The effect of exercise and training on VMA excretion

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THE EFFECT OF EXERCISE AND TRAINING ON VMA EXCRETION

By

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B.S., Northern State College, 1969

Presented in partial fulfillment of the requirements for the degree of

Master of Science

UNIVERSITY OF MONTANA

1970

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Date Aug 7, 1970
ACKNOWLEDGMENTS

The author wishes to express his gratitude and appreciation to Dr. Brian Sharkey, Dr. John Dayries, Dr. Galen Mell, Mr. Harley Lewis, Mr. Thomas Whidden, Mr. William Vogel, and Mr. Douglas Barnes for their contribution during the completion of this study.

The author is indebted to his wife, Smithe, for her exceptional sacrifice and understanding.

R.L.D.
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CHAPTER I

PROBLEM

I. INTRODUCTION

Driving a car through heavy traffic, working at a frustrating job, watching a child struggle with illness, quarreling with one's mate—the stress of life take infinitely varied form. And they can pose just as much of a challenge to health as bacteria, virus, malnutrition, or chemical and physical forces. (21)

Each man meets this challenge in his own way. Whatever the response, it involves the whole person; both body and mind play a part in dealing with the stresses of life. The entire organism reacts to an environment which it has interpreted as threatening.

These reactions have both physical and psychological aspects. The physical effects that result are dictated in part by the stimulus itself and in part by past experience.

Everyone sneezes if sufficient plant pollen is introduced into his nasal passages—this universal physical response has the effect of eliminating the pollen from the body. But in some people this response is very much exaggerated. Because of their genetic endowment and the effects of previous exposures, these people are allergic. They find pollen so threatening that they overreact to it, throwing up a defense that produces all the symptoms of a disease. . . . The symbolic meaning a situation has can also play a part in determining a physical response. (21)
Allergic people become so conditioned that they respond physically to the mere symbol of the thing they fear. The intensity of these conditioned responses depends not only on the conscious meaning that people assign to their experiences, but on their significance to the unconscious mind as well.

Throughout man's life most of the responses he makes are to such symbols and cues. No longer does he have to contend with the elements. Instead he must deal with schedules, traffic, noise, crowding, competition and other stressful situations imposed by man on man. Depending on the meaning of these stresses to him, man's reaction to stress may be out of proportion to the dangers it actually presents (21).

In any emergency, the overall response of the body generally falls into what has been called the "fight-or-flight" pattern, in which a series of internal changes occur. As described by Walter B. Cannon in 1915, the fight-or-flight response releases quantities of epinephrine into the bloodstream. This additional epinephrine serves a number of purposes; it calls forth stored carbohydrates from the liver, it mobilizes fats, it helps in distributing blood to the heart, lungs and central nervous system, it quickly abolishes the effects of muscular fatigue and it makes blood coagulate more readily (21).
In today's environment, some of the biological equipment that was necessary in earlier times may have become obsolete. As physiologist David A. Hamburg (21) puts it, "The contemporary human organism frequently gets mobilized for exertion but ends up doing little or nothing—preparation for action without action."

According to Selye (69), stress may be induced by physiological, psychological or sociological factors or by a combination of these factors. He further indicated that the body adjusts to these factors by discharging chemicals from the adrenal glands. These chemicals are corticosteroids. Cannon (14) believed that physiological, psychological, and sociological factors that stimulated epinephrine secretion were stressors. He introduced the idea that epinephrine serves the organism by aiding in the maintenance of homeostasis in emergencies.

The release of ACTH (Adrenocorticotrophic Hormone) is the basis for Ganong's (36) theory of stress. The release of increased quantities of ACTH stimulates the adrenal cortex to elicit the hormones specific for the stress response. Ganong's theory can be summarized to mean that stress is any stimulus that releases increased quantities of ACTH. Cannon's theory of stress included any stimulus that increased the activity of the adrenal medulla and Selye's theory included any stimulus which elicits the increased flow of
corticotropins. Figures 1, 2, 3 illustrate each theory.

Interpretations of the term stress have undergone many modifications. Presently any specific definition of stress depends upon the viewpoint of the researcher. Figure 4 is an illustration of Selye's, Cannon's and Ganong's theories as they relate to a working model that can account for each specific theory.

The validity of previous indexes of stress has posed many questions. Many of the early investigators used eosinophil count or the 17-Ketosteroid level as a measurement of stress. Yet, epinephrine can decrease the eosinophil count, whereas the secretion of androgens by testicular activity can influence the level of 17-Ketosteroids (81). Therefore, the index which appears to serve as a good indicator for the measurement of stress is epinephrine (29).

