. However, the pregnant group was housed in separate animal quarters and this last comparison would not be valid. Since exercised control animals were housed under the same conditions as were the control rats for exercise groups and the pregnant rats for exercise groups, then the effect of pregnancy on exercised rats as seen in net serosal accumulation is the only direct comparison to be made (Table IV). For all amino acids studied the 7 D and 21 D exercised control rats (Table IV) did not differ significantly from the control rats for exercise (Table III), even though they were usually one order of magnitude less in μ moles per gram dry gut weight. This indicates that exercise has no effect on net serosal accumulation of these substrates.

Examination of Table IV reveals that net serosal accumulation of alanine by the 14-20 D-P-E group was significantly less than that by the control rats which were exercised for 7 days ($P<0.05$). Net serosal accumulation of alanine by the 0-20 D-P-E group was greater than that of control rats exercised for 21 days ($P<0.05$) and nearly identical with control animals for exercise studies (Table III). The control rats exercised for 21 days showed less net serosal accumulation of alanine but were not significantly different from control rats exercised

TABLE IV

EFFECTS OF PREGNANCY ON EXERCISED RATS

AS SEEN IN NET SEROSAL ACCUMULATION

Group	Alanine	Lysine	Proline
Control Rats Exercised* 7 Days	5.15 ± 0.55[a] (26)[b]	1.63 ± 0.26 (11)	2.27 ± 0.33 (6)
Control Rats Exercised 21 Days	2.79 ± 0.43 (3)	——	——
Pregnant Rats Exercised on Days 0-6	5.30 ± 0.50 (9)	——	——
Pregnant Rats Exercised on Days 7-13	6.84 ± 0.69 (9)	——	——
Pregnant Rats Exercised on Days 14-20	3.05 ± 0.50[c] (12)	1.06 ± 0.25 (9)	2.19 ± 0.23 (9)
Pregnant Rats Exercised on Days 0-20	4.95 ± 0.51[d] (9)	——	——

*All rats ran on a treadmill at 15 M/min for 30 min/day for days indicated

a=Mean ± S.E.M. μ moles serosal accumulation per gram dry gut weight

b=Number of segments

c=$P < 0.05$ when compared with control rats exercised 7 days

d=$P < 0.05$ when compared with control rats exercised 21 days

7 days. There were no other significant changes in net serosal accumulation for alanine, lysine, or proline.

PERCENT RECOVERY OF AMINO ACIDS:

Percent recovery of amino acids was calculated as shown:

$$\frac{\text{Total } \mu \text{ moles amino acid in final serosal and mucosal solution}}{\text{Total } \mu \text{ moles amino acid in initial serosal and mucosal solution}} \times 100 = \text{percent recovery of amino acid}$$

Mean ± S.E.M. percent recovery values are reported in Table V for all groups and controls which showed significant net serosal accumulation changes. There were no significant differences except in the case of alanine when control rats for exercise experiments are compared with control rats for pregnancy experiments ($P<0.02$).

EFFECT OF PREGNANCY AND EXERCISE ON BODY WEIGHT:

Since normal growth processes cause an increase in body weight, changes in body weight were followed in several control virgin rats (which had similar starting weights) for 7, 14, and 21 days. Table VI indicates a significant increase in body weight change when 14 and 21 day control virgin rats are compared with 7 day control virgin animals ($P<0.01$ and $P<0.001$, respectively). The 21 day control virgin rats showed no significant difference from the 14 day virgin control rats in this respect.

The control group which was exercised for 7 days had starting weights similar to their respective experimental

TABLE V

PERCENT RECOVERY OF AMINO ACIDS

Group	Alanine	Lysine	Proline	AIB	Glutamic Acid
Virgin Control Rats for Pregnancy Experiments	90.8 ± 1.80[a] (12)[b]	94.7 ± 1.33 (24)	96.3 ± 0.69 (12)	97.3 ± 0.39 (14)	97.8 ± 2.17 (17)
Control Rats for Exercise Experiments	94.3 ± 0.42[c] (24)	95.6 ± 0.59 (6)	—	—	—
Control Rats Exercised* 7 Days	93.6 ± 0.28 (26)	—	—	—	—
Control Rats Exercised 21 Days	98.2 ± 1.28 (3)	—	—	—	—
7 Day Pregnant Rats	—	97.3 ± 0.76 (9)	—	95.3 ± 0.60 (9)	97.8 ± 0.77 (9)
14 Day Pregnant Rats	—	—	—	95.9 ± 0.37 (8)	98.0 ± 0.87 (6)
21 Day Pregnant Rats	—	—	95.9 ± 0.91 (12)	98.1 ± 0.60 (9)	—
Pregnant Rats Exercised Days 0-6	—	—	—	—	—
Pregnant Rats Exercised Days 7-13	93.6 ± 0.68 (9)	—	—	—	—
Pregnant Rats Exercised Days 14-20	94.7 ± 0.84 (12)	—	—	—	—
Pregnant Rats Exercised Days 0-20	93.2 ± 1.28 (9)	—	—	—	—

