



This is to certify that the

thesis entitled

EFFECTS OF DIETARY FATS AND CARBOHYDRATES ON THE
VOLITIONAL ACTIVITY IN THREE STRAINS OF RATS,
THE MONGOLIAN GERBIL AND THE DOMESTIC PIG

presented by

William David Hart

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Human Nutrition

Major professor

Date 9-11-78

EFFECTS OF DIETARY FATS AND CARBOHYDRATES ON THE
VOLITIONAL ACTIVITY IN THREE STRAINS OF RATS,
THE MONGOLIAN GERBIL AND THE DOMESTIC PIG

By

William David Hart

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

College of Human Ecology
Department of Food Science and Human Nutrition

1978

EFFECTS OF DIETARY FATS AND CARBOHYDRATES ON THE
VOLITIONAL ACTIVITY IN THREE STRAINS OF RATS,
THE MONGOLIAN GERBIL AND THE DOMESTIC PIG

By

William David Hart

This series of experiments looked at the relationship between dietary energy source and volitional activity in several animal species. Preliminary work with the Sprague-Dawley strain of rats showed that rats on a high fat diet were significantly less active than rats on a control diet, which was high in carbohydrate, after three weeks on the diet. Activity was measured with an open field test box.

The experiment was repeated with the Osborne-Mendel strain of rats. These animals did not show any decrease in activity on the high fat diet versus the high carbohydrate control diet at one, two, or three weeks. The animals did show significantly higher excretions of norepinephrine in their urine on the high fat diet as against the control diet. There was no significant difference in the urinary content of dopamine or in the brain and adrenal content of the catecholamines. Activity was again measured by the open field test box.

Young, growing domestic pigs were tested for activity on one of two semipurified diets; one high in fat and a control diet high in carbohydrate. The animals on the high fat diet showed a significant reduction of activity after eight and twelve days on the diets.

Activity was measured with commercial pedometers taped to the hind leg of the pig for a twenty-four hour period. Two attempts to repeat the experiment failed to find any significant differences in activity. This was due either to the change in diets from semipurified to grain rations more typical of a pig's normal diet or to the use of older pigs. The variance in activity was much higher in the second two experiments than in the first experiment.

The activity of the Mongolian Gerbil was examined with a radio field activity box. The animals were fed on either a barley control diet or a sunflower seed diet. There was no significant difference in activity between the two diets for the five-day test period; however, the gerbils on the sunflower seed diet showed a significant increase in activity over the five-day test.

The activity of two strains of rats, the Osborne-Mendel and the S5B/PL, and the urinary and brain contents of the monoamines, dopamine, norepinephrine and serotonin and the blood content of tryptophan were examined. Four diets were used; sucrose, corn starch, hydrogenated vegetable oil and corn oil provided the major source of calories in diets R-1, R-2, R-3 and R-4 respectively. Activity was measured with the radio field activity box. The animals on the high sucrose diet showed decrease in activity over the five-day test period and a significantly lower level of activity than the animals on either R-2, R-3 or R-4. The animals on diets R-2 and R-3 had essentially the same activity pattern while the animals on diet R-4 had initially higher activity which declined, then rose again over a four-day period.

Animals on diet R-3 had higher urinary levels of serotonin on day one of the experimental period than animals on the other diets. Similarly, the urinary levels of dopamine and norepinephrine for the animals on diet R-3 were higher at the five-day point than for animals on other diets.

Brain levels of serotonin were significantly higher on day one for animals on diet R-3 than for the animals on the other diets. Dopamine and norepinephrine levels did not vary significantly, but there was a paucity of samples.

Tryptophan levels in the blood did not show any effect of either diet or length of time on a diet.

DEDICATION

JMJ

ACKNOWLEDGMENTS

Completion of a doctoral dissertation is impossible without the help and guidance of a major professor. I was blessed with two very fine major professors to whom I owe much. Dr. Mickelsen first started me in the field of nutrition. His guidance, encouragement, and wisdom were very much appreciated. Dr. Schemmel was kind enough to take over the direction of my committee when Dr. Mickelsen departed. Her comments were helpful, her criticism was cogent, and her help was timely and much appreciated.

Dr. Van Huss was a friend as well as a source of ideas and suggestions. Dr. Dugan was often available for advice and comments as my work progressed. Dr. Miller helped review this manuscript as well as provided the materials and animals for some of the work. He also was always available for much needed help and suggestions.

Numerous other co-workers provided help both material and in the form of comments and criticisms. Dr. Wolterink provided both the radio field activity boxes and many discussions about the results as they unfolded. Dr. Kabara allowed the use of the equipment for the analysis of the catecholamines. Dr. Ullrey donated the use of the equipment for the analysis of the serotonin and tryptophan.

George Collings helped with support and comments which were always welcome.

My daughter, Patricia, was a constant joy who gave many hours of delight during the long haul. She also is rapidly becoming a very good little lab assistant.

Finally, my wife Mary Rose. She will never know how much this dissertation is also hers. Her love and encouragement kept me going through the disappointments. Without her, this never would have been finished.

TABLE OF CONTENTS

	Page
LIST OF TABLES	vii
LIST OF FIGURES	x
 Chapter	
I. INTRODUCTION	1
Foreword	1
Need	2
Problem	2
Theoretical Basis	3
Limitations and Scope of the Research	5
Research Questions	5
Overview of the Research	5
II. REVIEW OF LITERATURE	7
Introduction	7
Biochemical Aspects of Monoamines	8
Catecholamines	8
Synthesis	8
Location	10
Regulation	11
Serotonin	13
Biosynthesis	13
Location	18
Degradation	18
Interrelationships Between Monoamines	19
Hyperactivity	19
Symptoms	21
History	22
Treatment	23
Interactions of Monoamines and Activity	27
Diet and Activity	30
Monoamines and Diet	32
Summary	34

Chapter	Page
III. PRELIMINARY STUDIES OF DIETARY EFFECTS OF FAT AND CARBOHYDRATE ON ACTIVITY AND CATECHOLAMINES IN THE OSBORNE-MENDEL AND SPRAGUE-DAWLEY RATS	35
Objective	35
Materials and Methods	35
Experiment I	35
Chemical Analyses	39
Results and Discussion	40
Conclusions	44
IV. STUDIES OF DIETARY EFFECTS OF FAT AND CARBOHYDRATES ON ACTIVITY IN THE MONGOLIAN GERBIL AND DOMESTIC PIB	45
Objective	45
Part A--Gerbil	45
Materials and Methods	45
Activity Measurements	45
Results and Discussion	46
Conclusion	50
Part B--Domestic Pig	51
Materials and Methods	51
Results and Discussion	53
Conclusions	60
V. STUDIES OF DIETARY EFFECTS ON FAT AND CARBOHYDRATE ON ACTIVITY AND MONOAMINES IN THE OSBORNE-MENDEL AND S5B/PL RATS	61
Introduction	61
Materials and Methods	61
Results and Discussion	66
Conclusions	77
VI. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS	80
BIBLIOGRAPHY	89

LIST OF TABLES

Table	Page
2.1 Chief Complaints for Minimal Brain Dysfunction	22
2.2 Dr. Cott's Vitamin Therapy	25
3.1 Composition of High Carbohydrate and High Fat Diets . . .	36
3.2 Time Required to Reach a Corner for Sprague-Dawley Rats on Two Different Diets (Trial One)	38
3.3 Time Required to Reach a Corner for Sprague-Dawley Rats on Two Different Diets (Trial Two)	38
3.4 Time Required to Reach a Corner for Osborne-Mendel Rats on Two Different Diets (Trial Three)	41
3.5 Average Weight Gain on Rats Fed High Carbohydrate or High Fat Diets (Osborne-Mendel Rats)	41
3.6 Catecholamine Contents of the Organ (Osborne-Mendel Rats)	42
3.7 Change in Catecholamine Excretion From Period One to Period Three (Osborne-Mendel Rats)	43
4.1 Sources of Energy in Male Gerbil Diets	46
4.2 Activity Levels for Male Gerbils Fed Two Diets	47
4.3 Evaluation of Activity in Gerbils Kolmogorov-Smirnov Test for Goodness of Fit	48
4.4 Analysis of Variance for the Log Transform of the Activity Level	48
4.5 Analysis of Variance of the Activity Levels	49
4.6 Composition of Semipurified Rations Used for the First Study of Activity in Young Pigs	52

Table		Page
4.7	Composition of Grain Rations for Pig Studies	54
4.8	Activity of Growing Pigs Fed High Fat or High Carbohydrate Rations Measured with Pedometers (Four Pigs per Group)--Pig Experiment One	55
4.9	Activity of Growing Pigs Fed High Fat or High Carbohydrate Grain Rations Measured with Pedometers (Five Pigs per Group)--Pig Experiment Two	56
4.10	Activity of Growing Pigs Fed High Fat or High Carbohydrate Grain Rations Measured with Pedometers (Four Pigs per Group)--Pig Experiment Three	57
4.11	Body Weights of Pigs Used in Pig Experiment Two (Five Pigs per Group)	58
4.12	Body Weights Used in Pig Experiment Three (Four Pigs per Group)	58
5.1	Composition of High Carbohydrate and High Fat Diets . . .	62
5.2	Log Transformations of the Voluntary Activity of Two Strains of Rats Fed High Fat or High Carbohydrate Diets	67
5.3	Kolmogorov-Smirnov Goodness-of-Fit Statistics	68
5.4	Analysis of Variance for the Log Transform of the Activity Levels	68
5.5	Serotonin in Urine for Two Strains of Rats Fed High Fat or High Carbohydrate Diets	70
5.6	Analysis of Variance for the Urinary Serotonin Content	70
5.7	Mean Dopamine Excreted Over Twenty-Four Hours per Two Strains of Rats Fed High Carbohydrate or High Fat Diets	71
5.8	Analysis of Variance for the Urinary Dopamine Content	71
5.9	Mean Norepinephrine Excreted in Twenty-Four Hour Period for Two Strains of Rats Fed High Fat or High Carbohydrate Diets	72

Table		Page
5.10	Analysis of Variance for the Urinary Content of Norepinephrine	72
5.11	Mean Total of Serotonin in the Brains of Two Rat Strains Fed High Carbohydrate or High Fat Diets for Three Different Lengths of Time on the Diets . . .	73
5.12	Analysis of Variance for the Brain Content of Serotonin	73
5.13	Mean Total Dopamine in the Brain of Two Rat Strains Fed High Carbohydrate or High Fat Diets for Three Different Lengths of Time on the Diets	75
5.14	Analysis of Variance for the Brain Content of Dopamine	75
5.15	Mean Total Norepinephrine in the Brain of Two Rat Strains Fed High Carbohydrate or High Fat Diets for Three Different Lengths of Time on the Diets . . .	76
5.16	Analysis of Variance for the Brain Content of Norepinephrine	76
5.17	Mean Total Tryptophan in the Blood for Two Rat Strains Fed High Carbohydrate or High Fat Diets for Three Different Lengths of Time on the Diet	78
5.18	Analysis of Variance for the Blood Content of Tryptophan	78
5.19	Analysis of Variance of the Effect of Rat Strain on the Urinary Excretion of Dopamine	79

LIST OF FIGURES

Figure	Page
2.1 Biosynthesis of catecholamines	14
2.2 Degradation of catecholamines	15
2.3 Biosynthesis of serotonin	16
2.4 Proposed monoamine interactions	20

CHAPTER I

INTRODUCTION

. . . Diseases affecting the central parts of the brain, as the corpus striata, the optic thalami, and the upper portions of the medulla oblongata, invariably deranges the powers of voluntary motion and sensation. --Davis, 1845, pp. 241-242

Foreword

Since the 1930s, there has been an increasing concern with a derangement of the powers of voluntary motion and sensation in which an extremely heightened level of activity is seen to occur, especially in children. This aberration is commonly referred to as the hyperkinetic syndrome. Recently, the popular clinical observations of Feingold (1974) and Cott (1975) have focused interest on some dietary factors which they claim, when altered, can control the symptoms of this condition. Current treatment for the syndrome involves the use of drugs which are normally classed as stimulants, but which, in children with this syndrome, produce a paradoxical calming effect (Cantwell, 1975). The use of these drugs has been criticized because of their side effects and their potential for abuse (Connors, 1974). Additionally, there have been charges of the misuse of these drugs to control children who may or may not be hyperactive but who do not respond to a school's efforts to regulate the child's behavior (Connors, 1974).

If a dietary control for this syndrome can, in fact, be found for at least some subset of the total population of hyperactive children, two possible benefits may accrue. First, the side effects and misuse problems associated with the drugs in current use could be avoided. Second, dietary changes could add an additional tool to probe the underlying neurophysiological aberration of the syndrome.

Need

Although some clinicians have reported on the use of dietary modifications to treat hyperactivity, their work has been in the nature of clinical observations (Cott, 1975; Feingold, 1974). There has been a dearth of reports of investigations into the possible mechanisms by which dietary modification could alter activity. More work into the mechanisms underlying the syndrome and its symptoms is required. Baseline data should be accumulated so that future clinical trials can be organized in a more systematic manner.

Problem

This research will examine the changes in certain monoaminergic neurotransmitters and activity during dietary modifications. Changes in the primary energy source in the diet will be studied in three strains of rats, in one strain of gerbils, and in one breed of pigs.

Theoretical Basis

The first reports of an organic disorder characterized by the symptoms now associated with the hyperkinetic syndrome were in the late 1930s (Bradley, 1937). As awareness of this syndrome grew, methods of treatment were sought. Traditional methods of dealing with behavioral disorders in children, such as individual psychoanalysis and familial group therapy, proved to have mixed success. Dr. Bradley, in 1937, published the first evidence of a paradoxical tranquilizing effect of stimulant drugs in a group of children with behavioral disorders (Bradley, 1937). These stimulant drugs, amphetamines, ritalin and benzedrine, have become the treatment of choice for children with this syndrome. However, the use of these drugs has been criticized by several investigators (Conners, 1974; Fish, 1971; Strother, 1973). Most controversial have been charges that schools and physicians have been forcing parents to accept drug therapy for their children who may or may not be hyperactive, but who have been labeled as behavioral problems for the school.

In the last several years, some clinicians have reported that they were able to control the symptoms of this syndrome by dietary modification (Cott, 1975; Feingold, 1974). Dr. Benjamin Feingold attributed the apparent increase in the number of cases of hyperkinesis which were being reported to an increase in the consumption of food containing additives of all kinds, such as coloring agents, preservatives, flavoring agents, etc. He prescribed a treatment regimen

designed to eliminate all foods which contained these additives. However, there are no data to support his hypothesis and his theory needs investigation.

Dr. Allen Cott has proposed the use of a high protein-low carbohydrate diet, avoidance of all sugar, and pharmacological doses of niacinamide. Both his work and that of Dr. Feingold are based mainly on clinical observations.

Dr. Samuel Livingston, in his book on the treatment of epilepsy in children, has made some observations on the tranquilizing effects of a high fat diet. He reports that this diet, which is used to control seizures in some types of epilepsy, also controls the hyperactivity to which epileptic children are often prone (Livingston, 1972).

Schemmel (1967) has reported that the activity levels of strains of rats were lower when fed a high hydrogenated fat diet (60 w/w) than for littermate rats of the same strain fed a grain diet. Dr. Samuels and his group reported that rats on a high fat diet were less active than those on either a high protein or high carbohydrate diet (Samuels et al., 1948). Preliminary work with the Sprague-Dawley rat indicated that this strain was less active when fed a high fat diet rather than a high sucrose diet (Chapter Three).

In any disorder of activity, the monoaminergic neurotransmitters can be suspected to play some role. Preliminary work with Osborne-Mendel rats showed an increase in the excretion of norepinephrine in the urine, relative to dopamine, in animals fed a high fat diet versus a high sucrose control diet (Chapter Three). Some

researchers have reported increased levels of norepinephrine in the urine of hyperactive children and decreased levels of serotonin in the blood of hyperactive children (Rappaport et al., 1970; Stewart, 1971).

Limitations and Scope of the Research

The research will be limited to three types of animals, the albino rat, the Mongolian gerbil, and pigs from herds maintained at Michigan State University. The conclusions can be applied only to these species under the similar circumstances. The research attempted to establish some base line data in animals, thereby increasing interest, so that clinical trials with human subjects would be eventually conducted.

Research Questions

This research was designed to answer the following research questions:

1. Do dietary energy sources alter the levels of certain of the monoaminergic neurotransmitters in the blood, urine, and selected tissues?
2. Do dietary energy sources affect volitional activity?

Overview of the Research

This research study is divided into five parts. The setting for the study is presented in Chapter One. It includes an introduction to the study, the statement of need and the purpose of the study,

limitations of the study, and a statement of the research questions to be addressed.

The review of the literature is contained in Chapter Two. The review is divided into three general areas: (1) the biochemistry of the monoamines, (2) hyperactivity, and (3) interactions between monoamines, diet and activity.

Preliminary work with two strains of rats, Sprague-Dawley and Osborne-Mendel, is presented in Chapter Three. The methods of investigation, the results, and discussion of these results, is presented, along with a discussion of the limitations of these studies.

Chapter Four presents the effects of various sources of dietary energy on volitional activity of the gerbil and the pig.

Chapter Five presents volitional activity data and tissue and urine values for norepinephrine, dopamine, serotonin, and tryptophan as affected by dietary energy source in Osborne-Mendel and S5B/Pl rats.

A summary of significant findings, conclusions, implications, and recommendations for further study is presented in Chapter Six.

CHAPTER II

REVIEW OF LITERATURE

Introduction

This review of the literature is divided into three general areas. The first area covers the general biochemistry of the monoamines, the synthetic and degradative enzymes of these monoamines, and the location of the monoamines in the central nervous system.

The second part of the review covers the hyperkinetic syndrome as diagnosed in human beings. A brief history of the syndrome will be presented. Some of the diagnostic difficulties will be discussed as well as some of the controversies surrounding the diagnosis and treatment of children with the syndrome. Also, the current attempts to treat the syndrome with dietary manipulations are presented. This area is included since it represents a potential application of the animal work to human subjects.

The third part presents the literature on interactions between the monoamines and activity, the effects of diet on the general activity of animals and the effects of diet on monoamines in tissues, and urinary excretion.

Biochemical Aspects of Monoamines

Catecholamines

Synthesis

The two primary catecholamines in the central nervous system (CNS) are dopamine (DA) and norepinephrine (NE). They are both synthesized by the same biochemical pathway, norepinephrine being synthesized from dopamine. This pathway, with enzymes, is shown in Figure 2.1.

Tyrosine Hydrolase is the first enzyme in the pathway. The enzyme is found primarily in the synaptosomal fraction (Mandel et al., 1975). It is normally saturated with its amino acid substrate. The K_m , depending on the source of the enzyme, has been reported to be between 45 millimolar and 20 millimolar which, in any case, is well below the concentration of tyrosine in the brain which has been reported as 80 millimolar (Mandel et al., 1975; Carlsson, 1974). The enzyme requires oxygen, iron, and pteridine as co-factors (Hornykiewicz, 1971; Torre, 1972; Weiner et al., 1974). This step is regarded as the rate limiting step in catecholamine biosynthesis and is subject to feedback regulation by both dopamine and norepinephrine according to Carlsson (1974), Torre (1972), and Weiner et al. (1974). This opinion, however, is not universally accepted. Some investigators suggested that the catecholamines are certainly capable of inhibiting tyrosine hydrolase in vitro. Another mechanism normally regulates catecholamine synthesis in vivo. They postulate that "a feedback mechanism operating via the

degree of receptor activities at the synaptic cleft may control the synthesis" (Carlsson et al., 1972, p. 383). The enzyme is stereo specific for L-tyrosine and can be competitively inhibited by L-methyl-B-tyrosine (Carlsson, 1974). The product of this reaction is L-dopa.