Because epinephrine (or catecholamines) have short half-lives it has been suggested that more reliable results can be obtained by estimation of catecholamine metabolites (29). From Figure 5 one can see that the major metabolite of the catecholamines is vanilmandelic acid or 3-methoxy-4-hydroxymandelic acid (VMA) (29).

Goodall (40) has shown that any situation which activates the sympathetic nervous system and prompts the release of epinephrine and norepinephrine will cause an
Figure 1. Selye's Theory of Stress

Figure 2. Ganong's Theory of Stress

Figure 3. Cannon's Theory of Stress

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Figure: Catecholamine Degradation

Epinephrine

\[\text{Epinephrine} \rightarrow \text{Metaepinephrine}\]

\[\text{Norepinephrine} \rightarrow \text{Normetaepinephrine}\]

\[\text{Dihydroxymandelic Acid} \rightarrow \text{VMA}\]

Key:
1. Catechol-O-methyltransferase
2. Monoamine Oxidase
Therefore, if a stressful situation occurs it could be measured by following VMA excretion in the urine.

In previous research (8, 29, 40, 65), exercise has been indicated to be a stressor, thus probably accounting for an increased epinephrine secretion. It has recently been shown that epinephrine was associated with the perceptual response of an individual to heavy exercise (35). Muscular work, if it is perceived as unpleasant, may act as a mental stressor (35, 40).

II. PROBLEM

Statement of Problem

The problem of this investigation was to determine the effect of training on VMA excretion and the effect of training on perceived exertion.

Significance of the Problem

Exercise is often used as a therapy for individuals who have high tendency toward coronary problems. A knowledge of what exercise does to factors which are associated with this tendency would be most helpful in determining a program for such individuals. According to Byers (11), three factors are most generally associated with high incidences of coronary disease. These are increased VMA
excretion, high serum cholesterol, and hastened blood clotting. Hopefully, this study will aid in clarifying the response of one of these factors, VMA excretion.

**Limitations of the Study**

This study was limited to five subjects due to the time involved in training and VMA analysis. A possible weakness of the study included the validity and reliability of Sunderman's (75) VMA analysis. This is not an absolute measurement of VMA which occurs in the body. Moreover, it was impossible to control the outside activities of the subjects which may have influenced the amount of VMA excreted in the three-hour collection period following exercise.

**Definitions**

For the purpose of clarification and understanding, the following definitions are used:

1. *Epinephrine*—employed to designate the naturally secreted product, the physiological secretion from the adrenal medulla gland.
2. *Norepinephrine*—employed to designate the naturally secreted product, the physiological secretion from the adrenal medulla gland.
3. *Stress*—any stimulus which causes an increase in the secretion of the adreno-medullary hormones.
4. VMA—vanilmandelic acid or 3-methoxy-4-hydroxymandelic acid, the end metabolic product of epinephrine and norepinephrine metabolism.

5. Excretion—the act or process of eliminating from the body, as through the kidneys.

6. Secretion—the act or process of releasing a substance within the body.

*In this study epinephrine and norepinephrine will be used rather than adrenaline and noradrenaline.
CHAPTER II
REVIEW OF LITERATURE

I. BACKGROUND

More than one hundred years ago Vulpain demonstrated the presence in the adrenal glands of a material which gave a green color with ferric chloride (45). He also showed that this material was present in the adrenal venous blood. Unknowingly, he was demonstrating the storage and release of a catecholamine (epinephrine). The presence in the adrenal medulla of a pharmacologically active material and its release under conditions of stress were well known before the end of the last century (45). Only in the last 20-30 years have significant quantities of a second catecholamine, norepinephrine, been demonstrated in the adrenal gland (5, 50) and sympathetic nervous tissue (45). A third amine, dopamine (dihydroxyphenylethyamine), has also been identified in the adrenal glands (71), and dopamine has now been shown to constitute a large proportion of the catecholamine fraction of sympathetic nerve fibers (45).

The presence in mammalian tissue of the three catecholamines, epinephrine, norepinephrine, and dopamine,
has been established. The cells in which these compounds are found are principally the cells of the sympathetic nervous system, including the related chromaffin tissue (45). The precursors of these tissues are derived from the neural crest of the embryo as primordial sympathetic ganglion cells; only after migrating outside the central nervous system do some of the ganglion cells become further differentiated as adrenal medulla cells (45). Catecholamines have also been reported in other mammalian organs such as the spleen (30), lungs (45), heart (45), and blood vessels (31). There is no evidence that the catecholamines are outside the sympathetic nervous tissue of these organs (45).