*All rats ran on a treadmill at 15 M/min for 30 min/day for days indicated
a=Mean ± S.E.M. percent recovery of amino acid
b=Number of segments
c=$P \leq 0.02$ when compared with virgin rats for pregnancy experiments

group. The body weight changes for these animals were grouped and treated as such. This was done so that body weight changes of experimental groups of different duration could be compared to the same exercised control group and therefore to each other. As expected, the gain in weight of the control virgin rats exercised 7 days was significantly less than the 7 day control virgin rats (Table VI).

The 7 D-P group gained significantly more weight than both the 7 D exercised control and the 7 D control virgin rats ($P<0.001$ in both cases). Similarly, the 0-6 D-P-E group gained significantly more weight than their respective exercised and non-exercised control rats, $P<0.02$ and $P<0.05$, respectively; but they did not differ in this respect from the 7 D-P group. No effect of exercise was noted in this case, since comparison of the exercised pregnant rats with the pregnant rats did not indicate a significant change in weight.

The 14 D-P group gained significantly more weight than the 7 D exercised control virgin rats and more than its respective control group ($P<0.001$ in both cases). An effect of exercise on pregnancy is noted when one recognizes the fact that the 7-13 D-P-E group gained significantly more weight than the 7 D exercised control virgin rats ($P<0.001$) but not significantly more than the 14 D control virgin rats. In addition to this, the 7-13 D-P-E group gained less weight than did the 14 D-P group. Gain in body weight for the

TABLE VI

EFFECT OF PREGNANCY AND EXERCISE

ON BODY WEIGHT OF ADULT RATS

Group	7 Day[c]	14 Day	21 Day	0-20 Day
Control Rats	12.2 ± 1.6[a] (10)[b]	26.3 ± 5.1 h^{i} (7)	25.7 ± 2.3 h^{iii} (19)	25.7 ± 2.3 (19)
Exercised* Control Rats	5.9 ± 2.0 d^{i} (15)	——	——	23.0 (1)
Pregnant Rats	21.9 ± 2.1 $d^{\dot{v}},e^{\dot{v}}$ (16)	53.6 ± 1.2 $d^{\dot{v}},e^{\dot{v}}$ (15)	125.4 ± 5.8 $d^{\dot{v}},e^{\dot{v}},g^{iii}$ (14)	——
Exercised Pregnant Rats	22.3 ± 2.4 $d^{\odot},e^{ii}$ (3)	40.3 ± 5.8 $e^{\dot{v}}$ (3)	108.4 ± 4.5 $d^{\dot{v}},e^{\dot{v}},f^{ii},g^{iii}$ (10)	121.3 ± 9.0 $d^{\dot{v}},g^{ii}$ (3)

*All exercised rats ran on a treadmill at 15 M/min for 30 min/day for the days indicated

a=Mean ± S.E.M. body weight change in grams

b=Number of observations

c=Duration of condition with exercised pregnant rats running durging last trimester of period except 0-20 day which ran days indicated

d=Probability when compared with control rats of like duration

e=Probability when compared with 7 day exercised control rats

f=Probability when compared with pregnant rats of like duration

g=Probability when compared with control rats exercised on days 0-20

h=Probability when compared with 7 day control rats

i=$P<0.01$; ii=$P<0.05$; iii=$P<0.001$; $\dot{v}$=$P<0.001$; $\odot$=$P<0.02$

21 D-P and 14-20 D-P-E group was significantly greater than the gain for both 7 D exercised and 21 D non-exercised control virgin rats ($P<0.001$ for both).