L-Aromatic Amino Acid Decarboxylase, the second enzyme in the synthetic pathway is a fast and very efficient non-specific enzyme. It decarboxylates a wide range of aromatic amino acids including L-dopa, tyrosine, 5-hydroxytryptophan, histidine, L-methyldopa, m-tyrosine, and tryptophan (Goldstein et al., 1972). The enzyme requires pyridoxal phosphate (Hornykiewicz, 1971). The enzyme has a high affinity for L-dopa ($K_m = 500$ millimolar) and is found also in cytoplasm. Some authors have argued for the presence of two decarboxylases, one for L-dopa and one for 5-hydroxytryptophan (Sims and Bloom, 1973). Others have suggested that the evidence, rather than supporting two decarboxylases, supports the idea of two binding sites with a common catalytic site (Bender and Coulson, 1972).

Dopamine- β -Hydroxylase, the third enzyme in the pathway, catalyzes the conversion of dopamine to norepinephrine (Mandel et al, 1975; Torre, 1972). This enzyme is located in the cytoplasm primarily of adrenergic cells. It is found localized in the synaptic vesicles and is released in parallel with the catecholamines. It requires oxygen and ascorbic acid as co-factors and is a copper containing enzyme (Mandel et al., 1975). Much, if not all, of the norepinephrine synthesized in the central nervous system is stored immediately in vesicles or is degraded. It cannot penetrate the blood-brain barrier

as norepinephrine. Dopamine, on the other hand, is found to some degree in the cytosol (Mountcastle and Baldessarini, 1974; Laverty, 1963). The enzyme is a mixed function oxydase which functions via a ping-pong mechanism. The ascorbic acid is oxidized simultaneously with the reduction of the copper to Cu^+ . Then the dopamine is hydrolyzed (Mandel et al., 1975). The enzyme is not specific to dopamine. This enzyme has been found to have decreased activity in patients with Parkinson's disease; this activity is increased during treatment with L-dopa (Goldstein et al., 1972).

Location

Dopamine is located in several discrete areas of the brain. The major portion is found in nigro-striatal complex, particularly in the caudate nucleus and the putamen, but also in the substantia nigra and the globus pallidus (Hornykiewicz, 1971; Thompson, 1975). These areas are those that play an important role in muscle control (Schneider and Tarshis, 1975). Depletion of the dopamine from the substantia nigra has been associated with the symptomology of Parkinson's disease (Hornykiewicz, 1971; Nagatsu et al, 1977; Schneider and Tarshis, 1975).

Norepinephrine has been identified in several areas of the brain such as the medulla, the pons, the midbrain, the rectilinear formation, and the hypothalamus (Schneider and Tarshis, 1975). The norepinephrine neurons in the hypothalamus derive from fibers which have their origins in the pons and medulla oblongata (Fuxe and Hökfelt, 1970). Norepinephrine is found especially in systems which affect the

autonomic nervous system. It has been suggested that it is involved in the motivational and emotional aspect of behavior (Thompson, 1975). A single norepinephrine neuron normally gives rise to a large collateral network innervating many other areas in all parts of the brain and spinal cord (Fuxe and Hökfelt, 1970). Norepinephrine is confined to synaptic vesicles where it is synthesized from the dopamine which has been transported out of the cytosol (Mountcastle and Baldessarini, 1974; Töresjö, 1972). The norepinephrine which is released upon stimulation is either degraded or absorbed back into the storage vesicles. This conserves the available norepinephrine (Glowinski, 1970). Also, there appear to be two pools of norepinephrine, one more easily released than the other. These two features allow for a sustained response to stimuli (Mountcastle and Baldessarini, 1974).

Regulation

Both of the catecholamines are degraded using one or the other or both of two ubiquitous enzymes; monoamine oxidase or catechol-O-methyltransferase. The deamination occurs intraneuronally while the methylation occurs primarily extraneuronally (Glowinski, 1970).

Monoamine Oxidase is a widely distributed degradative enzyme requiring FAD, ascorbic acid, and molecular oxygen (Mandel et al., 1975). It functions by removing an amino group and replacing it with a carboxyl group. The enzyme is widely distributed throughout the organism and is found in conjunction with the other mitochondrial membrane (Torre, 1972). The enzyme itself is of high molecular weight; the active portion contains both a flavoprotein and copper (Mandel et

al., 1975). Monoamine oxidase appears to be more important for the degradation of the catecholamine in the central nervous system whereas catechol-o-methyltransferase is more important in the periphery (Glowinski, 1970).

Catechol-o-Methyltransferase functions in conjunction with monoamine oxidase to regulate and degrade the catecholamines; it does not appear to have a function in the degradation of serotonin. The enzyme is relatively nonspecific acting on almost any chemical with the catechol configuration, including such diverse compounds as ascorbic acid and 2-hydroxyestradiol (Guldborg and Marsdin, 1976). In the central nervous system, the enzyme is found primarily in the neurohypophysis, although activity is found in all regions of the brain (Guldborg and Marsdin, 1976). The enzyme is located both extraneuronally and in association with the mitochondria at the nerve endings (Guldborg and Marsdin, 1976; Mandel et al., 1975; Torre, 1972).

The O-methylation occurs primarily in the meta-position in vivo. In vitro, significant amounts of the para product have been reported with some assay systems (Guldborg and Marsdin, 1976; Mandel et al., 1975). The enzyme's activity is strongly affected by the in vivo concentration of Mg^{++} (Guldborg and Marsdin, 1976).

Other Minor Enzymes are present which act on the products of monoamine oxidase and/or catechol-o-methyltransferase action. There have been reported both an alcohol dehydrogenase (to form the alcohol) and an aldehyde oxidase (to form the acid), especially after monoamine oxidation (Mandel et al., 1975). Also, the sulfo- and Glycuromyl-conjugates have been reported.

These enzymes work usually in conjunction to form characteristic catabolic products for each species. For example, in man, the primary catabolic products are vanilmandelic acid and homo-vanillic acid. In the rat, on the other hand, the most important catabolic product is 3-methoxy-4-hydroxyphenylethylene glycol (Mandel et al., 1975).

Serotonin

Biosynthesis

The biosynthetic pathway for serotonin is similar to that of the catecholamines. The synthetic pathway is shown in Figure 2-3.

Tryptophan Hydrolase, the first enzyme in the pathway, is the point of regulation of serotonin synthesis. Unlike tyrosine hydrolase, tryptophan hydrolase does not appear to be regulated by feedback mechanisms. Indeed, the enzyme appears to be about half-saturated with the amino acid substrate under physiological conditions (Carlsson, 1974). This seems to indicate that the level of serotonin in the brain is regulated by the availability of substrate (Carlsson, 1974; Fernstrom et al., 1974; Fernstrom and Jacoby, 1975; Gessa and Tagliamonte, 1974; Moir, 1974). Thus, the plasma concentration of tryptophan becomes important to the synthesis of serotonin. Tryptophan is both free and bound to albumin in the plasma. Only the free tryptophan is available for transport to the brain. However, the bound tryptophan is apparently readily removed from the albumin, thus, increasing the effective concentration of the tryptophan available for passage into

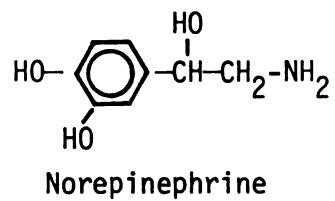
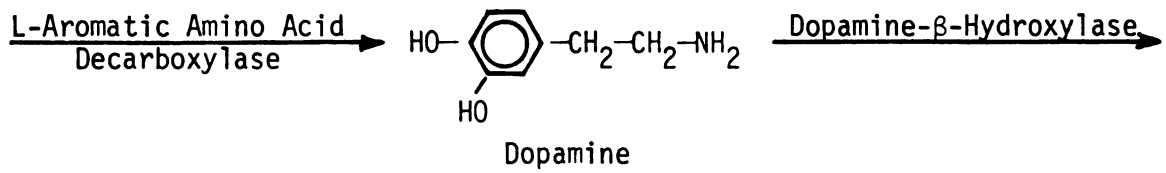
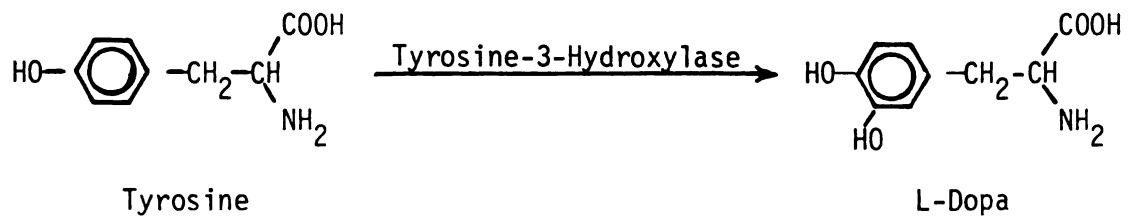


Figure 2.1 Biosynthesis of catecholamines.

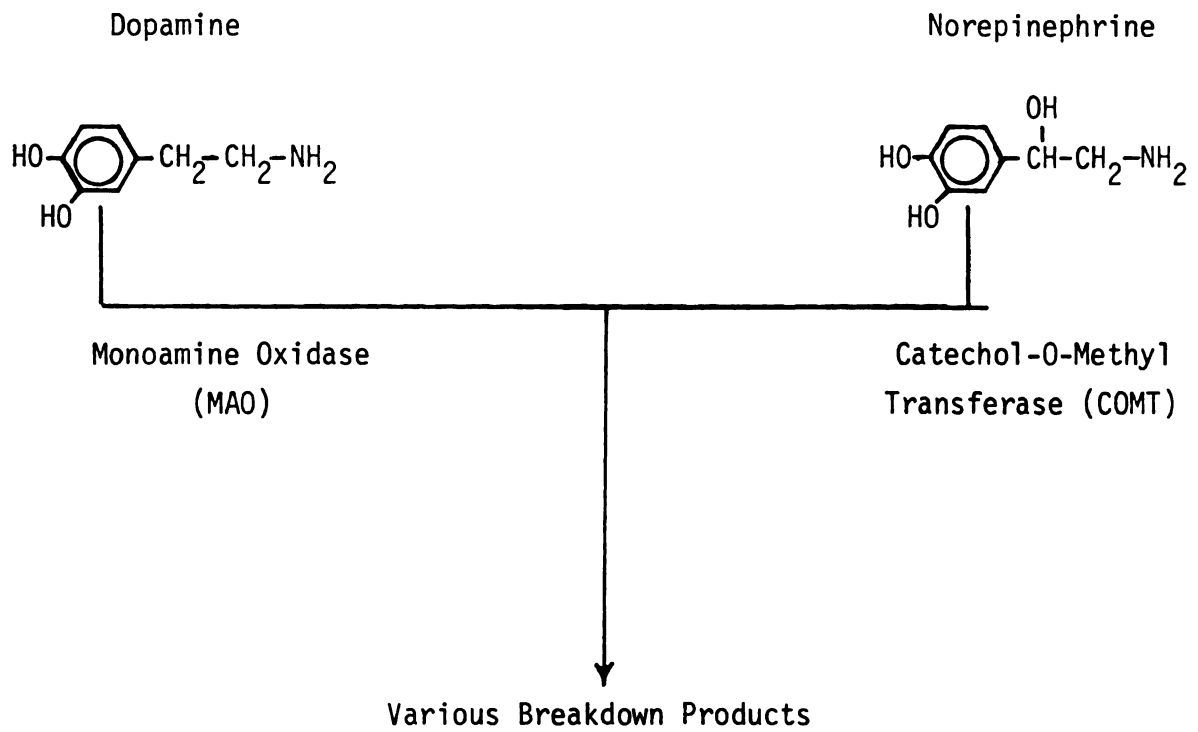


Figure 2.2 Degradation of catecholamines.

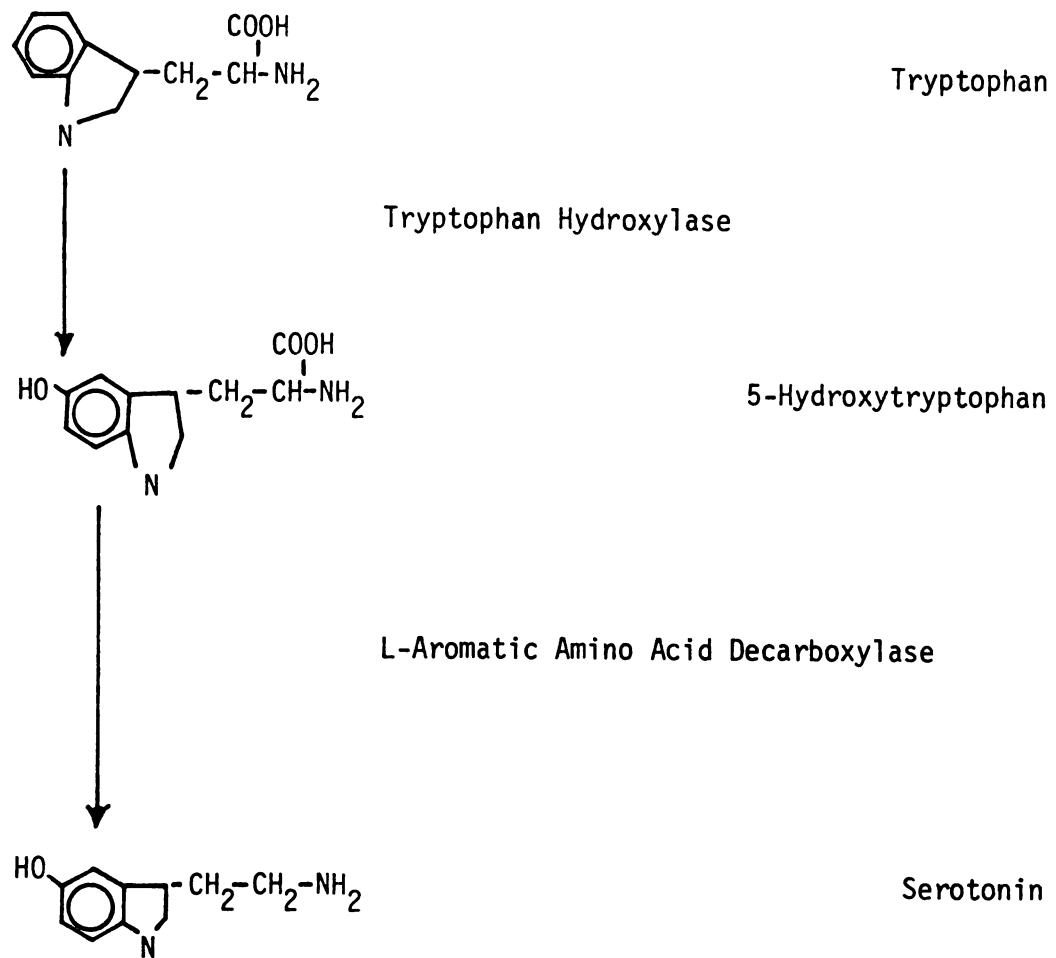


Figure 2.3 Biosynthesis of serotonin.

the brain. Transport across the blood-brain barrier is by an active carrier mechanism which is nonspecific. The tryptophan competes for this carrier with the other large neutral amino acids (Fernstrom et al., 1974; Fernstrom and Jacoby, 1975; Garattini and Valzelli, 1965). The albumin binding has been shown to be affected by the pH of the blood and by competitive binding of other substrates to the albumin, such as free fatty acids (Yuwiler et al., 1977). Madras et al. studied the effects of a high carbohydrate diet (72%) on the concentrations of tryptophan in the blood and brain. They found that the concentration of brain tryptophan did not change after two hours on the diet, although the blood concentrations of free tryptophan were increased. However, after three days, the brain concentration was about 1.5 times higher (Madras et al., 1974). Yuwiler et al. attributed the slow rise in the brain tryptophan concentration to the competition for the blood-brain barrier carrier (Yuwiler et al., 1977).

Insulin also affects the serum concentration of tryptophan as well as the synthesis of serotonin. This will be treated in the section of diet-monoamine interaction below. The enzyme is pterin-dependent and requires oxygen and 2-mercaptoethanol (Kaufman, 1974). Large amounts of the substrate have been shown to inhibit this enzyme but it does not appear to be inhibited by its end product, particularly under physiological conditions (Kaufman, 1974).

Aromatic L-Amino Acid Decarboxylase functions in the serotonin systems in a manner similar to that in the catecholamine systems. It decarboxylates the first product, 5-hydroxytryptophan, to 5-hydroxytryptamine, or serotonin. The enzyme is fast-acting; very little of

the 5-hydroxytryptophan can be detected under normal conditions in vivo (Carlsson, 1974; Fernstrom and Jacoby, 1975).

Location

Serotonin is found in distinct areas of the brain; these include the pineal gland and especially the raphe nucleus in the mid-brain (Thompson, 1975). Saavedra (1977) has published a more extensive list of the concentrations of serotonin in various areas of the rat brain. The raphe nucleus sends axons to many parts of the fore-brain, including the hypothalamus and limbic areas (Thompson, 1975). These limbic structures have been implicated in the regulation of several behavioral functions (Saavedra, 1977). Of special interest are the findings of inhibitory connections from the raphe nucleus to the caudate nucleus (Antelman and Caggiula, 1977; Mabry and Campbell, 1973). In these locations, serotonin plays a major role in regulating sleep and temperature and has been implicated in hallucinations (Schneider and Tarshis, 1975). In the hypothalamus, serotonin has been found in the median eminence and the subcommisural organ as well as several of the hypothalamus nuclei (Brownstein, 1977; Fuxe and Hökfelt, 1970).

Degradation

Serotonin is degraded primarily by monoamine oxidase to 5-hydroxyindoleacetic aldehyde. This is converted to the acid by an aldehyde dehydrogenase (Garattini and Valzelli, 1965; Torre, 1972). The primary excretion product in both man and rat is the 5-hydroxy-indolacetic acid but other products have been detected and may be of some importance in other species (Garattini and Valzelli, 1965).

Interrelationships Between Monoamines

These monoamines are closely related both in central nervous system location and in structure. It would be expected that they would have influences on each other. One known scheme for monoamine interactions is shown in Figure 2.4. As can be seen, serotonin has an inhibitory influence on the dopaminergic caudate nucleus (Antelman and Caggiula, 1977). It has been shown that specific destruction of the raphe nucleus leads to increased activity in the caudate nucleus and that conversely, stimulation of the raphe nucleus depresses neuronal activity in the caudate nucleus (Mabry and Campbell, 1973). Because serotonin levels are influenced by such dietary factors as tryptophan concentrations (Fernstrom, 1975), this represents a potential point whereby diet might influence activity. This caudate nucleus is implicated in neural control of movement, both postural and other (Schneider and Tarshis, 1975; Thompson, 1975). However, it has been hypothesized that perturbation in dopaminergic systems are, to some extent, compensated for by actions of norepinephrinergic systems (Antelman and Caggiula, 1977). These compensations have been postulated to be most functional in stress situations (Antelman and Caggiula, 1977).