All three catecholamines, epinephrine, norepinephrine, and dopamine, have been found in the adrenal medulla (45); the relative amounts of each vary according to species. In the adrenal glands of adult primates there is very little norepinephrine, whereas in birds half or more of the adrenal catecholamines may be norepinephrine (45). There is good evidence that in some mammals, including man, norepinephrine is the predominant adrenal catecholamine in the fetal life (45). The proportion of epinephrine stored in the adrenal medulla increases as the animal becomes older (45).

Blascho and Welch (7) have demonstrated that catecholamines are held largely within intracellular particles.
Recently, by centrifugation of the intracellular particles in a specific gravity gradient, it has been possible to obtain two different types of granules (7). In electron microscopy, the less dense granules remaining near the top of the gradient show the characteristic morphology of mitochondria (45). The denser granules, which settle to the bottom of the gradient, contain very large amounts of catecholamines and ATP. Evidence shows that the largest fraction of the catecholamines is in a bound state in combination with an equivalent amount of ATP (77). The granules can be released from the cell by:

1. stimulation of the splanchnic nerves;
2. changes in toxicity;
3. changes in pH (77).

Histologists and cytologists have described changes in shape, location, and composition of the chromaffin granules during secretion (45). DeRobertis and Vaz Ferreira (23) have made electron photomicrographs of the adrenal medulla of rabbits before and after strong stimulation of the splanchnic nerves. They observed a reduction in the density of the chromaffin granules and in some cases they observed changes in the relationship of the granules to the cell membrane to which they became attached (45). The natural history of the chromaffin granules might be that they begin as small vesicles derived from endoplasmic
reticulum, gather catecholamines and ATP, increase in size and finally excrete their contents at the cell surface (45).

The catecholamines in sympathetic nerves consist of dopamine and norepinephrine; dopamine is found principally in the nonparticulate cytoplasm whereas a large part of the norepinephrine is located in granules (45). Thus there is a difference from the adrenal medulla where dopamine is largely intragranular (23). Nothing is known concerning any morphological changes occurring during the release of the catecholamines at the sympathetic nerve endings (45).

Although the adrenal medulla can secrete both epinephrine and norepinephrine, it seems that adrenergic neurons release primarily, if not totally, norepinephrine (60). In addition to norepinephrine, dopamine can be found; however, there is no evidence for dopamine release (45).

Similarities and differences between the action of a pharmacological agent at the synapse between the pre-ganglionic fiber and the chromaffin cell of the adrenal medulla have been reviewed by many workers. There are differences in response of sympathetic nerve cells and adrenal medulla cells to pharmacological agents. Thus cyanide or anoxia can cause the release of catecholamines from the adrenal medulla but depress the excitability of the sympathetic ganglion cells to acetylcholine (45).
Stresses such as anoxia and sudden excitement are known to cause liberation of catecholamines from the adrenal medulla and sympathetic nerves (13, 14). Stressing factors involving the catecholamine-producing system may either induce a reaction from the adrenal medulla provoking an increased release of epinephrine, or activate the nor-epinephrine-producing nerves, or both (29).

In general, epinephrine release is the most common response to a variety of stressing factors, particularly those which involve a certain degree of emotional discomfort (29). Also, epinephrine secretion has been related to emotional states of apprehension, discomfort, pain, and unpleasant feeling (29).

II. EPINEPHRINE AS IT RELATES TO EXERCISE

Muscular work may act as a mental stressor, if, for example, it is perceived as unpleasant. Under such circumstances catecholamine output may be expected to increase (35).

Muscular exercise has long been viewed as a stressor. In 1922, Hartman, Waite and McCordock (49) found that cats forced to run on a treadmill excreted large amounts of epinephrine. In mild exercise the amount of epinephrine excreted increased much later.

On the basis of an extensive study comprising

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different kinds of physical activity, Euler and Hellner (1952), as reported by Frankenhauser et al. (35), concluded that a positive correlation appeared to exist between the degree of "stress" involved in the work and the catecholamine output. The results may be interpreted as an indication that an increase in adrenal-medullary secretion occurs during muscular work when the work grows so heavy that feelings of emotional stress and unpleasantness are evoked. In this same study, as reported by Vendsolu (77), Euler and Hellner demonstrated an increased secretion of epinephrine and norepinephrine in the urine of men during strenuous muscular work, while during slight or moderate work the increase was insignificant. They also reported no change in the ratio at rest or work between epinephrine and norepinephrine. Thus Euler (29) states that a gradual elevation of norepinephrine and epinephrine occurs at submaximal workloads followed by a much greater increase when energy expenditure is near maximum or at maximal levels.