An effect of exercise on pregnancy was noted in the third trimester group also. This is illustrated by the fact that the 21 D-P and 14-20 D-P-E groups gained significantly more weight than did the 0-20 D exercised control virgin rats ($P<0.001$), and the 14-20 D-P-E group gained significantly less weight than did the 21 D-P group ($P<0.05$).

Finally, the 0-20 D-P-E group gained more weight than its respective 0-20 D-E and non-exercised control rats ($P<0.05$ and $P<0.001$, respectively).

EFFECT OF PREGNANCY AND EXERCISE ON RAT ADRENAL GLAND WEIGHTS:

All control group adrenal gland weights were sufficiently uniform to consider them as one group. In a few instances experimental group data would be significantly different from control rats of the same duration but not from all control groups. These data are included in Table VII.

In general, adrenal gland weights did not exhibit significant changes in rats belonging to any of the groups having a duration of 7 days.

On an absolute weight basis the 21 D-P and 7-13 D-P-E group had significantly larger adrenal glands than did all of the control groups. The 14-20 D-P-E group had significantly

TABLE VII

EFFECT OF PREGNANCY AND EXERCISE ON RAT ADRENAL GLAND WEIGHT

Duration	7 Day		14 Day		21 Day		0-20 Day	
Condition	mg	mg/100 gm Body Weight	mg	mg/100 gm Body Weight	mg	mg/100 gm Body Weight	mg	mg/100 gm Body Weight
Virgin[h] Control Rats	60.5 ± 3.1[a] (10)[b]	28.4 ± 1.5[a] (10)	64.6 ± 4.4 (7)	28.8 ± 0.8 (7)	60.7 ± 1.9 (18)	26.5 ± 0.8 (18)	———	———
Exercised Control Rats	60.2 ± 1.6 (15)	27.4 ± 0.7 (15)	———	———	———	———	62.2 (1)	27.2 (1)
Pregnant Rats	59.2 ± 1.2 (16)	26.6 ± 0.6 (16)	65.5 ± 1.7 (16)	26.0 ± 0.7 d^{i} (16)	68.9 ± 2.0 c^{ii},d^{iii} (17)	21.4 ± 0.7 c^{ii},d^{ii} (17)	———	———
Exercised[j] Pregnant Rats	54.9 ± 4.5 (3)	25.1 ± 2.0 (3)	72.7 ± 3.0 $c,\dot{v}d^{i},e^{iii}$ (3)	31.4 ± 0.4 e^{i} (3)	65.7 ± 1.4 e^{i} (10)	21.4 ± 0.6 c^{ii},d^{ii},e^{ii} (10)	66.9 ± 0.6 g^{i} (3)	21.0 ± 0.4 $c^{iii},e^{\odot},g^{i}$ (3)

a=Mean ± S.E.M. rat adrenal gland weight
b=Number of observations
c=Probability when compared with all control animals
d=Probability when compared with control animals of like duration
e=Probability when compared with 7 day exercised control animals
f=Probability when compared with pregnant animals of similar duration
g=Probability when compared with 0-20 day exercised control animals
h=Total of controls (35) on mg basis = 61.4 ± 1.3; on mg/100 gm body weight basis = 27.3 ± 0.6
j=Exercised pregnant rats ran on days 0-6, 7-13, 14-20, and 0-20, respectively

i=P<0.05; ii=P<0.001
iii=P<0.01; $\dot{v}$=P<0.02
$\odot$=P<0.002

higher adrenal gland weights than did the 7 D exercised control virgin rats ($P<0.05$). On a mg basis, the 0-20 D-P-E group had significantly higher weights than did the 0-20 D exercised control virgin rats ($P<0.05$).

On a mg/100 gm body weight basis, the 21 D-P and 14-20 D-P-E group had significantly less adrenal gland weight than did all of the virgin control rats, their respective 21 D control group, and the 7 D exercised control virgin rats ($P<0.001$ in all cases). The 0-20 D-P-E group had significantly less adrenal gland weight than did all of the virgin control rats, the 7 D exercised control virgin rats, and the 0-20 D exercised control virgin rats on a mg/100 gm basis ($P<0.01$, 0.002, 0.05, respectively). The 7-13 D-P-E group had more adrenal gland weight than did the 7 D exercised control virgin animals ($P<0.05$).