Hyperactivity

Hyperactivity or hyperkinetic syndrome or minimal brain dysfunction is a complex, poorly understood, ill-defined and controversial entity. One definition for the syndrome is "chronic sustained excessive level of motor activity which is the cause of significant and

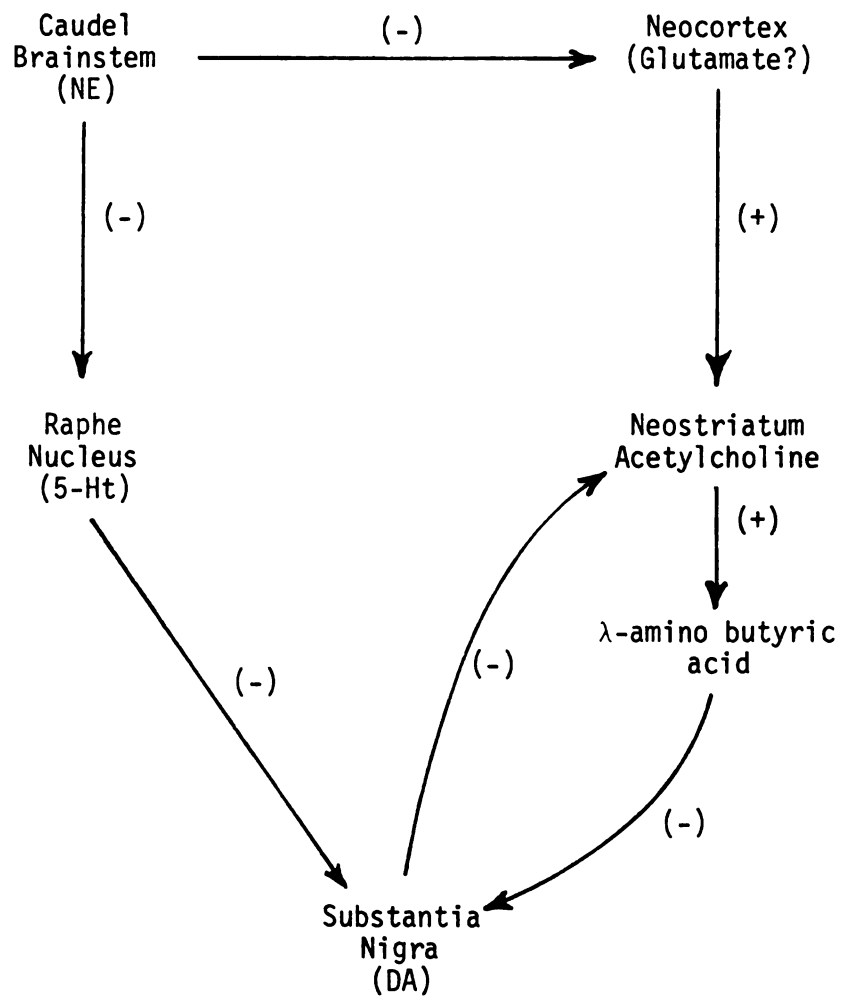


Figure 2.4 Proposed monoamine interactions.

Source: Antelman, S. M., and Caggiula, A. R. 1977. Science 195: 646-653.

continued complaint. . ." (Casey, 1977, p. 68). In many respects it is a "new" disease. However, it has been described as far back as 400 B.C. To many educators and physicians it is a specific, if undefined, disease state with an effective treatment and good prognosis for most sufferers. Others claim that it does not exist as a disease but is merely a catch-all for difficult psychiatric cases who would be better treated with conventional psychotherapy. Some have claimed that it is a sociological problem where medication is used as an adjunct to more conventional disciplinary tools of society. Recently, some clinicians have claimed that the food we eat may be responsible for the syndrome (Cott, 1975; Feingold, 1974).

Symptoms

Hyperkinetic syndrome is the official designation for a collection of symptoms for which, it is currently assumed, there is some undetected organic lesion (Conrad, 1976; Gross and Wilson, 1974). The children afflicted with this syndrome exhibit a cluster of symptoms (see Table 2.1) but not all children exhibit all the symptoms (Livingston, 1972). The most common symptoms are underachievement, hyperactivity, aggressiveness, and poor concentration (Gross and Wilson, 1974). The syndrome is found primarily in males (Conrad, 1976). Most estimates of the percentage of the population who exhibit these symptoms range around 3% to 5%, although some estimates are higher (Gross and Wilson, 1976). Heussy (1977) has stated that 20% of all school-age children are affected. Feingold (1974, p. 16) suggests that anywhere

Table 2.1 Chief Complaints for Minimal Brain Dysfunction

Complaint	Percentage of Patients
Underachievement, learning problems	75.4
Restlessness, hyperactivity	24.9
Temper outbursts	19.2
Aggressiveness, hostility	18.9
Distractible, poor concentration	17.0

Source: Gross, M. D., and Wilson, W. C. 1974 Minimal Brain Dysfunction. Brunner & Mazel:New York.

from 500,000 to 5,000,000 children may be affected. Often the hyperkinetic child is characterized as normal to bright in intelligence but is usually failing in school (Conrad, 1976; Livingston, 1972).

History

This syndrome was first reported in the early 1930s, although there is literature evidence for such a syndrome as early as 400 B.C. (Strother, 1973). In 1937, Bradley described the depressant effect of Benzidrine on a group of children with conduct disorders (Bradley, 1937). He referred to this as a paradoxical effect. In the 1950s and early 1960s, awareness of this syndrome grew both among physicians and clinicians and also among educators and parents (Conrad, 1976; Strother, 1973). In 1966, a task force was set up by the United States Public Health Service to clarify the ambiguous and confusing

welter of terminology and symptomology in the field (Strother, 1973). This task force was unable to agree on any subset or separate compartmentalization of symptoms, so it assigned the term minimal brain dysfunction to all separate descriptions, including the hyperkinetic syndrome (Strother, 1973).

This has led to a considerable degree of difficulty in establishing an underlying etiology for all as part of the children so classified. The possibility that undefined subsets with common symptoms and different causes are being lumped together under the rubric of the Hyperkinetic Syndrome is widely accepted (IFT report, 1976; Wender, 1973).

Treatment

Treatment for these children was initially by conventional psychotherapy. Indeed, some authors suggest that the delay in establishing the concept of the hyperkinetic syndrome was due to the tendency to see such problems only in light of psychological problems (Gross and Wilson, 1974). Bradley's findings of the paradoxical effects of benzedrine led to the increased use of this agent and, indeed, almost a circular diagnostic track whereby a child with hyperkinetic symptoms who responded to the treatment was considered to be hyperactive by virtue of this response (Conrad, 1976; Strother, 1973). Some have suggested that a large percentage of these children show various abnormalities in their EEG (Gross and Wilson, 1974). If this is so, it would greatly aid in the diagnostic process. Others have, however, disputed this evidence claiming either an inability to find such

abnormalities or claiming that the so-called abnormalities are so widespread as to make the term 'abnormality' ludicrous (Conrad, 1976). Duration of the illness is also in question due in part to the lack of large numbers of long-term studies. Most people feel that the patient outgrows his hyperactivity but Douglas has reported that some investigators feel that the hyperactive child tends to be a hyperactive adult (1974).

Another author (Conrad, 1976) has made an extensive study of the treatment of children who have been diagnosed as hyperactive. Conrad (1976) examined the sociological side of hyperactivity and concluded that too often there may be no other basis for the diagnosis than that of behavioral problems in school. He raises an issue which has been very strongly debated in recent years; that is, to what extent is nonconformation with expected behavioral norms being diagnosed as a disease and being medicated. His study of the way in which diagnoses were made in a large, well-equipped special clinic with a highly motivated staff led him to the conclusion that some significant portion of the children were thus misdiagnosed and also led him to postulate that this percentage was even higher among less well-trained clinicians in a more typical physician's setting. The whole issue of the use of potent medication on young, growing children for extended periods of time is one of the factors that has led investigators to look for other modes of treatment (Pelham, 1977).

Beginning in the late 1960s and into the early 1970s, several authors suggested that the hyperkinetic syndrome was related to an

allergenic reaction to some component of the environment. Mandell (1968, 1969) began reporting on the allergic reactions of people to many different environmental factors. These included many food items and the symptomology included hyperactive behavior. Although he did not deal directly with the hyperkinetic syndrome, his attribution of symptomology which could be diagnosed as a psychological illness to chemical 'contaminants' in the environment anticipated another allergist, Feingold, who did.

Feingold (1974, 1975) created a great deal of publicity about the hyperkinetic syndrome with his books and articles on the subject. His thesis was that the hyperactive child was hyperactive because of an allergic reaction to some part of his environment. Further, he specified that the reaction was most likely due to the "artificial" coloring agents, texturizers, emulsifiers and preservatives in commercial food products. He claimed a very high success rate (up to 80%) in treating these children by removing these irritants from their environment. His books and articles sparked a lively debate and caused the beginnings of investigations by both the Nutrition Foundation and Senator Kennedy into the efficiency of Dr. Feingold's treatments (Nut. Found. report, 1975).

Dr. Feingold's hypothesis is being tested at this time. A study at the University of Pittsburgh was somewhat supportive but methodological problems, as well as a small sample size, limit the conclusions which may be drawn (IFT report, 1976). A study by the University of Wisconsin was more negative in its conclusions but still

recommended further testing of the hypothesis (Harley et al., 1977). In general, an Experts Panel on Food Safety and Nutrition of the Institute of Food Technologies concluded that Dr. Feingold's concept was poorly tested and vaguely controlled but that there was some possibility that some subset of the population of children who were diagnosed as hyperactive could benefit from dietary treatment and that further testing was warranted (IFT report, 1976).

Another author, Cott (1973) has suggested that food may play some part in the development of hyperkinesis. Cott theorizes that the molecular environment of the brain is out of balance for hyperactive children. His therapy is in two parts: first, a regimen of massive doses of selected vitamins (Table 2.2); second, he uses a low carbohydrate diet specifically excluding the simpler sugars (Cott, 1973, 1976). He reports both a reduction in hyperactivity with these treatments and an increase in the excretion of 5-hydroxyindolacetic acid after treatment in the urine of his patients (Cott, 1976).

Livingston, in his treatise on the management of epilepsy in children, also commented on hyperactivity. During treatment of their epilepsy with a high fat diet, he found that this regimen had a tranquilizing effect and that removal of the child from the high fat diet often caused appearance of the hyperactive symptoms (Livingston, 1974, pp. 451-455).

Table 2.2 Dr. Cott's Vitamin Therapy

Vitamin	Recommended Daily Dose (mg)	RDA (mg)
Niacin	1,000-2,000	13-18
Ascorbic acid	1,000-2,000	55-60
Pyridoxine	200-400	2
Calcium pantothenate	200-600	10*

Source: Cott, A. 1974. Treatment of Learning Disabilities, J. of Orthomolecular Psychiatry, 3:1-13.

*Not yet established.

Interactions of Monoamines and Activity

The dopaminergic and norepinephrinergic neurons in the brain are closely associated with activity. Bartholini and his co-workers (1969) have presented evidence that the dopaminergic system is involved in the locomotor stimulation processes. Parkinson's disease, a disease which strongly affects activity, has been shown to affect these dopaminergic neurons and cause a depleted level of dopamine in them (Hornykiewicz, 1971). One of the standard treatments, the use of L-dopa, may function at least in part by increasing the level of dopamine in the brain (Hornykiewicz, 1971). This treatment decreases the severity of the symptoms of the disease.

L-dopa has several effects of interest to this discussion. It increases activity, reverses the action of reserpine, a drug which

inhibits the reuptake of the catecholamines by the storage vesicles, and lowers the threshold to electroshock or some types of drug-induced convulsions (Hornkiewicz, 1971).

The beneficial effects of L-dopa occur even though the dopaminergic neurons have degenerated; the dopamine is apparently formed in other parts of the brain and diffused to the site of postsynaptic dopamine receptors (Pelton and Chase, 1975). The central catecholaminergic neurons mediate behavioral arousal and their destruction lessens an animal's response to unfamiliar stimuli (Smith, 1976).

One investigator examined the brain levels of the catecholamines in normal and "aggressive" mice (Valzelli, 1974). He found that there was no significant difference in the concentration of the dopamine and norepinephrine but that the turnover of dopamine was increased in the brain while that of norepinephrine was decreased. The turnover of serotonin in brain was also increased in his aggressive mice (Valzelli, 1974).

Rapaport and his colleagues (1970) have also reported that there is a correlation between the hyperactive symptoms of a hyperkinetic child and the excretions of norepinephrine. The greater the activity, the greater the excretion of norepinephrine. They also found a decrease in the urinary excretion of norepinephrine when the hyperactive symptoms were controlled with medication. Stewart (1971), however, disputes the significance of these findings.

Oral ingestion of amphetamines, which normally increase activity in adults, seem to function by affecting the reuptake of dopamine in the

brain and by triggering its release from storage vessicles (Hornykiewicz, 1971). The drug also lowers the norepinephrine content of many parts of the brain. The amphetamine appears to lower the norepinephrine to a greater extent if a rat is with other rats as opposed to isolated rats (Estler, 1975). The lowering of the dopamine content of the rat brain by which amphetamines was greatest in the cortex and midbrain (Estler, 1975).

Serotonin is also implicated in activity alterations. It is known that serotonin is involved in the control of sleep; blocking the production of serotonin surgically or by drugs leads to various depths of insomnia which are relieved by administration of 5-hydroxytryptophan (Mountcastle and Baldessarini, 1974).

Serotonin also depresses the activity of the caudate nucleus and thus alters dopamine metabolism in the brain (Mabry and Campbell, 1973). This is, to some extent, compensated for by the actions of norepinephrine (Antelman and Caggiula, 1977). The level of serotonin in the brain also affects the sensory awareness level of the brain (Schneider and Tarshis, 1975). Several reports indicate that some hyperactive children show decreased blood levels of serotonin (Coleman, 1971; Coleman et al., 1976). One study tested six hyperactive children, with no observable organic reason for their symptoms, for response of the blood serotonin levels to pharmacological doses of vitamin B₆, or methylphenidate, a standard drug used in the treatment of hyperkinesis, versus a placebo. Evaluations were done at regular intervals on a double-blind cross over study. Children showed the most alleviation

of their hyperactive symptoms on vitamin B₆ therapy. This correlated very well with increased blood levels of serotonin (Coleman et al., 1976). Methylphenidate, a common drug prescribed for treatment of hyperactivity, was less effective than the vitamin B₆ therapy in controlling hyperactive symptoms and showed no effect on blood serotonin levels.

Bloxam et al. (1977) studied the effects of tryptophan and portocaval anastomosis on activity and brain tryptophan metabolism using either interperitoneal injections of either a 0.9% saline solution or a tryptophan solution providing 20 mg/kg. The results indicated that increased levels of tryptophan decrease the activity of the rats as compared to the rats given saline. The increased tryptophan did not affect rearing, turning, headlifting, or grooming. Activity was measured by an open-field box.

Diet and Activity

There are several reports (Bloxam et al., 1977; Schemmel, 1967; Samuals et al., 1948) in the literature that indicate that dietary components may affect activity. Preliminary work for this thesis suggested that young, growing Sprague-Dawley rats lowered their activity levels as measured by an open-field test box after they had been fed a high fat diet for three weeks as compared to a high carbohydrate control diet. Osborne-Mendel rats when changed from a high carbohydrate diet to a high fat diet showed a decrease in the amount of norepinephrine excreted in the urine relative to the amount of dopamine excreted per day.

Macnab and his associates (1965), in a longer term trial, did not see any significant differences in activity among rats fed a high carbohydrate diet, a high fat diet, or a 50/50 mixture of the two. However, their fat level was not as high as the diets used here (53% vs. 63% of calories) and they used different methods of activity evaluation (activity wheels) to measure the activity.

Schemmel (1967) estimated the activity of six strains of rats fed either a high fat or a grain diet by observation of the animals three times a day, 9:00 a.m., 4:00 p.m., and 9:00 p.m. Male and female rats fed a high fat diet (60% w/w) were about half as active as those fed a grain ration.

Moskowitz (1959) studied running wheel activity as a function of food and water deprivation. He found that food deprivation sufficient to maintain a rat at some set percentage of normal body weight led to an increase in activity and that the increase in activity was inversely related to the decrease in the percentage of the normal body weight. These animals would probably be motivated by a search for food; food or water deprivation has been found to cause increases in animal search patterns (Samuels et al., 1948).

Joosten and van der Kroon (1974) have reported that the genetically obese mouse (ob/ob) shows decreased locomotor activity relative to non-obese littermates as measured by an open-field test box. This occurs before the obese mice begin to show changes in adipocytes and while they are still similar in size to the non-obese littermates.

Samuels and his co-workers studied the effects of previous diet on the ability of rats to do work during subsequent fasting. The three diets used were high fat, high carbohydrate, or high protein. They found that, during feeding, the animals fed the high fat diet were the least active but during fasting, they were the most active (Samuels et al., 1948).

Uhleman found that a high fat diet protected mice from both maximal electroshock and hydration threshold electroshock. The diet did not protect against threshold electroshock or pentylenetetrazol induced convulsions. Reversion to a control diet removed the protective effects within 24 hours (Uhleman and Neims, 1972).

Chadwick et al. (1977) report that there is a large body of experimental evidence which suggests that alterations in serotonin in the brain is associated with an alteration of seizure threshold in an inverse relationship.

Monoamines and Diet

Several nutrients have been shown to affect the formation of monoamines in the brain. Sourkes (1972) reviewed the effects of four nutrients involved in monoamine synthesis and catabolism as co-factors; pyridoxine, riboflavin, copper, and iron. Pyridoxine deficiency decreases the activity of aromatic L-amino acid decarboxylase. This is not followed by a concomitant decrease in any of the monoamines or their breakdown products probably because the enzyme is normally found in excess.

Riboflavin deficiency decreases slightly the norepinephrine content of the brain beyond that which can be accounted for by the loss of tissue weight in the deficient state.

Copper deficiency induced by chelating agents tends to decrease brain levels of norepinephrine. Also, it has been shown that the lack of norepinephrine leads to increased activity of tyrosine hydrolase by removal of feedback inhibition.

Iron deficiency has been reported to decrease monoamine oxidase activity in the liver. Its effects in brain tissue were not studied.

Pilecki and his group (1975) studied the effect of an atherogenic diet where they added cholesterol (2.5%) to a control diet and "essential" phospholipids on the content of catecholamines in rat brain and on the rat's activity. They report that the atherogenic diet decreased the activity as measured by an open field test box.