Hale (47) used 10 members of a laboratory staff to study epinephrine and norepinephrine and their relationship to heat tolerance. The relatively high catecholamine excretion in heat-acclimatized men provided support to the theory that epinephrine and norepinephrine contribute to heat tolerance.
III. EPINEPHRINE AND OXYGEN CONSUMPTION

The discovery of an increased oxygen consumption (calorigenic effect) in response to epinephrine has been attributed to Belawenez in 1903. The original Belawenez study pointed out that large amounts of epinephrine reduced both oxygen consumption and body temperature (57).

Neal (63) measured VMA of 19 men at rest and in graded exercise to determine energy expenditure and VMA excretion. He collected the control urine sample 150 minutes prior to exercising. Subjects performed Balke’s standardized physical working capacity test up to a heart rate of 160 beats per minute. He found a mean increase of 44 ug. per hour during exercise. VMA excretion and oxygen consumption were found to be linearly related.

Marshall (58), using six experienced subjects, measured the oxygen saturation of the femoral artery during exercise. After an injection of epinephrine the saturation was 85 per cent during exercise. He concluded that epinephrine increased the blood flow, not by the normal circulation of muscles, but through vessels which are not accessible for metabolic exchanges. He based this on Griffin's histologic evidence for the presence of shunts in muscles. Thus, exercise would increase the flow of blood through "nutritive" vessels; epinephrine and other vasodilator substances would increase flow through "non-nutritive vessels,"
and a combination of exercise and epinephrine would increase flow through both systems of vessels by equivalent amounts. Thus increased muscular activity, as a result of the action of epinephrine on the nervous system, may be quite important to calorigenic action.

Epinephrine increased oxygen consumption and it is four to ten times more effective than norepinephrine in that regard. The mechanisms underlying this calorigenic effect of epinephrine are still not clear. The most recent research using striated muscle, heart, or liver, showed that epinephrine increased the activity of phosphorylase a (57). It has been suggested that epinephrine affects the cellular mechanism by increasing the concentration of active enzyme phosphorylase a (57). During a period of exercise, epinephrine did not influence glucose uptake. Exercise alone is known to increase glucose use much above the resting level. The effect of exercise on glucose uptake may be explained by permeability changes, but more attention should be given to variations in phosphorylase a activity (57).

Epstein (26) exercised 16 males on a treadmill to determine exercise endurance. He then administered a Beta adrenergic blockade; l-isopropylamino-3-(1-naphthyloxy)-2-propanol hydrochloride. He retested the subjects and found that a 40 per cent reduction in exercise endurance occurred between the original test and the administration of the drug.
He concluded from this that Beta adrenergic blockade impaired the circulatory response to exercise. He explained his conclusion on the basis that sympathetic stimulation of the heart plays a significant role in the circulatory response to exercise. Its contribution is not very great, yet it would appear that sympathetic stimulation of the heart is only one of a number of mechanisms by which the cardiac output is augmented during exercise (26).

In a study to measure cardiac output, Allwood (1) injected the forearm of eight subjects with epinephrine. After 60 seconds of exercise there was an increased blood flow four times that of resting values and the cardiac output increased by three liters (1). After five minutes the blood flow had reduced to less than two times the blood flow at rest, but the cardiac output had remained the same. Allwood attributed the 25 per cent stroke volume increase to be the effect of epinephrine, not the effect of the exercise. Thus an interesting question arises; does exercise by itself cause the increased cardiac output or is it due to the increased secretion of epinephrine?

Development of tolerance to the various effects of epinephrine has been reported. Investigators reported a good calorigenic response to a specific dose of epinephrine in mice while a similar group of non-tolerant mice was killed with the same dosage.
IV. EPINEPHRINE AND NON-EXERCISE STRESS

Increased epinephrine excretion is known to take place as a result of hypoglycemia (22, 29) and after exposure to cold (29). These conditions may be termed metabolic stresses and are presumably different in character from those in which the emotional factor is dominant (29).