EFFECT OF EXERCISE ON CONTENTS OF CONCEPTUS:

Average placental weights of the 7-13 D-P-E, 14-20 D-P-E, and 0-20 D-P-E groups were significantly lower than the pregnant animals for similar durations ($P<0.02$, $P<0.05$, $P<0.05$, respectively). There were no other significant differences. However, a trend was apparent in that weights of contents of the conceptus were lower in groups which had exercised during only one trimester of pregnancy. Exercise throughout pregnancy did not lower the weights of contents of the conceptus.

TABLE VIII

EFFECT OF EXERCISE ON CONTENTS OF CONCEPTUS

Group	Conceptus Data		Fetal Data		Number of Resorptions	Placental Average Weight (gm)
	Total Weight (gm)	Percent of Total Body wt.	Number	Average Weight (gm)		
7 Day Pregnant Rats	0.555 ± 0.04* (16)[a]	0.24 ± 0.01 (16)	10.2 ± 0.7 (16)	———	0.06 ± 0.06 (16)	———
14 Day Pregnant Rats	10.29 ± 0.8 (15)	4.09 ± 0.2 (15)	10.13 ± 0.7 (15)	0.261 ± 0.03 (15)	0.86 ± 0.36 (15)	0.219 ± 0.01 (15)
21 Day Pregnant Rats	73.91 ± 3.1 (14)	22.15 ± 0.8 (14)	11.57 ± 0.3 (14)	4.57 ± 0.15 (14)	0.36 ± 0.16 (14)	0.728 ± 0.03 (14)
Pregnant Rats Exercised on Days 0-6	0.534 ± 0.00 (3)	0.21 ± 0.01 (3)	10.0 ± 2.5 (3)	———	0 ± 0 (3)	———
Pregnant Rats Exercised on Days 7-13	8.09 ± 0.8 (3)	3.53 ± 0.4 (3)	9.33 ± 0.9 (3)	0.151 ± 0.00 (3)	1 ± 1 (3)	0.163[b] ± 0.00 (3)
Pregnant Rats Exercised on Days 14-20	67.76 ± 3.0 (10)	21.94 ± 0.8 (10)	10.80 ± 0.5 (10)	4.54 ± 0.88 (10)	0.30 ± 0.15 (10)	0.622 ± 0.04[c] (10)
Pregnant Rats Exercised on Days 0-20	76.69 ± 3.7 (3)	24.12 ± 0.9 (3)	11.33 ± 0.6 (3)	4.88 ± 0.02 (3)	0 ± 0 (3)	0.579 ± 0.01[c] (3)

*All Values reported as mean ± S.E.M.

a=Number of observations

When compared with pregnant rats of similar duration, b=P<0.02; c=P<0.05

EFFECT OF PREGNANCY AND EXERCISE ON GUT LENGTH:

Gut lengths of all control animals were sufficiently similar to warrant considering them as one group. The 21 D-P groups, whether or not 7 day or 21 day exercise had been incurred, had gut lengths which were significantly longer (at least 5.6 cm) than control groups. There were no other significant differences between groups.

RESULTS OF CHROMATOGRAPHIC ANALYSES:

Chromatographic analyses of serosal and mucosal aliquots indicated that proline, lysine, alanine, and AIB were not changed appreciably in crossing the gut. Radiochromatograms indicated that lysine was not appreciably changed in crossing the gut and are now in the process of being analyzed for the other amino acids. It is not expected that any difference will occur between the chromatograms and the radiochromatograms. It should be pointed out that this method only indicates the similarity of final serosal and mucosal samples and indicates the presence or absence of only a small segment of the total metabolism which may have taken place in the intestinal tissue.

TABLE IX

EFFECT OF EXERCISE AND PREGNANCY

ON GUT LENGTH OF ADULT RATS*

Duration[f]	7 Days	14 Days	21 Days	0-20 Days
Condition	cm	cm	cm	cm
Exercised Control Rats	114.2 ± 1.2[a] (16)[b]	———	———	110.0 ± 0.0 (1)
Pregnant Rats	114.4 ± 1.5 (16)	112.8 ± 2.4 (15)	121.7 ± 1.8[c,i] (17)	———
Exercised Pregnant Rats	112.6 ± 2.9 (3)	118.0 ± 2.9 (3)	120.1 ± 2.2[c,ii,d] (10)	118.0 ± 1.7[e] (3)