Serotonin in the brain has been shown, by numerous workers, to be affected by dietary factors such as tryptophan, carbohydrate and other large, neutral amino acids (Fernstrom, 1974; Fernstrom et al., 1974; Fernstrom and Jacoby, 1975; Gessa and Taglianolute, 1974; Moir, 1974; Wurtman, 1970). This is primarily because of two reasons. First, at the initial step in serotonin synthesis, the enzyme tryptophan hydrolase is not saturated with its amino acid substrate. Thus, increasing the level of substrate can increase either the level of serotonin, the level of 5-hydroxyindolacetic acid or both (Fernstrom et al., 1974). Second, the tryptophan is transported across the

blood-brain barrier by a mechanism which shows saturable kinetics and competitive inhibition from other large neutral amino acids. Thus, in serotonin synthesis, the concentration of the precursor, tryptophan, regulated the rate of synthesis (Gessa and Tagliamonte, 1974). This level is affected both by the protein in the diet and the carbohydrate. The protein in the diet provides a source not only of tryptophan but of competing amino acids, such as phenylalanine, tyrosine, and isoleucine, for the blood-brain barrier carrier.

Carbohydrate in the diet acts more indirectly by stimulating the flow of insulin. Insulin has been shown to decrease the blood levels of free tryptophan, increase the intracellular levels of free tryptophan, and have no effect on the levels of bound tryptophan in the blood (Parfitt and Grahame-Smith, 1974).

Summary

This review has covered material in three general areas. The general biochemistry of the monoamines was discussed. The hyperkinetic syndrome was presented with its history, diagnosis, and treatment. Last, a general overview of the interactions of the monoamines and activity, the effects of diet on activity, and the effects of diet on monoamines was presented.

CHAPTER III

PRELIMINARY STUDIES OF DIETARY EFFECTS OF FAT AND CARBOHYDRATE ON ACTIVITY AND CATECHOLAMINES IN THE OSBORNE-MENDEL AND SPRAGUE-DAWLEY RATS

Objective

The experiments that are discussed in this chapter were designed to provide preliminary data on the effects of carbohydrate or fat as the primary energy source on voluntary activity of rats.

Materials and Methods

Experiment I

For the experiment, 18 male Sprague-Dawley rats weighing approximately 200 grams each, were purchased from Spartan Animals, East Lansing, Michigan. Upon receipt of the rats, they were randomly assigned by weight to either one of two groups. After a three-day acclimatization period where the animals were fed the high sucrose control diet, one group of rats was fed the high fat diet while the other group was left on the control diet (Table 3.1).

The animals were fed the diets for three weeks, then were tested for their exploratory activity in an open-field test box. The open-field box consisted of a four by four feet sheet of plywood with sides 8 inches high painted white, ruled into six inch squares and then

Table 3.1 Composition of High Carbohydrate and High Fat Diets

Components	Diet	
	High Sucrose Control	High Fat
	----- Grams -----	
Crisco®	5	33
Casein (vitamin free)	21	21
Sucrose	67	5
Vitamin mix ¹	2	2
Mineral mix ²	5	5
% of calories as fat	11.33	74.06
% of calories as carbohydrate	88.66	22.41
Calories per gram of protein	18.90	19.10

¹Purchased from the Nutritional Biochemical Corporation, Cleveland, Ohio. The vitamin content is given below (in grams except as noted):

Vitamin A (200,000/gm)	4.50	Niacin	4.50
Vitamin D (400,000/gm)	0.25	Riboflavin . . .	1.00
Alpha tocopherol . . .	5.00	Pyridoxine HCL	1.00
Ascorbic acid	45.00	Thiamine HCL. .	1.00
Inositol	5.00	Ca Pantothenate	3.00
Choline chloride . . .	75.00	Biotin	20.00
Menadione	2.25	Folic acid . . .	90.00
PABA	5.00	Vitamin B-12 . .	1.35

²Wesson (1932) modified Osborne-Mendel mineral mix purchased from Teklad test diets, Madison, Wisconsin.

covered with a clear acrylic coat. Two tests were conducted, two days apart, between 8:00 and 10:00 p.m., during the animals' dark cycle. This was done to maximize their activity.

The test was conducted in a darkened room that was isolated from outside sounds. The animals to be tested was placed in one corner of the box with its nose into the corner. As the animal was released, the stopwatch was started. Each animal was allowed 180 seconds to explore the box. The time at which the animal reached each of the other three corners was noted. A score was assigned for each corner reached equal to the time required to reach that corner in seconds. A score of 180 was assigned for each corner that the rat did not reach. The box was cleaned rapidly between each animal with a cloth soaked in ethanol to obliterate any scent trails from the previous animals (Tables 3.2 and 3.3).

In the second experiment, 18 male Osborne-Mendel rats of about 100 grams weight, were assigned to one of two groups based on their activity as measured in the open-field test box. The animals in this experiment were housed in individual metabolism cages for the duration of the experiment. The rats were acclimated to these cages for three days and during this time, were fed the control diet. One group of rats was assigned to the control diet while the other group of rats was assigned to the high fat diet (for the composition of the diets, see Table 3.1). At one week (Period I), two weeks (Period II), and three weeks (Period III), a twenty-four hour urine collection was accomplished on each animal beginning just prior to the start of their dark cycle (7:00 p.m.).

Table 3.2 Time Required to Reach a Corner for Sprague-Dawley Rats on Two Different Diets (Trial One)

Corner	Control Diet N = 9	High Fat Diet N = 9
-----Time in Seconds-----		
1	15.4 ± 13.5 ¹	77.0 ± 70.8*
2	46.3 ± 56.5	140.1 ± 68.5*
3	101.9 ± 72.6	150.9 ± 57.3
Average	54.5 ± 63.0	122.7 ± 71.1*

Comparisons between rats fed the two diets from the same row are significant at:

*means differ at the $p < .025$ level.

¹mean ± standard deviation.

Table 3.3 Time Required to Reach a Corner for Sprague-Dawley Rats on Two Different Diets (Trial Two)

Corner	Control Diet N = 9	High Fat Diet N = 9
-----Time in Seconds-----		
1	37.8 ± 59.7 ¹	108.1 ± 83.81 ^a
2	71.3 ± 72.8	155.4 ± 48.61 ^b
3	109.4 ± 77.1	164.4 ± 29.2 ^a
Average	72.8 ± 73.5	142.6 ± 60.9

Comparisons between rats fed the two diets for the same row are significant at:

^ameans differ at the $p .05$ level.

^bmeans differ at the $p .01$ level.

^cmeans differ at the $p .005$ level.

¹mean ± standard deviation.

To protect the catecholamines in the urine, the collection bottles had 0.5 milliliter of 6 N hydrochloric acid added prior to the start of the collection. After the collection, the samples' volumes were determined and the samples were frozen. During the dark period immediately following the collection period, the animals were tested for their exploratory behavior in the 4 x 4' open-field test box. The tests were conducted in the first experiment above. At the end of the test period, after the last open-field test, the animals were sacrificed by ether anesthesia and the brains and the right and left adrenals were removed.

Chemical Analyses

Analysis of the catecholamines was accomplished by the method of Kissinger, as further modified by his group (Kissinger et al., 1975; Kissinger, 1976). The pH of the urine samples was adjusted with 3 N NaOH to pH 6.5. Five milliliters of the sample were added to ten milliliters of a phosphate buffer. The samples were centrifuged at 2,500 RPM for ten minutes to sediment any protein. The samples were then poured into a short gravity flow catecholamine column from Bio-rad Laboratories, Richmond, California. The column was washed with ten milliliters of distilled water. One milliliter of 0.7 N H_2SO_4 was added to the column to lower the pH and free the catecholamines from the column. The catecholamines were then eluted into five milliliter vials with four milliliters of 2 N ammonium sulfate. The vials contained approximately 70 to 80 milligrams acid-washed alumina, 0.1

milliliters 5% thioglycollic acid, and 0.5 milliliter of 3 m TRIS. The vials were capped and the samples were mechanically agitated for ten minutes. The eluent was removed from the vials with suction, and the vials were washed twice with distilled water.

One-half milliliter of 1.0 N acetic acid was added to each vial to elute the catecholamines from the alumina. If the samples were allowed to sit at this point for at least half an hour, the recovery of the catecholamines was improved. Recovery was approximately 45%. The samples were stable in the refrigerator for about one week. The samples were injected into a high-performance liquid chromatography unit with an electrochemical detection unit by Bioanalytical Systems, Inc. The machine has a constant volume injection loop. Standard solutions of the catecholamines were made up fresh every two weeks to contain 200 nanograms of each of the catecholamines. Analysis of the samples was made by peak height comparison to the standards (Kissinger et al., 1975).

The brain and adrenal samples were homogenized in five milliliters of 0.1 N HCl. The samples were treated as the urine samples above.

Results and Discussion

The results of the activity measurements are presented in Table 3.4. In none of the trials did the apparent differences between the two groups reach statistical significance. Possible reasons for this are discussed below. Table 3.5 details the weight gain pattern

Table 3.4 Time Required to Reach a Corner for Osborne-Mendel Rats on Two Different Diets (Trial Three)

Corner	Control Diet N = 6	High Fat Diet N = 6
	----- Time in Seconds-----	
1	63.2 ± 57.0 ¹	64.0 ± 52.3
2	89.1 ± 44.7	92.8 ± 68.5
3	130.1 ± 47.7	139.6 ± 40.9
Average		

Note: There were no significant differences between the rats on either of the two diets.

¹Mean ± standard deviation.

Table 3.5 Average Weight Gain on Rats Fed High Carbohydrate or High Fat Diets (Osborne-Mendel Rats)

	Weight Gain	
	Control Diet N = 6	High Fat Diet N = 6
	-----Grams-----	
Initial weight	153 ± 31 ¹	147 ± 24
Final weight (week three)	271 ± 70	248 ± 52
Weight gain	118 ± 42	102 ± 43

¹Mean ± standard deviation.

for the two groups. Over a three-week period there was no significant difference in weight gain between the two groups of rats. The dopamine contents of the brain was 1.33 and 1.34 for the control and high fat diets respectively (Table 3.6). No statistically significant differences were noted in either the brain or adrenal content of the catecholamines. No significant difference in the urinary excretion of the catecholamines was noted either. However, there was a significant difference in the change in the excretion of the catecholamines. Table 3.7 shows the range in the catecholamines in the urine from week one to week three.

Table 3.6 Catecholamine Contents of the Organ (Osborne-Mendel Rats)

Organ	Amounts of Catecholamines	
	Control Diet N = 6	High Fat Diet N = 6
-- Micrograms/Gram Wet Weight --		
Brain		
Dopamine	1.33 ± 0.12 ¹	1.34 ± 0.13
Norepinephrine	0.50 ± 0.01	0.55 ± 0.13
Adrenals		
Norepinephrine	0.43 ± 0.06	0.56 ± 0.38

¹Mean ± standard deviation.

Table 3.7 Change in Catecholamine Excretion From Period One to Period Three (Osborne-Mendel Rats)

Catecholamine	Change in Catecholamine	
	Control Diet N = 6	High Fat Diet N = 6
	----- Microgram/Day -----	
Dopamine	+0.19	+0.36
Norepinephrine	-0.27	+0.54 ^a

^aComparison between diets is significant at the $p < .02$ level.

These experiments suggest that if the dietary energy source is primarily fat, the activity of the rats was reduced compared to controls fed high carbohydrate diets, and that the change in the activity may be reflected in the urinary catecholamine patterns. The excretion of norepinephrine in urine was increased in conjunction with feeding a high fat diet. In the first experiment, the reductions in activity with high fat feeding were statistically significant, while in the second experiment, there were no significant differences at week one, week two, or week three. Two points must be noted. First, the standard deviations for the activity measurements are very large, in some cases exceeding the means for the measurements. Second, in the latter experiment, the level of norepinephrine excreted in the urine declined in the rats on the high sucrose diet, although the measured activity did not change. Stewart (1971) has noted that the

urinary content of norepinephrine varies inversely with the activity of the levels of the animal tested.

It can be argued that the activity as measured by the open-field test box is not an adequate measure of the voluntary activity of the animal, at least for the strain of rat examined. It should be noted that many different methods have been used to study activity and that there is some evidence that different types of activity may be controlled by different centers in the brain. The problem of measuring activity will be discussed further in Chapter Five.

Conclusions

It appears that increasing the level of fat in the diet of the Sprague-Dawley rat decreases its exploratory behavior as measured in the open-field test box. It appears that in the Osborne-Mendel rats, the activity levels may be reduced by feeding a high fat diet as suggested by the increase in the urinary excretion of norepinephrine. The exploratory behavior may not be affected but need not reflect voluntary activity. There is a large variation among rats, even within the same experimental dietary group, in their exploratory behavior patterns.

CHAPTER IV

STUDIES OF DIETARY EFFECTS OF FAT AND CARBOHYDRATES ON ACTIVITY IN THE MONGOLIAN GERBIL AND DOMESTIC PIG

Objective

The experiments described in this chapter were designed to supplement the work done with the different rat strains as described in Chapters Three and Five. This work examined the effects of dietary energy source on the voluntary activity of the Mongolian gerbil and the domestic pig.

Part A--Gerbil

Materials and Methods

Activity Measurements

Activity measurements for the gerbils were accomplished with a radio-field activity box, Columbus Instruments, Columbus, Ohio. This device works by generating a weak radio field. The gerbils, during the measurement of their activity, were housed six to a cage in plastic cages seated on the radio generator. As the gerbils moved through the radio field, they altered the capacitance of the field. A detection device senses the changes in capacitance. It registers these changes as a digital count. The strength and duration of the capacitance change

that will activate the detector are adjustable. Conditions within the cages, depth of bedding, number and placement of food cups, etc., were kept constant. The limitations of this method of measuring activity will be discussed in the Discussion Section of this chapter. Twelve four-week old gerbils were assigned, upon receipt, to one of two dietary groups. The six gerbils in group two were fed barley for three days while being acclimated to their cage, then were fed sunflower kernels for either one or five days (Table 4.1).

Table 4.1 Sources of Energy in Male Gerbil Diets

Component	Barley	Sunflower Seed Kernels
	----- G/100 Gm -----	
Protein	8.21	24.00
Carbohydrate	78.89	19.90
Fat	1.07	47.30
Cal/gram of protein	43.61	23.33

Results and Discussion

The significance of the activity and the logarithm of the activity between gerbils fed barley and those fed sunflower kernels were tested with the SPSS statistical program at Michigan State University Computer Center (Nie et al., 1975). Mathematical transforms of the raw activity data were recognized as valid if they increased the normality of the distribution of the data (Wolterink, 1978).

Both activity levels and their log transform were tested with a nonparametric analysis for the normality of their distribution. The means and standard deviation for the average activity per hour is in Table 4.2. The Kolmogorov-Smirnov test for goodness of fit was used (Nie et al., 1975). The results of this test for the activity levels and their log transform are given in Table 4.3. Both the activity levels and the log transforms were then analyzed for the effects of diet and length of time on the diet with MANOVA analysis of variance package (Nie et al., 1975). The F table for the log transform of the activity level is given in Table 4.4.

Table 4.2 Activity Levels for Male Gerbils Fed Two Diets

Days on Diet	Activity	
	Barley Diet	Sunflower Seed Diet
	-----Counts/Hour-----	
1	2,826.83 ± 2,280.93 ^a	1,939.09 ± 2,052.74 ^a
2	2,996.13 ± 1,985.82 ^a	2,539.13 ± 2,075.22 ^{a,b}
3	2,611.43 ± 1,870.90 ^a	3,226.87 ± 2,067.75 ^b
4	3,465.00 ± 2,255.42 ^a	4,242.00 ± 2,476.54 ^c
5	2,768.87 ± 1,754.90 ^a	4,189.17 ± 2,707.66 ^c

Note: There were no significant differences between diets. Different superscripts within a column differ at the $p < .05$ level.

Table 4.3 Evaluation of Activity in Gerbils Kolmogorov-Smirnov Test for Goodness of Fit

Activity Measure	Mean	Standard Deviation	K-S Z
Activity levels	3084.45	2235.82	1.7852
Log of activity	7.6169	1.3482	2.4571

Note: The distribution approaches normality as the K-S Z statistic approaches zero.

Table 4.4 Analysis of Variance for the Log Transform of the Activity Level

Source of Variation	DF	MS	F	Significance of F
Within cells	220	1.7985	--	--
Diet	1	0.1122	0.0624	.80299
Time on diet	4	3.2440	1.8038	.12910
Diet by time on diet	4	1.8737	1.0418	.38648

The results of the analysis of variance for the activity levels are tabulated in Table 4.5. It appears that for the gerbil, the activity level is a better measure than the log of the activity as indicated by the K-S goodness of fit test. The contrast of the cells via a Tukey confidence interval is given in Table 4.2.

Table 4.5 Analysis of Variance of the Activity Levels

Source of Variation	DF	MS	F	Significance of F
Within cells	220	4707355.8	--	--
Diet	1	5230347.2	1.1111	.29300
Time of diet	4	15648095.5	3.3242	.11430
Diet by time on diet	4	10329027.3	2.1941	.07060

Activity measurements are a difficult task because of the uncertainty of what is actually being measured. One measure of activity, the open-field test box, indicated activity levels were higher in SD rats fed high carbohydrate diets than in the same rats fed high fat diets but was not observed in Osborne-Mendel rats. However, in the latter strain of rats, there was biochemical evidence from the urinary norepinephrine which indicated that there may have been differences in activity levels which were not reflected in the open-field test results. The radio-field test apparatus was selected for the studies reported in this and the following chapter because it appeared to offer a better measure of the actual voluntary activity of the animal. This device had several limitations. Any metal, such as the cage top, will alter the radio-field geometry and cause the machine to 'see' a given level of movement as being larger or smaller depending on where the movement occurs in the cage. Also, such things as the depth of bedding and the number of food cups can affect the readings. The device attempted to convert an analog function into

a digital representation. That is, movement tended to be more or less continuous once it is nonzero, but the machine converted it into a discontinuous, digital form. An analogous situation to this would be attempting to measure the kidney's output of urine by monitoring the bladder's output of urine. Over a long time span, the total volume would be measured correctly but smaller variations on a short-time scale would be lost. Also, during the course of the measurements, the electronic components shifted in their response envelope so that a given level of movement was recorded as larger or smaller than previously. That happened with the machines that were being used in these experiments. When the response curves shifted, they did not do so in a linear fashion. This increased the difficulty in comparing the activity recordings for different groups. This, in turn, caused the loss of two records in this experiment. The records which were available did show some effect as was shown in Table 4.4.

Conclusion

As can be seen in Table 4.2, there was no effect on activity from diet one. On diet two, the sunflower seeds, the animals were initially less active and gradually increased their activity levels throughout the experimental period. It thus appeared that for the gerbil, activity was affected by the diet. It may be that the change in diet was responsible for the observed differences rather than the differences in dietary composition. This experiment cannot distinguish between those two possibilities.