Goodall (41) tested subjects in a three-hour pre-run, a one-hour run followed by a one-hour post-run, and a three-hour post-run centrifugation to determine the adrenal medullary reaction to centrifugation. The male subjects showed marked elevation of urinary epinephrine and norepinephrine. Goodall related the epinephrine increase to anxiety of anticipating the centrifugation. He further stated that anxiety associated with riding was not due to physiological changes produced by the ride itself.

In a similar study Berman (6) used VMA to serve as an indirect measure of the sympatho-adrenal response to stress. Six military pilots were studied under various conditions. In forward acceleration and altitude tests, VMA increases of 123 per cent and 54 per cent were noted. There was no significant change in VMA when subjects were exposed to heat, isolation, and psychological testing. From these results Berman concluded that increased VMA reflected sympatho-adrenal discharges in response to emotional factors the subjects experienced on being exposed to acceleration.
Differentiation of responses is clearly discernible; thus, some stress conditions appear to involve only the adreno-medullary system without causing any marked reaction from the pituitary-adrenocortical axis and vice versa (29).

It has been demonstrated that the effect of ACTH on epinephrine synthesis is mediated by an action on the adrenal cortex. The effect of ACTH on epinephrine synthesis is not direct, but depends upon its ability to stimulate the secretion of glucocorticoids from the adrenal cortex. This suggests that PNMT (phenylethanolamine-N-methyl transferase) (Figure 6) activity might be controlled not by the levels of the glucocorticoids in general circulation, but by those present in the adrenal venous blood (84).

The total amount of epinephrine stored in the adrenal medulla depends upon several factors in addition to its rate of synthesis. These include the rate at which it is liberated and the rate at which it is destroyed by intra-adrenal enzymes. Thus, it seems likely that changes in adrenal epinephrine result from either decreased formation of the amines, or enhanced release, or both (84).

Using 12 men with behavior patterns associated with high incidences of coronary artery diseases (pattern A) and 11 men with behavior patterns associated with low incidences of coronary artery diseases (pattern B), Byers (11) demonstrated in increased excretion of VMA during the working
hours in men exhibiting pattern A as compared to those exhibiting pattern B. This strengthens the view that pattern A actually discharges more VMA and norepinephrine during the working day than pattern B. The VMA values were reported to be within the normal range for VMA excretion.

V. NOREPINEPHRINE

Ganong (38) states that norepinephrine is increased by emotional stresses with which the individual is familiar, whereas epinephrine secretion rises when the individual faces a situation in which he does not know what to expect.

Pineius (65) reported that subjects who were familiar, with the nature of a hospital test were quiet and demonstrated normal epinephrine and norepinephrine excretion, whereas those who were unfamiliar with the situation had an increased excretion of epinephrine and norepinephrine. The latter were also restless, asked many questions, and were anxious about the experiment. To further show the effects of the sympathetic aspect, Pineius collected urine samples 10 to 30 minutes before a hockey game and then three hours after the game. There was a sixfold increase in norepinephrine and a moderate increased in epinephrine before the game. Two players who did not play had no increase in norepinephrine whereas they had an appreciable increase in epinephrine.

Norepinephrine plays a dual role as a substance with
an independent biological action and as an intermediate in epinephrine formation (77). The steps for the formation of epinephrine according to Vendsolu (77) are shown in Figure 6.

Euler (29) has shown norepinephrine increases in the blood during heavy muscular work. However, Zuberk (85) has shown that increases in norepinephrine, not in epinephrine, occurred after severe restriction of the visual, auditory, and kinesthetic-proprioceptive stimulation.

As a rule norepinephrine release is less affected by emotional stimuli than epinephrine secretion. However, marked increases in norepinephrine liberation were observed in a situation involving strain on the blood pressure-homeostatic system (29).

VI. SUMMARY

Epinephrine has been shown to be affected by hypoglycemia, exposure to cold and heat. It also affects oxygen consumption either by increasing it or decreasing it. Several investigators have shown that epinephrine excretion increases during heavy exercise, whereas there is only a slight increase during moderate exercise.

Norepinephrine is generally associated with situations where the individual is unfamiliar and in exercise that places a strain on the blood pressure-homeostatic system.
Figure 6. Synthesis of Catecholamines

1. Phenylalanine hydroxylase
2. Tyrosine hydroxylase
3. Aromatic L-amino acid decarboxylase
4. Dopamine-Beta-oxidase
5. Phenylethanolamine-N-methyltransferase (PNMT)
CHAPTER III

PROCEDURE

I. THE SUBJECTS

Eight volunteer subjects were selected during winter quarter from general physical education classes in swimming, basketball, and physical conditioning. The criteria used in the selection were:

1. willing to partake in a training program
2. willing to collect urine samples for three hours a week
3. willing to follow the guidelines of no smoking, no alcohol, and no foods containing vanilla for a period of two days each week.