*Value of virgin control rats = 112.5 ± 1.2 for 39 observations
a=Mean ± S.E.M.
b=Number of observations
c=Probability when compared with control rats
d=P<0.02 when compared with 7 day exercised control rats
e=P<0.01 when compared with 0-20 day exercised control rats
f=Exercised pregnant rats ran on days 0-6, 7-13, 14-20, and 0-20, respectively
i=P<0.001
ii=P<0.05

V. Discussion

PRELIMINARY STUDIES:

The preliminary studies on the effect of exercise on body weight and active transport in terms of serosal accumulation of lysine were performed under conditions which deserve special mention. While technique studies had been performed on the treadmill before any rats were exercised, the element of learning by the technician could not be ruled out. As a result, it is probable that rats exercised in the preliminary studies were stressed more than rats exercised later and that this may have caused the decrease in net serosal accumulation of lysine not seen in the later study. It is probable that differences in technique were ruled out since later studies performed on effects of exercise spanned a period of 12 months.

The decrease in weight gain of the rats exercised in the preliminary study seems to have been the natural result of a changed metabolic state which resulted in an increased caloric output which was proportional to the amount of work performed by the animals.

The progressive decrease in net serosal accumulation of lysine as intensity of exercise increased can be attributed to the increased work load experienced by these rats. It is a matter of speculation as to whether active transport decreased or metabolism by the gut increased. The latter appears most likely since overall metabolism increases with adaptation of the body to exercise.

Before attempting a discussion on the effects of exercise and/or pregnancy on net serosal accumulation by the gut, several assumptions will be made. The first of these is that the following deductions indicate the maximum amount of amino acid which could have been lost from the serosal compartment due to metabolism: (A) The maximum amount of amino acid not accounted for by the percent recovery studies is 10% (3.2 μ moles); (B) at least 75% (2.4 μ moles) of the amount of amino acid in (A) is present in the free amino acid pool of the tissue being studied at the end of the incubation period (unpublished data); and (C) of the remaining 0.8 μ moles, a maximum of 50% (0.4 μ moles) could have been lost due to metabolism by the serosal surface. From these deductions one can observe that the maximum amount of amino acid which could have been lost from the serosal compartment due to metabolism was 12.5% of the amount unaccounted for by percent recovery calculations.

The second assumption is based on the following deductions: (A) The calculated amount of maximum serosal metabolism of any group is added to the mean net serosal accumulation value of that group to yield the "corrected" mean net serosal accumulation value; (B) the corrected mean net serosal accumulation value of one group is subtracted from that of a group from which it differs; and (C) if this value yields a significant "p" value in the Student's t-test, the difference in net accumulation between the two groups is assumed to be

due to factors other than metabolism (i.e. active transport).

Using the above method, one can quickly assess the changes that occurred in net serosal accumulation between controls housed in Animal Quarters I and II. Values of net serosal accumulation of alanine were 5.89 ± 0.61 for "controls for pregnant" (I) and 4.36 ± 0.43 for "controls for exercise" (II). These animals were housed in Animal Quarters I and II, respectively. The values for percent recovery, 90.8 ± 1.80 (I) and 94.3 ± 0.42 (II), yield "corrected" mean net serosal accumulation values which are significantly different ($P<0.05$). Accordingly, it appears that the animals of Category I had a higher rate of active transport of alanine than did animals of Category II. The fact that gut lengths did not show any significant differences (1.2 cm S.E.M. for all control rat guts) indicates that the active transport rate of at least this neutral amino acid is susceptible to change.[42, 43, 44]

The same statements and conclusions can be made about differences in net serosal accumulation of lysine. There were significant differences in net serosal accumulation and "corrected" net serosal accumulation values of the differently housed control groups. It can be seen, therefore, that differences in net serosal accumulation of lysine were due to differences in intestinal active transport rates of the two groups of animals.

EFFECT OF PREGNANCY ON NET SEROSAL ACCUMULATION OF AMINO ACIDS:

The very significant increase in net serosal accumulation

of lysine by the 7 D-P group, and the significant decrease in net serosal accumulation of proline by the 21 D-P group, when compared with their respective control groups, was associated with an equally significant change of like magnitude when "corrected" net serosal accumulation values were used in the t-test. It appears, therefore, that the change in net serosal accumulation of lysine was due to an increase in the active transport rate of the intestine of the 7 D-P group. Similar deductions indicate that the decrease in net serosal accumulation of proline was due to a decrease in the intestinal active transport rate of the 21 D-P group.