Part B--Domestic Pig

Materials and Methods

In this experiment, three different groups of domestic pigs from a crossbred herd of Yorkshire-Hampshire pigs, maintained at the Michigan State University Swine Research Station, were tested for the effects of dietary energy source on their voluntary activity. The first group of eight pigs were housed in stainless steel pens, two pigs to a 6' x 3' pen. The pigs were assigned to one of four pens by weight so that the total weight of the two pigs in each pen was similar to the total weight of both pigs in each of the other three pens. After an initial period of four days in the pens during which time the pigs were fed a semipurified control ration (Table 4.6). the activity levels of all pigs were measured over a twenty-four hour period with a commercial pedometer (K. R. Pedometer, MC Sporting Goods, Lansing, Michigan). The pedometer was taped to the hind leg of each pig. At this time, the pedometer was removed and the pig was bled. Bleeding was accomplished by placing the pig on its back in a wooden "v" shaped trough and inserting a needle to one side of the upper end of the sternum into the vena cava. This procedure allowed for the rapid collection of up to 10 milliliters of blood without harming the animal. Blood was frozen and saved for analysis of catecholamines. Based on an initial twenty-four hour pedometer reading, the pens were assigned to either one of two diets. The composition of these rations was recorded in Table 4.6. The activity measurements and bleedings were repeated three

Table 4.6 Composition of Semipurified Rations Used for the First Study of Activity in Young Pigs

Component	Rations	
	Control Diet	High Fat Diet
	----- Grams-----	
Casein ¹	1,000	1,000
Crisco ® ²	250	1,040
Alpha cellulose	250	250
Corn starch, ²	3,150	1,650
Mineral mix ²	300	300
Copper mix ²	50	50
Corn oil ²	50	50
Fat soluble vitamin mix ³	50	50
Water soluble vitamin mix ³	100	100
% calories as fat	14.0	48.1
% calories as carbohydrate	65.3	32.3
Calories per gram of protein	19.3	20.4

¹Teklad test diets, Madison, Wisconsin.

²Purchased from the Michigan State University food stores.

³See Miller et al., 1964.

more times at four-day intervals. A second (with different pigs) experiment resembled the first experiment except for two modifications. The ration was changed to a grain type which resembles more closely the usual ration of pigs (Table 4.7). Second, the pigs were housed in two larger 10' x 10' pens. This was done to minimize the problems that had been encountered in the first experiment with animals jumping out of their pens. It was felt that with increased numbers of animals and a larger area, the pigs would have less incentive to jump out of their pens. The pigs were again tested for activity and bled as before. The experiment was repeated a third time, with a third group of eight pigs.

Results and Discussion

The activity levels for the first experiment (Table 4.8) were significantly reduced by 60% when pigs were changed from control to high fat diets. The variances tended to be large in relation to the means. The results of the first measurement in experiment one were lost when the animals managed to remove the pedometers in five of the eight cases. Activity for pigs as measured in artificial units ranged from 2.31 to 4.30 for controls and 2.41 to 3.39 for those fed high fat. No significance was found (Table 4.9). The results of the last set of trials is found in Table 4.10. Table 4.11 records the weight gains for the second group and Table 4.12 records the weight gains and feed efficiencies for the last group.

Table 4.7 Composition of Grain Rations for Pig Studies

Component	Rations	
	Control Diet	High Fat Diet
	----- Pounds -----	
Ground shell corn	71.15	0.0
Corn starch	0.0	7.10
Dehulled soybean meal	25.00	37.62
Defluorinated phosphate	1.50	1.50
Calcium carbonate	0.60	0.60
Salt	0.50	0.50
MSU vitamin and mineral mix ¹	0.50	0.75
Vitamin E and selenium premix ¹	0.50	0.50
Antibiotic premix ¹	0.25	0.25
Alpha-cellulose	0.0	1.40
Crisco® ²	0.0	12.06
% calories as fat	5.30	48.10
% calories as carbohydrate	73.50	30.70
calories per gram of protein	22.60	23.00

¹See Nutrition: Swine Feeds and Feeding, Extension Bulletin 537, Michigan State University, September 1975.

²Purchased from the Michigan State University food stores.

Table 4.8 Activity of Growing Pigs Fed High Fat or High Carbohydrate Rations Measured with Pedometers¹ (Four Pigs per Group)--Pig Experiment One

Trial	Activity	
	High Carbohydrate Diet	High Fat Diet
One	4.75 ± 1.32^a ³	4.42 ± 0.63^a
Three ²	1.83 ± 0.34^b	4.24 ± 1.09^a
Four	1.58 ± 0.52^b	4.25 ± 1.30^a

Note: Comparisons between pigs fed high fat and high carbohydrate rations were statistically significant ($p < 0.025$) if they have different superscript letters. If they have the same superscript letter, they are not significantly different.

¹The pedometers are designed for a human stride. The pig's stride is much shorter. Therefore, the actual 'milage' registered has no meaning except as a comparative measure of the movement of the pigs.

²After trial one, Group II was fed the high fat ration (Table 4.6).

³Mean \pm standard deviation.

Table 4.9 Activity of Growing Pigs Fed High Fat or High Carbohydrate Grain Rations Measured with Pedometers (Five Pigs per Group)--Pig Experiment Two

Trial	Activity ¹	
	High Carbohydrate Diet	High Fat Diet
One	3.80 ± 0.83 ³	3.39 ± 1.57
Two ²	2.31 ± 2.16	2.41 ± 1.12
Three	3.88 ± 0.85	2.42 ± 1.18
Four	4.30 ± 2.31	2.46 ± 1.20

¹See Table 4.8.

²After trial one, the pigs in Group II were fed a high fat ration (Table 4.7).

³Mean ± standard deviation.

Table 4.10 Activity of Growing Pigs Fed High Fat or High Carbohydrate Grain Rations Measured with Pedometers (Four Pigs per Group)--Pig Experiment Three

Trial	Activity ¹	
	High Carbohydrate Diet	High Fat Diet
One	2.08 ± 5.97 ³	3.64 ± 3.47
Two ²	2.50 ± 5.84	1.00 ± 2.92
Three	0.35 ± 0.11	3.34 ± 3.54
Four	2.78 ± 4.65	3.31 ± 0.68
Five	1.88 ± 2.92	1.59 ± 1.30

¹See Table 4.8.

²After trial one, the pigs in Group II were fed a high fat ration (Table 4.7).

³Mean ± standard deviation.

Table 4.11 Body Weights of Pigs Used in Pig Experiment Two (Five Pigs per Group)

Trial	Body Weight (Kg)	
	Group I	Group II
One	10.73 ± 1.98 ²	9.93 ± 1.59
Two ¹	11.60 ± 3.03	10.93 ± 1.84
Three	13.73 ± 4.02	13.00 ± 2.21
Four	15.30 ± 5.43	14.40 ± 2.38

¹After trial one, Group II was placed on a high fat ration (Table 4.7).

²Mean ± standard deviation.

Table 4.12 Body Weights Used in Pig Experiment Three (Four Pigs per Group)

Trial	Body Weight (Kg)	
	Group I	Group II
One	14.12 ± 1.94 ²	13.87 ± 0.98
Three ¹	25.29 ± 3.03	24.15 ± 1.75
Four	28.07 ± 3.16	28.46 ± 1.37
Five	30.84 ± 3.57	29.71 ± 1.36
Weight gain efficiency in lb gain/lb food	0.34	0.32

¹After trial one, Group II was placed on a high fat ration (Table 4.7).

²Mean ± standard deviation.

In Experiment 1, the data suggest that the diet which was higher in fat did indeed decrease the activity of the pigs tested, at least as measured by the pedometers. In the second experiment, the new high fat ration would not feed down through the throat of the feeder; even with two people monitoring the feeder to push the feed through the throat. The pigs in this group were often without food for periods of up to four hours during the day and up to eight hours at night. Presumably this caused the pigs to be more active as they were continually searching for food. Moskowitz (1959) has reported that food deprivation increases activity. Even when the pigs had food, they spent more time at the food trough and in fighting with each other for a place at the trough than the pigs fed the usual grain ration.

In the last experiment, the feed problem was solved by the use of a different feeder. The pigs did not show any difference in their activity levels as had the first group. This may be due to several reasons. Perhaps most likely was the possibility that, for some reason, the semipurified diet had a greater effect on the animals than does the grain type of ration. Another possibility related to the fact that these animals were larger and older than the animals in the first experiment. Pigs tend to decrease their activity as they grow older if they are housed inside, as these were (Miller, 1978). When the activity data for these latter pigs were examined, it appeared that the pigs varied greatly in their activity both between animals and in the same animal from one period to another. The data indicated that the pigs tended to be quiescent with occasional outbursts of activity,

perhaps related to fighting or some challenge. Under these circumstances, it would be less likely that there would be a discernible effect from the increased levels of fat in the diet. It was also apparent that the high fat diet was less efficient in terms of weight gain.

Conclusions

It appeared that there may be an effect of dietary energy source on the volitional activity for both the younger pig and for the gerbil. The effect for the gerbil may only be an effect of changing the diet. In the pig, the decrease in activity observed seemed to be confined to the younger pigs, at least in the pen environment. More work should be done in both of these species to clarify the exact nature of the effects and to see whether they can be duplicated.

CHAPTER V

STUDIES OF DIETARY EFFECTS ON FAT AND CARBOHYDRATE ON ACTIVITY AND MONOAMINES IN THE OSBORNE-MENDEL AND S5B/PL RATS

Introduction

In this experiment, two strains of rat, Osborne-Mendel and S5B/PL, were tested for the effects of the dietary energy source, fat or carbohydrate, on their volitional activity. The catecholamines, dopamine and norepinephrine, have been implicated in the neural control of locomotion (Bartholini et al., 1969; Stewart, 1971). These were examined in the brain and the urine of the rats. Serotonin has also been suggested as a neurotransmitter which may vary inversely with activity (Coleman et al, 1976; Valzelli, 1974). The serotonin content of the brain and urine were determined. Fernstrom et al. (1974) have found that the concentration of tryptophan directly affects the brain concentration of serotonin. Tryptophan levels in the blood were determined.

Materials and Methods

Osborne-Mendel and S5B/PL rats were used in these experiments. The Osborne-Mendel rats were obtained from a colony of rats maintained at Michigan State University and offspring from NIH stock. These

Table 5.1 Composition of High Carbohydrate and High Fat Diets

Component	Weights			
	R-1	R-2	R-3	R-4
	-----Grams-----			
Lact albumin ¹	20.0	20.0	20.0	20.0
Vitamin mix ²	2.0	2.0	2.0	2.0
Mineral mix ²	5.0	5.0	5.0	5.0
Sucrose ³	58.0	10.0	0.0	0.0
Corn starch ³	10.0	58.0	10.0	10.0
Corn oil ³	5.0	5.0	5.0	20.0
Hydrogenated vegetable oil ³	0.0	0.0	25.8	10.8
% of calories as fat	11.33	11.33	69.79	69.79
% of calories as carbohydrates	88.66	88.66	30.21	30.21
Calories per gram of protein	19.85	19.85	19.86	19.86

¹Teklad diets, Madison, Wisconsin.

²The vitamin and mineral mix are the same as those used in Chapter Three.

³Purchased at the MSU food stores.

animals were relatively disease free and came from a colony which had been maintained in the Department of Food Science and Human Nutrition for over fifteen generations. The second animal strain to be used was the S5B/PL rat. This rat was developed as a cross between the Sprague-Dawley and NIH Black rat by Poily at DHEW (Harris et al., 1977). It is highly resistant to obesity when fed a high fat diet ad lib, whereas the Osborne-Mendel will become obese if fed a diet which is high in fat (Thiel et al., 1972).

The S5B/PL rat was obtained by the establishment of a breeding colony from male and female rats from a breeding colony previously maintained at the Department of Food Science and Human Nutrition, Michigan State University, for at least fifteen generations.

The basic experimental diets were given in Table 5.1. One of four diets was fed to the rats for periods of one, three, or five days. The experimental period was preceded by a three-day acclimatization period during which the rats were maintained on diet R-1. The four diets and their compositions are given in Table 5.1. Four diets were thus fed for a period of either one, three, or five days for a total of twelve blocks. The experiment was repeated four times with a urine collection study and an activity study for each strain of rats. This gave a grand total of 48 blocks of six rats each for the study. Each strain of rats had 24 blocks of six rats in each block. Because the animals were being drawn from a small breeding colony, the blocks were run individually as soon as there were six males of either strain of approximately the same weight (100 grams). In the activity studies,

the animals were housed in plastic cages, six rats to a cage. In the urine collection studies, the animals were housed individually in metabolism cages.

Urine samples were collected during the last twenty-four hours of the experimental period for the groups in the metabolism cages. After each group of six rats had been fed the diet for the duration of the experimental period, the rats were sacrificed with ether. The brain, tongue, heart, two adrenals, two kidneys, and liver were removed, weighed, and frozen on dry ice. These samples were collected by severing the vena cava above the diaphragm after opening up the chest cavity. Blood was removed from the lung cavity and stored in capped tubes until it had clotted. The serum was then frozen. An additional blood sample was taken by removing 20 microliters of blood with a micro pipette with disposable tips and spotting it on a circle of coarse filter paper.

Analysis of the tissues and the urine for norepinephrine and dopamine was done by the method previously detailed in Chapter Three. The samples were assayed for serotonin by the method of Welch and Welch (1969) with the following modifications. It was determined that the serotonin in the samples, which had been prepared according to the method of Kissinger et al. (1975), migrated with the catecholamines as far as the step where the catecholamines had been eluted from the Bio-rad column into vials containing alumina to which the catecholamines were then absorbed. At this point, the serotonin was present in the supernatant but did not absorb into the alumina. Thus, after the vials had been shaken for the prescribed

length of time, the eluent was removed and frozen. Injection of this eluent on the high pressure liquid chromatograph gave a single peak at the same point as an injected standard. The sample was analyzed by mixing 0.5 milliliters of the sample with 0.5 milliliters of a 6 N solution of hydrochloric acid immediately prior to reading the sample on a fluorometer. The excitation wavelength was 295 nm and the emission wavelength was 535 nm. The amount of serotonin in the sample was then determined by comparing the reading to a standard curve prepared by processing 5 milliliters of several dilutions of a standard solution of reagent grade serotonin (Sigma). Since the solutions had been processed in the same way as the standards, no correction factor was required in this determination. Analysis of a standard solution which was not treated as a sample indicated that the recovery was approximately 40%.

Tryptophan was analyzed by the method of Wapnir and Stevenson (1969). The dried spots of blood were cut out of the rest of the filter paper, cut into quarters, and placed in a small test tube. One milliliter of 78% ethanol was added to each tube and the samples were allowed to incubate for one hour. Five-tenths milliliters of the alcohol-tryptophan eluent was transferred to a clean test tube. Immediately prior to reading the solution on the fluorometer, 3.0 milliliters of a 0.02 molar solution of TRIS base were added. The solution was mixed and transferred to a standard cuvette for reading. A standard curve was prepared by making up four dilutions of a standard solution and spotting 20 microliters of the solution into coarse filter

paper and treating them as the samples had been treated. A blank was prepared using distilled water. Adding twenty microliters of the standard solution directly to the ethanol and treating it like a sample indicated that the method recovered 85% of the tryptophan.

Hydroxyindole acetic acid was assayed by the method of Welch and Welch (1969). The wash from the first part of the catecholamine assay, where the sample had been applied to the Bio-rad column, was frozen. Conversation with people at Bioanalytical Systems, West Lafayette, Indiana, had indicated that this is the point at which the 5-HIAA eluted from the column. The indication has been made that the compound was stable when frozen. Attempts at analysis showed that the compound was decayed. Attempts to locate the point at which the compound had begun to decay established that the compound had indeed been washed through the column, but had decayed at the pH of the solution.

Results and Discussion

It appears that, from the data in Table 5.2, there is indeed an effect of dietary energy source on activity in the rat strains that were used in these experiments. The activity measurements were those obtained from the combination of the results from both strains of the rats. This was done because the devices used to measure the activity had failed for a number of the blocks. Analysis of variance for the effect of rat strain showed that there was no statistically significant difference between the two strains ($F = 1.0825$, $\text{Sig} = .3043$). It appears

Table 5.2 Log Transformations of the Voluntary Activity of Two Strains of Rats Fed High Fat or High Carbohydrate Diets

The Time Fed Diet	Activity			
	R-1 ¹	R-2	R-3	R-4
----- Average Counts per Hour -----				
Day one	6.5706 ² a1.7651 ^a n = 138	6.6472 a1.5645 ^a n = 92	6.5696 a1.7046 ^a n = 92	7.2641 b1.4052 ^{ab} n = 69
Day two	6.1305 a1.6816 ^{ab} n = 92	6.4364 ab1.5007 ^a n = 46	6.8279 bc1.5899 ^a n = 69	6.9930 c1.4097 ^a n = 46
Day three	5.7829 a1.7927 ^{bc} n = 9]	6.4573 b1.5282 ^a n = 46	6.8086 b1.5849 ^a n = 69	6.7994 b1.4329 ^a n = 46
Day four	5.4724 a2.1235 ^c n = 46	6.6094 b1.5795 ^a n = 23	6.4999 b1.5706 ^a n = 23	7.9493 c0.7643 ^b n = 23
Day five	5.4471 a2.0943 ^c n = 46	6.5026 b1.8244 ^a n = 23	6.2810 b1.8135 ^a n = 23	-- --

Different superscripts to the right of the SD indicate differences at the $p < .05$ level between different days on the same diet.

Different superscripts to the left of the SD indicate differences at the $p < .05$ level between different diets on the same day.

¹See Table 5.1 for the dietary composition.

²Mean \pm standard deviation.

Note: Because of the uneven n's in all of these tables, the s(D) for the Tukey (Cress, 1978) interval were calculated with the following formula:

$$s(D) = \sqrt{MSE (n_1 + n_2 / n_1 n_2)}$$

Table 5.3 Kolmogorov-Smirnov Goodness-of-Fit Statistics

Measurement	Mean	Standard Deviation	K-S Z
Activity levels	1607.38	1631.07	5.3992
Log transform of the activity levels	6.4937	1.7242	4.5634

Note: The distribution approaches normality as the K-S Z test statistic approaches zero.

Table 5.4 Analysis of Variance for the Log Transform of the Activity Levels

Source of Variation	DF	Mean Square	F	Significance of F
Error term	1085	2.76215	--	--
Diet	3	57.66284	20.8760	.00001
Time on a diet	4	9.46385	3.42626	.00859
Diet by time on a diet	11	6.47288	2.34342	.00753

that the rats were less active on the diet which was high in sucrose, R-1. It also appears that the rats fed diet R-1 decreased their activity with increasing time on the diet. Diets R-2 and R-3 seemed to have the same effect on the animals with no changes in the activity over the course of the experiment. The rats fed diet R-4 were more active than the animals on any other diet, gradually lowered their activity levels to the norm, and then increased them.

Urinary values of serotonin did not differ significantly from period to period (Tables 5.5 and 5.6). There was, however, an effect within one period, period one. The levels of serotonin in the urine of the animals on diet R-3 were significantly higher than those on diet R-1. Diet R-2 animals gave results that were intermediate between these two.

Urinary values of dopamine also varied within a period (Tables 5.7 and 5.8). During period three, the animals on diet R-3 were significantly higher in their levels of dopamine excretion than the animals on any of the other diets.

The urinary values for norepinephrine showed the same pattern as the ones for dopamine (Tables 5.9 and 5.10). The rats fed on diet R-3 secreted significantly higher amounts of norepinephrine than the animals on any other diet.

The brain levels of serotonin showed an effect both for the period of time on the diet and for the diet within each period (Tables 5.11 and 5.12). Diets R-1, R-2, and R-4 did not show any differences during the experiment within the diets. Diet R-3 showed

Table 5.5 Serotonin in Urine for Two Strains of Rats Fed High Fat or High Carbohydrate Diets

Sample Day	Serotonin			
	R-1 ¹	R-2	R-3	R-4
----- Micrograms per Twenty-Four Hour Period -----				
Day one	3,468.95 ² 2,885.52 ^a n = 11	4,349.98 2,379.05 ^{ab} n = 11	5,598.18 1,818.44 ^b n = 12	-- --
Day three	5,882.57 1,122.44 ^a n = 7	-- --	-- --	7,107.94 3,459.40 ^a n = 11
Day five	5,302.10 1,185.73 ^a n = 10	4,699.13 2,487.89 ^a n = 8	5,215.34 789.15 ^a n = 3	6,143.31 2,516.41 ^a n = 11

Different superscript letters indicate differences at the $p < .05$ level between diets for a day.

¹Dietary composition is in Table 5.1.

²Mean \pm standard deviation.

Table 5.6 Analysis of Variance for the Urinary Serotonin Content

Source of Variation	DF	Mean Square	F	Significance of F
Within cells	75	5653581.18	--	--
Diet	3	20274390.02	3.58611	.01758
Day	2	13090093.09	2.31536	.10575
Diet by day	3	330624.62	0.58479	.62684

Table 5.7 Mean Dopamine Excreted Over Twenty-Four Hours per Two Strains of Rats Fed High Carbohydrate or High Fat Diets

Sample Day	Dopamine			
	R-1 ¹	R-2	R-3	R-4
	----- Nanograms per Twenty-Four Hour Period-----			
Day one	298.85 ² 259.51 ^a n = 11	404.71 168.04 ^a n = 11	398.51 154.77 ^a n = 12	-- --
Day three	322.46 72.09 ^a n = 7	-- --	-- --	239.05 101.06 ^a n = 11
Day five	365.99 123.82 ^a n = 10	299.15 125.51 ^a n = 8	829.47 240.86 ^b n = 3	388.07 175.97 ^a n = 11

Different superscript letters indicate differences at the $p < .05$ level between diets for a day.

¹Dietary composition is found in Table 5.1.

²Mean \pm standard deviation.

Table 5.8 Analysis of Variance for the Urinary Dopamine Content

Source of Variation	DF	Mean Square	F	Significance of F
Within cells	75	27020.49270	--	--
Diet	3	102694.25298	3.80061	.01357
Day	2	80775.30601	2.98941	.05635
Diet by day	3	160640.80684	5.94515	.00108

Table 5.9 Mean Norepinephrine Excreted in Twenty-Four Hour Period for Two Strains of Rats Fed High Fat or High Carbohydrate Diets

Sample Day	Norepinephrine			
	R-1 ¹	R-2	R-3	R-4
	----- Nanograms per Twenty-Four Hour Period -----			
Day one	99.00 ²	110.12	126.91	--
	101.45 ^a	60.59 ^a	47.43 ^a	--
	n = 11	n = 11	n = 12	
Day three	97.96	--	--	74.90
	44.64 ^a	--	--	44.29 ^a
	n = 7			n = 11
Day five	86.27	75.86	244.88	119.66
	47.38 ^a	24.02 ^a	101.25 ^b	64.85 ^a
	n = 10	n = 8	n = 3	n = 11

Different superscript letters indicate differences at the $p < .05$ level between diets for a day.

¹Dietary composition is in Table 5.1

²Mean \pm standard deviation.

Table 5.10 Analysis of Variance for the Urinary Content of Norepinephrine

Source of Variation	DF	Mean Square	F	Significance of F
Within cells	75	3770.1598	--	--
Diet	3	12422.1770	3.2949	.02502
Period	2	2874.4460	0.76242	.47012
Diet by period	3	15029.9472	3.98655	.01084

Table 5.11 Mean Total of Serotonin in the Brains of Two Rat Strains Fed High Carbohydrate or High Fat Diets for Three Different Lengths of Time on the Diets

Sample Period	Serotonin			
	R-1 ¹	R-2	R-3	R-4
	-----Milligrams per Gram Wet Weight-----			
Day one	710.05 ² a665.88 ^a n = 9	999.47 a1,043.74 ^a n = 9	1,748.63 a819.97 ^b n = 8	850.74 a421.49 ^a n = 5
Day three	484.81 a567.85 ^a n = 10	620.75 a413.61 ^a n = 4	308.49 a360.49 ^a n = 4	500.08 a350.32 ^a n = 3
Day five	548.69 a330.42 ^a n = 16	630.97 a168.17 ^a n = 9	832.58 b782.53 ^a n = 5	868.00 a782.19 ^a n = 7

Different superscript letters to the left of the standard deviation differ within diets at the $p < .05$ level.

Different superscript letters to the right differ within periods at the $p < .05$ level.

¹Dietary composition is found in Table 5.1

²Mean \pm standard deviation

Table 5.12 Analysis of Variance for the Brain Content of Serotonin

Source of Variation	DF	Mean Square	F	Significance of F
Within cells	77	385900.06	--	--
Diet	3	1237300.89	3.20627	.02771
Period	2	2066627.22	5.35534	.00664
Diet by period	6	562097.60	1.45659	.20439

a significant drop from the measurements at period one to period two. It appeared that the levels were again rising but they did not reach significance for period one. Diet R-3 was significantly higher than any of the other diets. At no other period were there significant differences between the serotonin contents of the brain for the animals on any of the four diets.

Because of the paucity of samples of the brains that give good results for the catecholamines, it is difficult to assign any significance to the results that were obtained (Tables 5.13 to 5.16). However, although there were no differences between the diets in the dopamine levels excreted in a twenty-four hour period, there was a significant difference between the excretion by the S5B/PL and the Osborne-Mendel rats. An analysis of variance of the urinary dopamine excretion versus the rat strain showed a significantly lower level of excretion for the S5B/PL rats (see Table 5.19). Neither the norepinephrine nor the serotonin excretion showed this difference. Because the samples may have been damaged when the freezer in which they were stored failed, it is difficult to decide whether or not these results are real or are artifacts of the partial decomposition of the samples. Samples which gave analytical results that were clearly defective (one of the peaks missing) were not included in the analysis of variance. However, it is possible that there was partial loss of the catecholamines from the sample and that the catecholamines are lost at different rates. No such pattern was observed in the analysis of the brain contents of the monoamines.

Table 5.13 Mean Total Dopamine in the Brain of Two Rat Strains Fed High Carbohydrate or High Fat Diets for Three Different Lengths of Time on the Diets

Sample Period	Dopamine			
	R-1 ¹	R-2	R-3	R-4
	----- Nanogram per gram wet weight -----			
Day one	--	1.6859 ² 5.0577 ^a n = 9	10.5464 15.5044 ^{ab} n = 8	9.8697 13.5189 ^b n = 5
Day three	-- --	16.6181 11.3738 ^{ab} n = 4	8.3092 16.6184 ^a n = 4	23.5558 7.4477 ^b n = 3
Day five	5.0517 11.1484 ^a n = 16	-- --	2.2006 4.9208 n = 5	15.1642 12.3337 ^b n = 7

Different superscript letters indicate differences at the $p < .05$ level.

¹Dietary composition is found in Table 5.1.

²Mean \pm standard deviation.

Table 5.14 Analysis of Variance for the Brain Content of Dopamine

Source of Variation	DF	Mean Square	F	Significance of F
Within cells	77	88.5688	--	--
Diet	3	622.1838	7.02486	.00031
Period	2	109.7118	1.23872	.29546
Diet by period	6	232.9932	2.63064	.02253

Table 5.15 Mean Total Norepinephrine in the Brain of Two Rat Strains Fed High Carbohydrate or High Fat Diets for Three Different Lengths of Time on the Diets

Sample Period	Norepinephrine			
	R-1 ¹	R-2	R-3	R-4
-----Nanogram per Gram Wet Weight-----				
Day one	--	275.968 ²	431.687	371.553
	--	827.903 ^a	801.532 ^a	667.827 ^a
		n = 9	n = 8	n = 5
Day three	--	1,605.569	776.031	1,116.871
	--	1,339.606 ^a	1,552.062 ^a	1,239.279 ^a
		n = 9	n = 8	n = 5
Day five	410.751	--	180.110	1,053.939
	886.284 ^{ab}	--	402.739 ^a	967.019 ^b
	n = 16		n = 5	n = 7

Different superscript letters indicate differences at the $p < .05$ level.

¹Dietary composition is in Table 5.1.

²Mean \pm standard deviation.

Table 5.16 Analysis of Variance for the Brain Content of Norepinephrine

Source of Variation	DF	Mean Square	F	Significance of F
Within cells	77	591559.06	--	--
Diet	3	1498021.58	2.53276	.06313
Period	2	1089065.03	1.84132	.16552
Diet by period	6	1519380.14	2.56887	.02541

There were no significant differences in the tryptophan levels in the blood of any of the animals on any of the diets (Tables 5.17 and 5.18).

It may be that since the animal blocks were run at different times the gradual shift in the machine response curve could account for the differences. Much more work needs to be done on the measurement of activity itself before these results can be interpreted more fully.

Neither the serotonin, the dopamine, nor the norepinephrine content of the brain or the urine appear to vary in any fashion with the activity variations. With the limited number of samples, it is difficult to interpret this. It may be that one or more of the other neurotransmitters in the brain is varying with the activity levels. It may also be that analysis of different areas of the brain would show variations not detected on whole brain analysis.

Conclusions

It appears that volitional activity in Osborne-Mendel and S5B/PL rats, over 1-5 days, was altered by whether or not the major energy source was fat or carbohydrate as well as the source of fat and carbohydrate such as sucrose versus cornstarch or hydrogenated versus liquid fat. Rats fed liquid corn oil (R-4) as a primary source of energy had lower volitional activity but it should be reevaluated. It may be that the drop in activity levels on diet R-4 represent a depression in activity for those animals.

Table 5.17 Mean Total Tryptophan in the Blood for Two Rat Strains Fed High Carbohydrate or High Fat Diets for Three Different Lengths of Time on the Diet

Sample Period	Tryptophan			
	R-1 ¹	R-2	R-3	R-4
Day one	1.2400 ²	0.8608	0.8873	3.1542
	0.8987	0.5034	0.4367	6.3964
	n = 3	n = 12	n = 15	n = 12
Day two	1.0025	1.6300	1.1300	1.0650
	0.4930	0.3461	0.5643	0.6706
	n = 16	n = 6	n = 9	n = 12
Day five	--	0.3550	0.9933	1.3567
	--	0.0354	0.3878	0.4160
		n = 2	n = 6	n = 6

¹Dietary composition is found in Table 5.1.

²Mean \pm standard deviation.

Table 5.18 Analysis of Variance for the Blood Content of Tryptophan

Source of Variance	DF	Mean Square	F	Significance of F
Within cells	88	5.3464	--	--
Diet	3	6.2336	1.16595	.32738
Period	2	3.0551	0.57144	.56679
Diet on period	5	5.3371	0.99827	.42365

Table 5.19 Analysis of Variance of the Effect of Rat Strain on the Urinary Excretion of Dopamine

Subject Strain	N	Mean	Standard Deviation
S5B/PL	43	316.12	88.33
Osborne-Mendel	41	405.49	249.13

CHAPTER VI

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

In these studies, the effects of dietary sucrose, cornstarch, hydrogenated fat and corn oil as the major energy source on the activity of rats, gerbils, and pigs were studied. The results supported the hypothesis that increasing the level of fat in the diet decreased the volitional activity of the animal for the pig and the Sprague-Dawley strain of rat. But rats fed primarily sucrose also had lower activity in comparison to those fed cornstarch.

The entire field of activity measurement and the interrelationship and the precision of these measurements needs to be looked at more closely. Three means of examining activity were used in the studies considered here, the open-field test box, the radio-field activity box, and the commercial pedometer. The open-field test box measures the exploratory behavior of the animal being studied. Simonson et al. used this method to study the offspring of underfed mother rats (Simonson et al., 1971). The severely undernourished mother rats had offspring which were much more active during part of the post-weaning growth phase. The work presented here found that increased levels of dietary fat decreased exploratory activity for Sprague-Dawley rats studied after several weeks on the test diet. Osborne-Mendel rats did not show any dietary-dependent effect on their exploratory behavior.

These differences could be due to the time of year that the trials were run, or to the fact that the second trials were made under slightly different conditions. First, the animals were housed in metabolism cages in the second trial. It was noted that the animals in the metabolism cages appeared to be more nervous, possibly due to the expanse of open space beneath them. Also, the lighting in the room where the studies were conducted had been altered between the two studies so that the room could not be made as dark as before.

The second method of studying activity that was used in this study was the radio field test box. The operation of this machine is such that a digital representation is made of an analog function. Also, the device is subject to unforecastable shifts in its performance envelope. These shifts do not appear to be linear so that the data obtained from different parts of the envelope cannot be easily compared with one another. Different machines may be at different parts of their performance envelope at the same time, making it difficult to compare the results of two machines. Wolterink used this device to study the long-term effects of drugs on a single animal (Wolterink, 1978). When one animal is compared to itself over a longer time frame than was used in these experiments, many of the problems can be avoided. Under these circumstances, this method may be of greater value than it was in some of these experiments.

Analysis of the activity of the pigs was made using a commercially available pedometer. This device was calibrated for a human stride and could not be used to measure the actual distance traveled

by the animal. But it did provide an estimation of the relative activity of the animals. The pedometers were easily damaged and the pigs were often able to remove them until a suitable tape could be found. In relation to its cost, this was the most effective device. It was not able to measure activity that did not involve movement of the leg to which the device was attached. Also, the animal to be tested had to be big enough to activate the pendulum in the device. If the animal was too small, the pendulum would not be activated or would be activated intermittently. More studies need to be done with this method to correlate it with other methods of activity measurement in large animals, such as radio transmitters installed subcutaneously.

Other methods have been used to study activity in animals. These include direct monitoring of the animals, activity-wheel devices, and tracing plotters which use the breaking of an infra-red beam to locate the animal in two dimensions and plots the movement around the cage. Direct monitoring has been used to detect differences in activity due to diet (Schemmel, 1967). This method requires a large expenditure of time. Also, if the animal being studied is a nocturnal animal, it would be desirable to obtain some measurements from the animal's dark period. This could require rather specialized animal facilities. Running-wheel devices have been used by several investigators to study activity. Samuels et al. studied the effect of previous diet on an animal's ability to do work during starvation with running wheels (Samuel et al., 1948). However, many factors can stimulate an animal to run in the wheel. Fright, loss of water or food (Moskowitz, 1959),

presence of a female in estrus, or a simple liking for the running itself, can motivate the animal to run. Also, some animals run the cage up to a high rate of speed and then ride the wheel until it slows back down. The tracing plotter restricts the activity measurement to one animal at a time and does not detect some types of motion, such as rearing and grooming. All of the activity measurement methods that have been discussed have been used to study activity in animals, but there is some question as to what aspect of activity they were measuring.

Different aspects of activity appear to be under the control of different centers of the brain. For example, Estler said that low doses of some of the amphetamines completely suppress rearing activity in the rat without altering exploratory behavior (Estler, 1975). Bartholini et al. have reported that small amounts of the dopa-decarboxylase inhibitor, R04-4602, increase locomotor activity by the selective increase in dopamine in the brain and a slight decrease in serotonin (Bartholini et al, 1969). Hornykiewicz has reported that 6-hydroxy-dopamine, in low doses, decreases exploratory behavior in the rat while increasing grooming and gnawing (Hornykiewicz, 1971).

All of this suggests that different aspects of what is classed as behavior may be under the control of different centers in the brain, and possibly under the control of different neurotransmitters. Different devices may then be measuring different aspects of behavior, making the comparison of their result difficult. More work needs to be done comparing different measuring systems to each other so that better understanding of what is being measured can be reached.

Some of the work presented here does indicate that increasing the level of fat in the diet decreases some aspect of activity. Mechanisms by which this could occur are not clear-cut. With an adequate diet, most of the fat will not reach or affect the brain. One possible point of interaction is in the albumin carrier system in the blood. Both tryptophan and free fatty acids are carried on the same site of the albumin. They bind in a competitive manner. If the increasing levels of fat in the diet increase the levels of the free fatty acids, then they would increase the level of free tryptophan in the blood and could possibly increase the rate at which the tryptophan crosses the blood-brain barrier and thus increase the level of tryptophan in the brain. Because the synthesis of serotonin is determined by the concentration of serotonin in the brain, this would lead to an increase in the concentration of serotonin. The primary serotonin containing neurons in the brain send inhibitory fibers into the dopaminergic centers which have been implicated in locomotor stimulation. This could then lead to a decrease in locomotor activity, assuming that the amount of tryptophan which is released to the brain is sufficient to cause a significant increase in serotonin. Some of the tryptophan is always found free in the plasma. Also, there is some evidence that the blood albumin can lose some of its bound tryptophan to the blood-brain barrier so that the amount available to the brain is not restricted to the amount found free in the plasma.

Another possible mechanism is related to the way in which the body controls its adipose deposits. Mrosovsky (1974) has shown that,

in hibernators, a given level of adiposity will be maintained which is appropriate to the time of year for that animal, even if the animal is prevented from hibernating. The weight loss associated with hibernating is maintained even though the animal is not sleeping and is eating. This weight loss is achieved by increased activity on the part of the hibernator. Mrosovsky proposes that hibernators utilize the same neuronal mechanisms for the control of body size as do other mammals; they simply use them at their extremes. He further proposes that some humoral factor expressed by the adipose in some way lets the brain sense how much adipose there is on the organism. It would not be unlikely that this proposed humoral factor would either be a fat or fat derivative or would be influenced by the levels of fat in the incoming diet. Thus, it could be possible that increasing the levels of fat in the diet would send a false signal to the brain telling it that the body adipose stores were low and leading to decreased activity to conserve the remaining stores.

All of the above has assumed that the measures which indicated that fat tended to decrease activity were the correct ones. However, the last rat experiment gave some indications that increased fat increased the activity of the rats and that the high sucrose diet led to both lower levels of initial activity and to decreasing activity. Sucrose diets have been known to cause greater fat deposits than cornstarch (Harris et al., 1977) diets. The problem of resolving these findings may lie in the resolution of the problems associated with measuring and defining activity that were mentioned above. If

the assumption is made that the results as presented are correct, then some explanation for them must be found. It should be noted that the studies which showed a decrease in activity for increased fat in the diet were all longer in time than the last rat study.

There is also the possibility that sucrose actually had a tranquilizing effect on the activity of the rats in the last experiment. It can be postulated that this effect may be a short term effect operating on a scale of days and being replaced by a neutral effect or a stimulatory effect on time scale of weeks. Schemmel et al. (1970) have suggested that sucrose is the sugar which is most capable of generating obesity in rats. Joosten and van der Kroon (1974) had noted that decreases in activity occur in obese mice prior to the onset of any anatomical differences in body composition. It would be worthwhile to examine the effects of sucrose versus cornstarch and fat on longer experiments and to examine the effects of different simple sugars.

It was assumed that the phenomenon was in operation from the start of the diet. This may not be true. It may be necessary to postulate an initial increase in activity from the higher fat levels or to postulate an initial calming effect from the high sucrose diet. An initial calming effect can be postulated to operate through the tryptophan increase method that was mentioned above. Increased loads of simple sugars will increase the flow of insulin to the system. Parfitt and Grahame-Smith have reported that increased insulin will decrease the plasma levels of free tryptophan and will increase the intracellular levels of free tryptophan (Parfitt and Grahame-Smith,

1974). This could provide an immediate postmeal increase in brain tryptophan, and thus, serotonin, but may not be effective in time scales of a few days. The high fat diet itself could cause an increase in the activity as the animals spend more time searching for a more familiar diet. The rat has been known for its distrust of unfamiliar foods. Again, there would be a question as to how long a time scale this initial distrust of a new diet would operate under.