Analysis of effects of pregnancy on AIB and glutamic acid net serosal accumulation is difficult to make. Active transport of AIB by the rat gut has been established, repudiated, and established again by others.[16] In view of such findings and other findings reporting no metabolism in one case and metabolism in another case,[39] any conclusions drawn relative to net serosal accumulation of AIB are tenative. Findings in this study indicate negative net serosal accumulation of AIB and glutamic acid which are indicative of a very small or no active transport rate.

The 14 D-P and 21 D-P groups showed no significant difference from control net serosal accumulation of AIB when "corrected" net serosal accumulation values were used. The uncorrected net serosal accumulation of these two groups were significantly different from the control group. It

appears that the differences between these groups and the control group were due to differences in metabolism and/or tissue uptake by the intestine. The 7 D-P group had a "corrected" net serosal accumulation which was significantly different from the control group ($P<0.001$). This, together with the fact that negative net serosal accumulation values were obtained, indicates that, while the active transport rate of AIB of the 7 D-P group was greater than the control rate, it was not a large one.

Data of the net serosal accumulation study of glutamic acid indicate that the transport rate of this amino acid is also small. "Corrected" net serosal accumulation values tended to cancel the effects noticed, indicating differences in metabolism by the intestines of the various groups.

It is obvious that little knowledge can be gained from the study on AIB and glutamic acid on the effect which pregnancy has on active transport in terms of net serosal accumulation.

EFFECT OF EXERCISE AND PREGNANCY ON NET SEROSAL ACCUMULATION OF AMINO ACIDS:

The results from the effect of exercise superimposed on pregnancy as measured by net serosal accumulation yielded no new information. The highly significant increase in net serosal accumulation of alanine by the 7-13 D-P-E group when compared with its respective control group appears to have been due to an increase in active transport. This increase is associated with an equally significant increase in the

"corrected" net serosal accumulation of alanine by this group.

Apparently exercise during the second trimester of pregnancy magnifies and makes significant the expected increased need for this amino acid at this time, since this increased need was not found when 14 D-P animals were compared with their respective control group.

It should be pointed out that exercise initiated in the first or second trimester of pregnancy had a tendancy to increase net serosal accumulation of alanine. Exercise initiated in the last trimester of pregnancy tended to cause a decrease in net serosal accumulation, but exercise initiated in the first trimester and carried through the second and third trimester of pregnancy shows no trend of an effect on net serosal accumulation.

EFFECT OF PREGNANCY ON EXERCISED RATS AS MEASURED BY NET SEROSAL ACCUMULATION:

In order to separate the effects of pregnancy on net serosal accumulation from those due to exercise, it was necessary to compare pregnant exercised animals with exercised control rats. In doing this it was found that the increase in active transport of alanine by the 7-13 D-P-E group was due to effects which exercise had on pregnant animals. This is concluded by the observation that comparison of the 7-13 D-P-E group with control rats exercised for 7 days negates the increase. This conclusion is strengthened by the fact that exercise for 7

days caused a slight increase in net serosal accumulation of alanine in exercised control rats over control rats for exercise.

The opposite interrelation is seen in the 14-20 D-P-E group. That is, the decrease in net serosal accumulation of alanine by the 14-20 D-P-E group can be thought of as being due to the effect of pregnancy on exercised rats, since comparison with the control group exercised 7 days did not negate the effect. It can be seen, therefore, that the accumulative effects of exercise and pregnancy were greater than those of exercise alone. It can be observed from the "corrected" net serosal accumulation data that the decrease in net serosal accumulation of alanine by the 14-20 D-P-E group was due to a decrease in intestinal active transport.

The 0-20 D-P-E group showed a significant increase in net serosal accumulation of alanine over the control rats exercised 21 days but not over the control rats for exercise. Thus, it appears that it is accumulative effect of pregnancy and exercise overriding the effect of exercise alone. "Corrected" net serosal accumulation data indicate that the active transport rate of the 0-20 D-P-E group is the parameter which was affected.

From the above observations one can conclude: (1) that exercise during the second trimester of pregnancy increases the active transport rate of alanine by the intestine; (2) exercise incurred during the last trimester of pregnancy in some way causes a decrease in active transport of alanine;

and (3) exercise throughout the term of pregnancy causes no observable effects on alanine transport. In other words, the alanine transport is adaptive to the physiological status of the animal.