There may be some factor in the way the animal responds to changing levels of stimulus. In Chapter Four, it was noted that the gerbil's activity was more normally distributed than the log transform of the activity. In the case of the rat, the log transform was more normally distributed, but neither the log transform nor the activity levels were as normally distributed as those of the gerbil. The rat is a nocturnal animal and thus, has its activity unevenly distributed on a twenty-four hour time scale. The gerbil, on the other hand, tends to be active throughout the whole twenty-four hours with periods of activity interspersed with periods of quiescence. The fact that the log transform is more normal is consistent with the theory by Wolterink that the center in the brain which responds to outside stimulus does so in a logarithmic fashion (1978). Thus, small levels of stimuli result in relatively larger responses than larger levels.

It would appear that the work presented here supports the concept of an effect of dietary energy source on activity. There appears to be a decrease in activity from increasing fat levels on a time-scale of a week or more. On shorter time-scales, there may

be an increase or no effect from the increased levels of fat. There is some biochemical evidence to support the idea that the monoaminergic neurotransmitters are involved in the changes in activity levels caused by dietary energy sources, but the evidence is limited by the problems mentioned earlier that occurred during data collection. The implications for the study of hyperactivity are also not clear cut because of these same problems.

Future work should focus on two areas. First, there needs to be more work done in the area of activity measurement. Different methods need to be compared to each other and more exact analysis of what method measures needs to be done.

Second, the biochemical data suggest that future analysis should look at the tryptophan-serotonin-5-HIAA system in more detail. Also, other possible neurotransmitters should be examined for a possible role in the control of activity. Finally, the variations of the neurotransmitters in specific brain locations need to be examined.

Davis, in the quote that begins Chapter One, suggests that certain areas of the brain are implicated in the control of activity in the mammal. We may be to the point where we can begin to pose the right questions so as to examine his hypothesis in detail.

BIBLIOGRAPHY

BIBLIOGRAPHY

- Aghajanian, G. K., and Graham, A. 1970. Serotonin Containing Neurons in the Brain: Inhibition of Firing by Monoamine Oxidase Inhibitors. Federation Proceedings. Abstract #26:251.
- Alexander, G. J., and Kopeloff, L. M. 1971. Induced Hypercholesteremia and Decreased Susceptibility to Seizures in Experimental Animals. Experimental Neurology, 32:134-40.
- Allen, A., Friedman, B., and Weinhouse, S. 1955. Tissue Preferences for Fatty Acid and Glucose Oxidation. I.B.C., 212:921-33.
- Amino Acids and Serotonin. 1964. Nutrition Reviews, 22:23.
- Anden, N. E. 1975. On the Function of the Nigro-Neostriatal Dopamine Pathway. Advances in Pharmacology and Chemotherapy, Garrattini, S., Golden, A., Hawkins, F., and Kopin, I. J., Eds., Academic Press:New York-San Francisco-London.
- Antelman, S. M., and Caggiula, A. R. 1977. Norepinephrine-Dopamine Interaction and Behavior. Science, 195:646-53.
- Axelrod, J. 1972. Dopamine- β -Hydroxylase: Regulation of Its Synthesis and Release from Nerve Terminals. Pharm. Rev., 24:233.
- Bachelard, H. S. 1975. Energy Utilized by Neurotransmitters. Brain Work, Ingvar, D. H., and Lassen, N. A., Eds. Muskagaard: Copenhagen.
- Bechtereva, N. P., Kambarova, A. K., and Pozdeev, V. K. 1975. Functional Interrelationships of Principal Catecholaminergic Centers in the Brain. Catecholamines and Behavior, Friedhoff, A. J., Ed. Plenum Press:New York, London.
- Bender, D. A., and Coulson, W. F. 1972. J. Neurochemistry, 19:2801.
- Bloxam, D. L., Curzon, G., Kantamaneni, B. D., and Tricklebank, M. D. 1977. Effects of Tryptophan and Protocarval Anastomosis on Activity and Brain Tryptophan Metabolism. Brit. J. Pharm., 60:277.
- Bradley, C. 1937. The Behavior of Children Receiving Benzedrine. Am. J. Psychiat., 94:571-585.

- Brooks, C. M., and Koizumi, K. 1974. The Hypothalamus and Control of Integrative Processes. Medical Physiology. Mountcastle, V. B., Ed. C. V. Mosby Co.:St. Louis.
- Brownstein, M. 1977. Neurotransmitters and Hypothalamic Hormones in the C.N.S. Federation Proceedings, 36:1960-63.
- Butcher, L. L. 1977. Nature and Mechanism of Cholinergic Monoaminergic Interactions in the Brain. Life Science, 21:1207-26.
- Camien, M. N., Dunn, M. S., Malin, R. B., Reiner, P. J., and Tarbet, J. 1949. Percentages of 12 Amino Acids in Swine Organs, Univ. Calif. Pub. in Psl., 8:328-32.
- Campbell, J., and Best, C. H. 1956. Physiologic Aspects of Ketosis. Metabolism, 5:95-107.
- Carlsson, A. 1974. The In Vivo Estimation of Tryptophan and Tyrosine Hydroxylation. Aromatic Amino Acids in the Brain. Ciba Foundation Symposium 22. Associated Scientific:Amsterdam, London, New York.
- _____. 1975. Physiology and Pharmacology of the Release of Monoamine in the CNS. Advances in Pharmacology and Chemotherapy. Vol. 13. Garattini, S., Goldin, A., Hawkins, F., and Kopin, I. J. Eds. Academic Press:New York, San Francisco, London.
- Carlsson, A., Kehr, W., Lindquist, M., Magnusson, T., and Atack, C. V. 1972. Regulation of Monoamine Metabolism in the CNS. Pharm. Rev., 24:371.
- Cartwell, D. P. 1975. Epidemiology, Clinical Picture and Classification of the Hyperactive Child Syndrome. The Hyperactive Child. Cartwell, D. P., Ed. Spectrum Pub., Inc.:New York.
- Casey, P. 1977. The Hyperactive Child. Review and Suggested Management. Texas Medicine, 73:68-75.
- Cavanaugh, J. J. A. 1975. Hyperkinesis and the Learning Disability Child. Pediatric Basics, 13:5-10.
- Chadwick, D., Jenner, P., and Reynolds, E. H. 1977. Serotonin Metabolism in Human Epilepsy: The Influence of Anticonvulsant Drugs. Ann. Neurol., 1:218-224.
- Chadwick, D., Jenner, P., and Reynolds, E. H. 28 June 1975. Amines, Anticonvulsants, and Epilepsy. The Lancet, pp. 1425-1426.
- Chernick, S. S. 1960. Developmental Aspects of Lipid Metabolism. Symposium of the Czechoslovak Academy of Sciences:Prague.

- Coleman, M. 1971. Serotonin Concentrations in Whole Blood of Hyperactive Children. J. Pediat., 78:985-90.
- Coleman, M., Greenberg, A., Bhavavan, H., Steinberg, G., Tippet, J., and Connors, O. 1976. The Role of Whole Blood Serotonin Levels in Monitoring Vitamin B₆ and Drug Therapy in Hyperactive Children. Monog. Neur. Sci., 3:133-36.
- Connors, C. K. (Ed.) 1974. Clinical Use of Stimulant Drugs in Children. American Elsevier Pub. Co., Inc.:New York.
- Conrad, P. 1976. Identifying Hyperactive Children. Lexington Books:Lexington, Toronto, London.
- Coppen, A., and Wood, K. 8 January 1977. Total and Non-bound Plasma. Tryptophan in Depressive Illness. Lancet, p. 94.
- Costa, E., and Trabacchi, M. 1975. Regulation of Brain Dopamine Turnover Rate. Catecholamines and Behavior, Friedhoff, A. J., Ed. Plenum Press:New York, London.
- Cott, A. Treatment of Learning Disabilities. J. Orthomolecular Psychiatry, 3:1-13.
- _____. 4 May 1976. Pers. letter to Dr. Mickelsen.
- Cross, B. A. 1974. Functional Identification of Hypothalamic Neurones. Recent Studies of Hypothalamic Function, Lerer, K., and Cooper, K. E., Eds. S. Karger:Basel, Munchen, Paris, London, New York, Sidney.
- Crow, T. J. 1976. Catecholamines-Containing Neurones and the Mechanisms of Reward. Mental Health in Children, Vol. 3, Sivasankar, O. V., Ed. PJD Pub. Ltd.:Westbury.
- Curzon, G., and Knott, P. J. 1974. Fatty Acids and the Disposition of Tryptophan. Aromatic Amino Acids in the Brain. Ciba Foundation Symposium 22. Associated Scientific Publishers: Amsterdam, London, New York.
- Davenport, V. D., and Davenport, H. W. 1948. The Relationship Between Starvation Metabolic Acidosis and Convulsive Seizures in Rats. J. Nut., 36:139.
- Davis, J. M., and Himwich, N. A. 1973. Amino Acids and Proteins of Developing Mammalian Brains. Biochemistry of the Developing Brain, Himwich, N. A., Ed. Marjell Dekker, Inc.:New York.

- Davis, N. S. 1845. Importance of a Correct Physiology of the Brain. Am. J. Insanity, 1:235-43.
- Dekirmenjian, H., and Maas, J. W. 1970. An Improved Procedure of 3-Methoxy-4-Hydroxyphenyl Ethylene Glycol Determination by Gas-Liquid Chromatography. Anal. Bch., 35:113-22.
- Denizeau, F., and Sourkes, T. L. 1977. Regional Transport of Tryptophan in Rat Brain. J. Neurochem., 28:951-959.
- Deuel, H. J. 1955. Fat as a Required Nutrient of the Diet. Fed. Proc., 14:639-49.
- Diet and Hyperactivity: Any Connection? April 1976. Institute of Food Technologists.
- Dobbing, J. 1974. Prenatal Development and Neurological Development. Early Malnutrition and Mental Development. Cravioto, J., Hanbraeres, L., and Vahlqvist, B., Eds. Almqvist & Wiksell: Stockholm.
- Douglas, V. I. 1974. Differences Between Normal and Hyperkinetic Children. Clinical Use of Stimulant Drugs in Children. Conners, C. K., Ed. American Elsevier Publishing Co., Inc.: New York.
- Dunn, M. S., Camien, M. N., Malin, R. B., Murphy, E. A., and Reiner, P. J. 1949. Percentages of 12 Amino Acids in Blood, Carcass, Heart, Kidney, Liver, Muscle and Skin of Eight Animals. University California Pub. in Psy., 8:293-326.
- Estler, C. J. 1975. Effect of Amphetamine-Type Psychostimulants on Brain Metabolism. Advances in Pharmacology and Chemotherapy. Vol. 13. Garattin, S., Goldin, M., Hawkins, F., and Kopin, I. J., Eds. Academic Press: New York, San Francisco, London.
- Experts Call for Study of Hyperkinesis and Food Additives. Special Report. 7 July 1975. The Nutrition Foundation, Inc.
- Eyzaguirre, C., and Fidone, S. J. 1975. Physiology of the Nervous System. Year Book. Medical Publishers, Inc.: Chicago.
- Feingold, B. F. 1974. Why Your Child is Hyperactive. Random House: New York.
- _____. 1975. Hyperkinesis and Learning Disabilities Linked to Artificial Food Flavors and Colors. American Journal of Nursing, 75:797-803.
- Fernstrom, J. D. 1974. Modification of Brain Serotonin by the Diet. American Review of Medicine, 25:1-8.

- Fernstrom, J. D., Madras, B. K., Medino, H. N., and Wurtsman, R. J. 1974. Nutritional Control of the Synthesis of 5-Hydroxytryptamine in the Brain. Aromatic Amino Acids in the Brain. Ciba Foundation Symposium 22. Associated Scientific Publishers:Amsterdam, London, New York.
- Fernstrom, J. D., and Jacoby, J. H. 1975. The Interaction of Diet and Drugs in Modifying Brain Serotonin Metabolism. General Pharmac., 6:253-58.
- Ferrendelli, J. A. 1975. Hypoglycemia and the C.N.S. Brain Work. Ingvar, D. H., and Lassen, N. A., Eds. Musksgaard:Copenhagen.
- Fetter, D., and Neidle, E. A. 1959. Effect of a High Fat Diet on Growth and Blood Sugar of the Rat. Metabolism, 8:763-8.
- Fish, B. 1971. The 'One Child, One Drug' Myth of Stimulants in Hyperkinesis. Arch. Gen. Psychiat., 25:193-203.
- Forbes, E. B., Swift, R. W., Elliott, R. F., and James, W. H. 1946. Relation of Fat to Economy of Food Utilization. J. Nut., 31: 203-12.
- Forbes, E. B., Swift, R. W., James, W. H., Bratzler, J. W., and Black, A. 1946. Further Experiments in the Relation of Fat to Economy of Food Utilization. J. Nut., 32:387-96.
- Forbes, R. W., and Rao, T. 1959. The Effect of Age on the Net Requirement for Nitrogen, Lysine, and Tryptophan by the Well-Fed Rat. Arch. Bch. BPY., 82:348-54.
- Fruits, Serotonin and Catecholamines. 1959. Nutrition Reviews, 17:284-5.
- Fuller, R. W., and Wong, D. T. 1977. Inhibition of Serotonin Reuptake. Fed. Proc., 36:8, 2154-2158.
- Fuxe, K., and Hökfelt, T. 1970. Central Monoaminergic Systems and Hypothalamic Function. The Hypothalamus. Martin, M., and Fraschini, F., Eds. Academic Press:New York and London.
- Garattini, S., and Valzelli, L. 1965. Serotonin. Elsevier Publishing Co.:Amsterdam, London, New York.
- Garten, C. T. 1977. Relationships Between Exploratory Behavior and Genetic Heterozygosity in the Oldfield Mouse. Animal Behavior, 25:328-32.
- Gazzaniga, M. S. 1973. Brain Theory and Minimal Brain Dysfunction. Ann. N.Y.A.S., 205:89-92.

- Gessa, G. L., and Tagliamonte, A. 1974. Serum Free Tryptophan. Aromatic Amino Acids in the Brain. Ciba Foundation Symposium 22. Associated Scientific Publishers:Amsterdam, London, New York.
- Gianutsos, G., and Moore, K. E. 1977. Effects of Pre- or Post-natal Dexamethasone, Adrenocorticotrophic Hormone and Environmental Stress on Phenylethanolamine-N-Methyl-Transferase in Sympathetic Ganglia of Neonatal Rats. J. Neurochem., 28:935-40.
- Glowinski, J. 1970. Metabolism of Catecholamines in the CNS and Correlation with Hypothalamic Functions. The Hypothalamus. Martin, L., Motta, M., and Fraschini, F., Eds. Academic Press:New York and London.
- Goldberg, H. C., and Marsden, C. A. 1976. Catechol-O-Methyl Transferase: Pharmacological Aspects of Physiological Role. Pharmacological Review, 27:135-306.
- Goldstein, M., Fuxe, K., and Hökfelt, T. 1972. Characterization and Tissue Location and Catecholamine Synthesizing Enzymes. Pharm. Rea., 24:293-309.
- Gross, M. D., and Wilson, W. C. 1974. Minimal Brain Dysfunction. Brunner/Mazel:New York.
- Habicht, J. P. 1974. Human Implication of Animal Studies in Prenatal Nutrition and Neurological Development. Early Malnutrition and Mental Development. Cravioto, J., Hanbraes, L., and Valquist, B., Eds. Almqvist and Wiksell:Stockholm.
- Haigler, H. J., and Aghajanian, G. K. 1977. Serotonin Receptors in the Brain. Fed. Proc., 26:8, 2159-2164.
- Hardebo, J. E., Edvinsson, L., Owman, C., and Rosengren, E. 1977. Quantitative Evaluation of the Blood-Brain Barrier Capacity to Form Dopamine from Circulating L-Dopa. Acta Physiol. Scand., 99:377-84.
- Harley, J. P., Mathews, C. G., and Eichman, P. L. 1977. Hyperkinesia and Food Additives, Pers. Comm., Dr. Mikkelsen.
- Harris, G. J., Stone, M., Czajka-Narins, D., Merkel, R. A., and Schemmel, R. 1977. Growth and Development of Gastrocnemius Muscle in S5B/PL and Osborne-Mendel Rats Overfed During Nursing. Growth, 41:305-314.
- Hartsoch, E. W., Hershberger, T. V., and Nee, J. C. N. 1973. Effects of Dietary Protein Content and Ratio of Fat to Carbohydrate Calories and Energy Metabolism and Body Composition of Growing Rats. J. Nut., 103:167-78.

- Heussy, Hans R. September 1977. Minimal Brain Dysfunction in Children (Hyperkinetic Syndrome): Recognition and Treatment. Drug Therapy, pp. 52-63.
- Himwich, H. E. 1958. Psychopharmacologic Drugs. Science, 127:59-72.
- _____. 1973. Early Studies of the Developing Brain. Biochemistry of the Developing Brain. Vol. 1. Himwich, W., Ed. Marcel Dekker, Inc.:New York.
- Hornykiewicz, O. 1971. Dopamine: Its Physiology, Pharmacology and Pathological Neurochemistry. Biogenic Amines and Physiological Membranes in Drug Therapy, Part B. Biel, J. H., and Abood, L. G., Eds. Marcel Dekker, Inc.:New York.
- Hyperactivity in Children. 18 October 1975. British Med. J., pp. 122-124.
- Hyperkinesis: Does Subtracting Additives Help? 20 September 1976. Medical World News, p. 50.
- Johnston, P. V. 1974. Nutrition and Neural Development. Food and Nutrition News, 45:1-4.
- Joosten, H. F. P., and Von der Kroon, P. H. W. 1974. Growth Pattern and Behavioral Traits Associated with the Development of the Obese-Hyperglycemic Syndrome in Mice (ob/ob). Metabolism, 23: 1141-7.
- Kaufman, S. 1974. Properties of Pterin-dependent Aromatic Amino Acid Hydroxylases. Aromatic Amino Acids in the Brain. Ciba Foundation Symposium 22. Associated Scientific Publisher:Amsterdam, London, New York.
- Kissinger, P. T. 1976. Personal communication.
- Kissinger, P. T., Riggin, R. M., Alcorn, R. L., and Rahe, C. D. 1975. Estimation of Catecholamine in Urine by High Performance Liquid Chromatography with Electrochemical Detection. Biochemical Medicine, 13:299-306.
- Klein, D. F., and Gittelman-Klein. 1974. Diagnosis of MBD and Hyperkinetic Syndrom. Clinical Use of Stimulant Drug in Children. Conners, C. K., Ed. American Elsevier Pub. Co., Inc.:New York.
- Kopin, I. J. 1975. Biochemical Aspects of Storage and Release of Biogenic Amines from Sympathetic Nerves. Advances in Pharmacology and Chemotherapy. Vol. 13. Garattini, S., Goldin, A., Hawkins, F., and Kopin, I. J., Eds. Academic Press:New York, San Francisco, London.