The fact that in the 21 D-P, 14-20 D-P-E, and 0-20 D-P-E groups the intestine was significantly longer in length than the respective control group indicates that this was an adaptation of the pregnant rat to increase dietary uptake. When one considers the fact that the exercised and non-exercised 21 D-P groups showed significant increases in intestinal length which was not shown by the control rats exercised 21 days, it is clear that pregnancy *per se* was the influencing factor on gut length.

EFFECT OF EXERCISE AND PREGNANCY ON BODY AND ADRENAL GLAND WEIGHT:

Pregnancy causes an increase in body weight. This increase is due in part to the added weight of the conceptus and in part to weight gained by the expectant rat. If for any one trimester of pregnancy the weight gained by the control group is added to the weight of the conceptus and this sum subtracted from the total weight gained by the pregnant rat, then it is clear that a gain in weight by the maternal tissue above that of control animals occurred. If the same is done for the pregnant-exercised rats, it can be seen that except in the case of the 0-6 D-P-E group the amount of weight gained

by the maternal tissue is in all cases less than the pregnant groups of similar duration. Furthermore, except in the case of the 0-20 D-P-E group, the smaller increase in body weight of the pregnant exercised groups was reflected by a similar smaller increase in total conceptus weight when compared with pregnant groups of the same duration. Thus, it can be seen that exercise during the second or third trimester only exerts a "steadying effect" on maternal weight gain resulting in a lower conceptus weight. Once again it appears that exercise throughout the term of pregnancy has a desirable effect on the parameters studied in that it reduces body weight gain by the maternal tissue while causing no ill effect on conceptus weight. This stabilization effect concept is strengthened by the finding that the conceptus weight as a percent of total body weight increased in this group. The fact that the 7-13 D-P-E group did not show a significant weight gain change when compared to 14 day control group but that the 14 D-P group did is seen to be due to the effect of exercise on maternal tissue.

Only in the last trimester of pregnancy was there a difference in absolute weights of the adrenal gland when compared with control rats of the same duration and to all control groups. This indicates that the physiological burden of pregnancy does not cause a significant stress until the last trimester. It can be seen from Table VII that there is a significant increase in absolute adrenal gland weights of

the 7-13 D-P-E group. There was no significant change in absolute adrenal gland weight of the 7 day exercised controls. This would tend to indicate that while the condition of pregnancy or exercise alone is not enough to stress the animal during the second trimester, the additive effects of the two conditions during this period are a definite physiological stress. It is worthwhile noting that in only one instance (14 D-P-E) was an increase in the adrenal gland weight on a mg/100 gm basis found in any experimental group when compared with appropriate control groups.

EFFECT OF EXERCISE ON CONTENTS OF THE CONCEPTUS:

Total conceptus weight, number of fetuses, average fetal weight, and fetal/conceptus weight ratio indicated a small but insignificant decrease in all exercised groups except the 0-20 D-P-E group where a slight insignificant increase occurred (except for fetal numbers). This may once again be an indication of the "sparing effect" mentioned before. In all instances the average placental weight of exercised groups showed a significant decrease. The meaning of this is not clearly understood.

VI. Summary

Amino acid active transport rates of the intestine of the rat have been shown to change in the face of different environmental conditions, pregnancy, and the accumulative condition of exercise and pregnancy. In addition to this, the rat intestinal length increased during pregnancy, but this increase was apparent only in the last trimester of pregnancy.

It appears that the increased nutritional demands of pregnancy in the rat may be compensated for by: (1) an increased intake of food; (2) an increased surface area of the intestine; (3) an increased intestinal active transport rate of amino acids; and (4) a shifting of the maternal protein mass to the developing fetuses.

Pregnancy appears as a "stress" only in the last trimester of pregnancy as evaluated by its effects on adrenal gland weight. Exercise potentiates the stressful condition of second trimester pregnancy and makes the physiological stress apparent. Exercise during the last trimester of pregnancy appears to reduce the stress of pregnancy; exercise throughout the term of pregnancy appears to "condition" the animal to the increased metabolic and tissue demands of pregnancy.

Exercise carried out during any one trimester of pregnancy, and lasting for the duration of that trimester only, was detrimental to the rat in nearly all parameters measured. However, exercise throughout the term of pregnancy (day 0-20) was beneficial in that no detrimental effects were noticed,

while maternal weight decreased and active transport rates adapted.

VII. Bibliography

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