- Krauthamer, G. M. 1975. Catecholamines in Behavior and Sensimotor Integration. Catecholamines and Behavior. Friedhoff, A. J., Ed. Plenum Press:New York, London.
- Lehninger, A. L. 1970. Biochemistry. Worth Publishers, Inc.:New York.
- Lennan, J. A. R., Turnbull, M. J., Reid, A., and Fleming, A. M. 1977. Urinary Monoamine Metabolite Excretion in Disorders of Movement. J. Neur. Sci., 32:219-25.
- Lennox, W. G., and Cobb, S. 1923. Epilepsy: From the Standpoint of Physiology and Treatment. Medicine, 7:108-289.
- Leonard, B. E. 1973. A Study of the Effects of Some Amphetamines on Brain Monoamines and Their Precursors. Symposium for Pharmacological Agents and Biogenic Amines in the C.N.S. First Congress of the Hungarian Pharmacological Society. Knoll, J., and Magyar, K., Eds. Akademiai Kiado:Budapest.
- Lipton, J. M. 1969. Effects of High Fat Diets on Caloric Intake, Body Weight and Heat Escape Responses in Normal and Hyperphagic Rats. J. Comp. and Ps1. Psychol., 68:507-15.
- Livingston, S. 1972. Comprehensive Management of Epilepsy in Infancy, Childhood and Adolescence. Charles C. Thomas, Pub.:Springfield.
- Lloyd, K. G., and Hornykiewics, O. 1975. Catecholamines in Regulation of Motor Function. Catecholamines and Behavior. Friedhoff, A. J., Ed. Plenum Press:New York and London.
- Lopez, A., Vial, R., Balart, L., Arroyave, G. 1974. Effect of Experience and Physical Fitness on Serum Lipids and Lipoproteins. Atherosclerosis, 20:1-9.
- Lowry, O. H. 1975. Energy, Metabolism of the Brain and Its Control. Brain Work. Ingoar, D. H., and Lasson, N. A., Eds. Musksgaard: Copenhagen.
- Luse, S. A., Blank, W., and Mettler, F. A. 1974. L-Dopa and Hyperkinesis. Federal Proceedings, Abstract #1489:512.
- Macnab, R. B. J., Reineke, E. P., and Montage, H. J. 1965. The Effect of High Fat and High Carbohydrate Diets on Spontaneous Activity in Albino Mice. Res. Quarterly, 36:449-63.
- Madras, B. K., Cohen, E. L., Munro, H. N., and Wurtman, R. J. 1974. Adv. Biochem. Psychopharm., 11:143-151.

- Mandel, P., Mack, G., and Morilis, C. 1975. Function of the Central Catecholaminergic Neuron. Catecholamines and Behavior. Friedhoff, A. J., Ed. Plenum Press:New York and London.
- Mandell, A. J., and Knapp, S. 1977. Regulation of Serotonin Biosynthesis in Brain: Role of the High Affinity Uptake of Tryptophan into Serotonergic Neurons. Fed. Proc., 26:8, 2142-2148.
- Mandell, M. 1968. Cerebral Manifestation of Hypersensitivity to the Chemical Environment. Rev. Allergy, 22:787-8.
- _____. 1969. Cerebral Reactions in Allergic Patients. Second International Congress of Social Psychiatry.
- _____. 1971. Allergic Headaches and Allergic Headache Syndromes. Abstract \$47. Twenty-seventh Annual Congress American College of Allergists.
- _____. 23 August 1971. Pollution Solutions. The Hartford Courast.
- _____. 30 January 1977. Allergies Alleged to be Causes of Psychoses. M.W.N.
- _____. Reprint. Neurotic Symptoms Can be Due to Allergy. Prevention Magazine.
- Martini, L., and Meites, J., Eds. 1970. Neurochemical Aspects of Hypothalamic Function. Academic Press:New York and London.
- McNamee, H. B. 2 November 1974. Tryptophan in Depression. Letters. The Lancet, p. 1086.
- Meister, A. 1965. Biochemistry of the Amino Acids. Academic Press: New York and London.
- _____. 31 May 1968. Mental State Reflects Biochemical Ups and Downs. M.W.N.
- Miller, E. 1978. Personal communication.
- Miller, E. R., Ullrey, D. E., Zutaut, E. L., Baltzer, B. V., Schmidt, D. A., Vincent, B. H., Hoefer, J. A., and Luecke, R. W. 1964. Vitamin D₂ Requirement of the Baby Pig. J. Nut., 83:140-148.
- Moir, A. T. B. 1974. Tryptophan Concentration in the Brain. Aromatic Amino Acids in the Brain. Ciba Foundation Symposium 22. Associated Scientific Publishers:Amsterdam, London, New York.

- Moskowitz, M. T. 1959. Running Wheel Activity in the White Rat as a Function of Combined Food and Water Deprivation. J. Comp. and Physiol. Psychol., 52:621-5.
- Mountcastle, V. B., and Baldessarini, R. J. 1974. Synaptic to Medical Physiology. Mountcastle, V. B., Ed. C. V. Mosby Company:St. Louis.
- Mrosovsky, N. 1974. Natural and Experimental Hypothalamic Changes in Hibernation. Recent Studies of Hypothalamic Function. Lederis, K., and Cooper, K. E., Eds. S. Karger:Basel, Munchen, Paris, London, New York, Sidney.
- Mukaida, C. S., and Lichton, I. J. 1971. Some Dietary Influences on the Excretion and Biological Activity of an Anorexigemic Substance in the Urine of Rats. J. Nutrition, 101:767-74.
- Munro, H. N. 1974. Control of Plasma Amino Acid Concentrations. Aromatic Amino Acids in the Brain. Ciba Foundation Symposium 22. Associated Scientific Publishers:Amsterdam, London, New York.
- Nagatsu, F., Kato, T., Nunata, Y., Ikata, K., Sano, M., Nagatsu, I., Kondo, Y., Inagaki, S., Iizuka, R., Hori, A., and Narabayashi, H. 1977. Phenyletherolamine N-Methyltransferase and other Enzymes of Catecholamine Metabolism in Human Brain. Clinica Chinica Acta, 75:221-32.
- The National Advisory Committee on Hyperkinesis and Food Additives. 1 June 1975. Report to the Nutrition Foundation.
- _____. 1977. Nutrition and Transmitter Amines in Rat Brain. Nutrition Review, 35:283.
- Nutrition: Swine Feeds and Feeding. Extension Bulletin 537. Michigan State University, September 1975.
- Palmer, S., Rapoport, T. L., and Quinn, P. O. 1975. Food Additives and Hyperactivity. Clinical Pediatrics, 14:56-9.
- Paoletti, R., Parcellati, G., and Facini, G., Eds. 1976. Lipids. Vol. 1. Biochemistry. Raven Press:New York.
- Parfitt, A., and Grahame-Smith, D. G. 1974. The Transfer of Tryptophan Across the Synaptosome Membrane. Aromatic Amino Acids in the Brain. Ciba Foundation Symposium 22. Associated Scientific Publishers:Amsterdam, London, New York.
- Pelham, W. E. 1977. Withdrawal of a Stimulant Drug and Concurrent Behavioral Intervention in the Treatment of a Hyperactive Child. Behavior Therapy, 8:463-9.

- Pelton, E. W., and Chase, T. N. 1975. L-Dopa and the Treatment of Extrapramidal Diseases. Advances in Pharmacology and Chemotherapy. Vol. 13. Garattini, S., Goldin, A., Hawkiny, F., and Kopin, I. J., Eds. Academic Press:New York, San Francisco, London.
- Phillips, F., Crist, A. H., McGuinness, B., Kalucy, E. C., Chen, C. N., Koval, J., Kalucy, R. S., and Lucey, J. H. 18 October 1975. Isocaloric Diet Changes and Electroencephalographic Sleep. The Lancet, pp. 723-5.
- Pilecki, R., Sanochowiec, L., and Szezka, K. 1975. The Influence of Atherogenic Diet and Essential Phospholipids Upon the Content of Noradrenalin and Dopamine in the Brain of Rats and Their Exploratory Activity. Atherosclerosis, 22:404-10.
- Pletscher, A. 1972. Regulation of Catecholamine Turnover by Variations of Enzyme Levels. Phar. Dev., 24:225.
- Porcellati, G. 1974. Lipid Metabolism and Its Regulation in Brain Tissues. Central Nervous System. Genazzain, E., and Herkins, H., Eds. Springer-Verlag:New York-Heidelberg, Berlin.
- Quik, M., and Sourkes, T. L. 1977. Central Dopaminergic and Serotonergic Systems in the Regulation of Adrenal Tyrosine Hydrolase. J. Neurochem., 28:137-47.
- Ramsey, R. B., and Nicholas, H. J. 1972. Brain Lipids. Advances in Lipid Research. Vol. 10. Paoletti, R., and Kaitchevsky, D., Eds. Academic Press:New York and London.
- Randrup, A., and Muskvad, I. 1973. Roles of Brain NE and DA in Pharmacologically Induced Aggressive Behavior. Symposium on Pharmacological Agents and Biogenic Amine in the C.N.S. First Congress of the Hungarian Pharmacological Society. Knoll, J., and Magyar, K., Eds. Akademiai Kiado:Budapest.
- Rapaport, J. L., Lott, I. T., Alexander, D. F., and Abramson, A. U. 1970. Urinary Noradrenalin and Playroom Behavior in Hyperactive Boys. Lancet, 3:1141.
- Refshange, C., Kissinger, P. T., Dreiling, R., Blank, L., Freeman, R., and Adams, R. N. 1974. New High Performance Liquid Chromatographic Analysis of Brain Catecholamines. Life Science, 14: 311-22.
- Rouser, G., Kritshevsky, G., Yamamoto, A., and Baxter, C. F. 1972. Lipids in the Nervous System of Different Species as a Function of Age. Advances in Lipid Research. Vol. 10. Paoletti, R., and Kritchevsky, D., Eds. Academic Press:New York and London.

- Saavedra, J. M. 1977. Distribution of Serotonin and Synthesizing Enzymes in Discrete Areas of the Brain. Fed. Proc., 36:8, 2134-3141.
- Samuels, L. J., Gilmore, R. C., and Reinecke, R. M. 1948. The Effect of Previous Diet on the Ability of Animals to Do Work During Subsequent Fasting. J. Nutrition, 36:639.
- Sanders-Bush, E., and Massari, V. J. 1977. Actions of Drugs that Deplete Serotonin. Fed. Proc., 36:8, 2149-2153.
- Schemmel, R. 1967. The Effect of a High Fat Ration on Body Weight, Body Composition and Adipose Tissue Weights of Rats as Influenced by Age, Strain and Weight Reduction of Obese Rats. M.S.U. Ph.D. dissertation.
- Schenider, A. M., and Tarskis, B. 1975. An Introduction to Physiological Psychology. Random House:New York.
- Schubert, W. K. 1973. Fat, Nutrition and Diet in Childhood. Am. J. Cardiol., 31:581-7.
- Sheffner, A. L., Kirsner, J. B., Palmer, W. L. 1948. Studies on Amino Acid Excretion in Man: I. Amino Acids in Urine. J.B.C., 175:107-115.
- _____. 1948. Studies on Amino Acid Excretion in Man: II. Amino Acids in Feces. J.B.C., 176:89-93.
- Shoemaker, W. J., and Wurtman, R. J. 1970. Effect of Perinatal Undernutrition on Development of Brain Catecholamine. Fed. Proc., 29:2496.
- Simonson, M., Stephan, J. K., Hanson, H. M., and Cow, B. F. 1971. Open Field in Offspring of Underfed Mother Rats. J. Nutrition, 101:331-6.
- Sims, K. L., and Bloom, F. E. 1973. Brain Res., 49:165.
- Smelik, P. G. 1970. Integrated Hypothalamic Responses to Stress. The Hypothalamus. Martini, L., Motts, M., and Franchini, F., Eds. Academic Press:New York and London.
- Smith, G. P. 1976. The Arousal Function of Central Catecholamine Neurons. Ann. NYAS, 270:45-56.
- Sokoloff, L. 1975. Influence of Functional Activity on Local Cerebral Glucose Utilization. Brain Work. Ingvar, D. H., and Larsen, W. A., Eds. Musksgaard:Copenhagen.

- Sourkes, T. L. 1972. Influence of Specific Nutrients on Catecholamine Synthesis and Metabolism. Pharm. Rev., 24:451-477.
- Spector, S., Tarver, J., and Berkowitz, B. 1972. Effects of Drugs and PSL Factors in the Disposition of Catecholamines in Blood Vessels. Pharm. Rev., 24:191.
- Statinsky, F. S. 1970. Hypothalamic Neurosecretion. The Hypothalamus. Martini, L., Motta, M., and Fraschini, F., Eds. Academic Press: New York and London.
- Stein, W. H., and Moore, S. 1954. The Free Amino Acids of Human Blood Plasma. J.B.C., 211:915-926.
- Steinhart, H. 1977. Determination of Tryptophane in Plasma with a Spectrofluorometric System. Anal. Chem., 49:950-3.
- Stewart, M. A. 1971. Urinary Noradrenalin and Playroom Behavior in Hyperactive Children. Lancet, 1:140.
- Strother, C. R. 1973. Minimal Cerebral Dysfunction: A Historical Overview. Ann. YASA, 205:6-19.
- Sunderlin, S., and Wills, B., Eds. 1969. Nutrition and Intellectual Growth in Children. Bulletin 25A. Association for Childhood Education International: Washington, D.C.
- Svennerbrolin, L. 1974. Lipid Biochemical Changes of Brain During Development. Early Malnutrition and Mental Development. Craviote, J., Hambraeres, L., Valquist, B., Eds. The Swedish Nutrition Foundation. Almqvist and Wiksell: Stockholm.
- Tang, L. C., and Cotzins, G. C. 1977. L-3,4-Dihydroxyphenylamine-Induced Hypersensitivity Stimulating Features of Denervation. PNAS, 74:2126-9.
- Thiel, A., Schemmel, R., Mickelsen, O., Johnson, J. T., and Gill, J. L. 1972. Free Choice Intakes by Two Strains of Rats Offered High Fat and High Carbohydrate Rations. Nut. Reports International, 5:101-10.
- Thompson, R. F. 1975. Introduction to Physiological Psychology. Harper and Row: New York, Evanston, San Francisco.
- Thompson, R. H. S., and Webster, G. R. 1960. Neurochemistry. Ann. Rev. Bch., 29:365-90.
- Torre, J. C. de la. 1972. Dynamics of Brain Monoamines. Plenum Press: New York and London.

- Twarog, B. M., Muneoka, Y., and Ledgeres, M. 1977. Serotonin and Dopamine as Neurotransmitters in Mytilus. J. Pharm. & Exp. Therap., 201:350-6.
- Udenfriend, S. 1972. Molecular Biology of the Sympathetic Nervous System. Pharm. Rev., 24:165.
- Udenfriend, S., Weissbach, H., and Mitoma, C. 1960. Metabolism of Amino Acids. Ann. Rev. Bch., 29:207-60.
- Uhlemann, E. R., and Neims, A. H. 1972. Anticonvulsant Properties of the Ketogenic Diet in Mice. J. Pharm. & Expt. Therap., 180:231-7.
- Undernutrition and Development of the CNS in the Pig. 1967. Nutrition Review, 25:185-6.
- Valzelli, L. 1974. Neurochemical Aspects of Behavior Central Nervous System. Genogani, E., and Herken, H., Eds. Springer-Verlag:New York, Heidelberg, Berlin.
- Vergroesen, A. J., and Gottenbos, J. J. 1975. The Role of Fats in Human Nutrition: An Introduction. The Role of Fats in Human Nutrition. Vergroesen, A. J., Ed. Academic Press:New York, San Francisco, London.
- Vogt, M. 1973. Release of Transmitters from Caudate Nucleus and Adjacent Regions. Symposium on Pharmacological Agents and Biogenic Amines in the C.N.S. First Congress of the Hungarian Pharmacological Society. Knoll, J., and Magyar, K., Eds. Akademiai Kiado:Budapest.
- von Euler, U. S. 1975. Foreword. Catecholamines and Behavior. Friedhoff, A. J., Ed. Plenum Press:New York and London.
- Webster, G. R. Some Aspects of Lipid Metabolism in Nervous Tissue.
- Wedeson, H., and Epstein, R. 1976. Intrapsychic Effect of Ephedamine in Hyperkinesis as Revealed Through Art Productions. Mental Health in Children. Vol. 3. Sivasankar, D. V., Ed. PJD Publishers, Ltd.:Westbury.
- Weiner, N., Lee, F. L., Waymire, J. C., and Posiviata, M. 1974. The Regulation of Tyrosine Hydrolase Activity in Adanergic Nervous Tissue. Aromatic Amino Acids in the Brain. Ciba Foundation Symposium 22. Associated Scientific Publishers: Amsterdam, London, New York.

- Weiss, G., and Minde, K. K. 1974. Follow-Up Studies of Children Who Present Symptoms of Hyperactivity. Clinical Uses of Stimulant Drugs in Children. Conners, C. K., Ed. American Elsevier Pub. Co., Inc.:New York.
- Welton, R. F., Martin, R. J., and Baumgardt, B. R. 1973. Effects of Feeding and Exercise Regimency on Adipose Tissue Glycerokinase Activity and Body Composition of Normal and Obese Mice. J. Nutrition, 103:1212-19.
- Wender, P. H. 1973. Some Speculations Concerning a Possible Biochemical Basis of Minimal Brain Dysfunction. Ann. NYAS, 205:18-28.
- Wesson, L. G. 1932. A Modification of the Osborne-Mendel Salt Mixture Containing Only Inorganic Constituents. Science, 75:339-340.
- Wie, Hull, Jenkins, Steinbrenner, and Bent. 1975. Statistical Package for the Social Sciences. McGraw-Hill Book Co.: New York.
- Wilk, S., and Stanley, M. 8 January 1977. Dopamine and Schizophrenia. Letter. Lancet, pp. 94-95.
- Wolterink. 1978. Personal communication.
- Woodson, H. W., Hier, S. W., Solomon, J. D., and Bergein, O. 1948. Urinary Excretion of Amino Acids by Human Subjects on Normal Diets. J.B.C., 172:613-18.
- Wool, I. G., Goldstein, M. S., Raney, E. R., and Levine, R. 1954. Role of Epinephrine in the Physiology of Fat Mobilization. Am. J. Ps1., 178:427-32.
- Wurtman, R. J. 1970. The Effects of Endocrine, Synaptic and Nutritional Inputs on Catecholamine Containing Neurons. Biochemistry of Brain and Behavior. Bowman, R. E., and Datta, S. P., Eds. Plenum Press:New York and London.
- Yager, J. D., Michtenstein, J., Bonney, R. J., Hopkins, H. A., Walker, P. R., Dorm, L. G., and Potter, V. K. 1974. Effects of Various Feeding and Exercise Regimens on Rat Growth and Survival. J. Nutrition, 104:273-86.
- Yuwiler, A., Oldendorf, W. H., Geller, E., and Braun, L. 1977. Effect of Albumin Binding and Amino Acid Competition on Tryptophan Uptake Into Brain. J. Neurochem., 28:1015-1023.

MICHIGAN STATE UNIV. LIBRARIES



31293102041740