The subjects were given a demonstration and explanation of the research, its purpose, its importance, and their role as participants. They were instructed on the guidelines they were to adhere to and each received a printed copy of those guidelines (Appendix A). The subjects' physical characteristics appear in Table 1.
Table 1
Physical Characteristics of Subjects\(^a\)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Height (inches)</th>
<th>Weight (pounds)</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.F.</td>
<td>66.0</td>
<td>134</td>
<td>19</td>
</tr>
<tr>
<td>M.P.</td>
<td>68.0</td>
<td>158</td>
<td>18</td>
</tr>
<tr>
<td>R.S.</td>
<td>72.0</td>
<td>179</td>
<td>19</td>
</tr>
<tr>
<td>J.T.</td>
<td>65.5</td>
<td>142</td>
<td>19</td>
</tr>
<tr>
<td>R.V.</td>
<td>70.5</td>
<td>160</td>
<td>19</td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>68</td>
<td>154</td>
<td>19</td>
</tr>
</tbody>
</table>

\(^a\)Three subjects were eliminated from the study because of inconsistent urination or illness.

II. EQUIPMENT

Treadmill

Subjects were trained on a motor-driven treadmill located in the Human Performance Laboratory at the University of Montana. The speed of the treadmill was adjusted to 3.5 miles per hour.

Tektronix 410 Physiological Monitor

Heart rate was monitored on a Tektronix 410 Physiological Monitor. It is a portable, battery-operated oscilloscope with a trigger sweep on heart signals and direct heart rate readout.
Electrodes

The electrodes consisted of a patch-type moleskin adhesive with a small silver electrode. Electrode paste was applied to the skin and to the electrodes to insure conduction. In order to minimize muscle noise, the two electrodes were placed on the left and right fifth ribs, slightly lateral to and below each nipple.

The Spectronic 20 Colorimeter

The concentration of VMA was measured with a Spectronic 20 colorimeter. Light at a wavelength of 495 millimicrons was passed through each sample tube. The waves which were not absorbed were amplified and recorded on a meter indicating absorbency of the sample. Colorimeter test tubes were matched by filling with distilled water and a tube was randomly picked for a reference. Each tube was then compared to the reference tube.

III. TESTS

Estimation of VMA

The estimation of VMA was determined by the Sunderman Method (75). A slight modification of this method was used. The amount of reagents as prescribed by Sunderman were reduced by one-half. The modified procedure that was followed may be found in Appendix B.
The urine was first acidified by addition of 6N HCL. This was accomplished by adding the acid to containers before subjects were to fill them. Upon filling the containers, subjects were requested to store them in a cool place. Samples were collected on Wednesday and refrigerated until the following Friday and Saturday when the VMA analysis was completed.

The interfering constituents in the urine were removed by adsorption with Forisil and HCL. The actual extraction of VMA was first accomplished by the use of ethyl acetate followed by potassium carbonate. After the extraction of VMA, it was oxidized by a potassium ferrous cyanide complex and zinc sulphate to vanillin. Extraction of vanillin was accomplished by toluene and potassium carbonate. Color was developed by the reaction of vanillin and indole in an acidic solution (Figure 7).

**Modified Balke Treadmill Test**

The cardiovascular system seems to suffer some limitations when the heart rate reaches 180 beats per minute (81). Therefore, the exercise performed on the treadmill was terminated when the heart rate reached 180 beats per minute. The speed of the treadmill was set at 3.5 miles per hour. The grade was started at zero per cent for one minute, then was raised to two per cent at the beginning of the second minute. Thereafter it was
Figure 1. Color Reactions for VMA

Ferricyanide Oxidation

\[ \text{VMA} + 2K_3[Fe(CN)]_6 + 3H_2O \rightarrow \text{VMA} + 3K^+ + 6[Fe(CN)]_3^3- \]

Vanillin

Indole Color Reaction

\[ \text{Vanillin} + \text{Indole} + H_2PO_4^- \rightarrow \text{Indole} + \text{Vanillin} + HPO_4^{2-} + H^+ \]

Carbonium Salt (Salmon color)
raised one per cent every minute until the desired heart rate was reached. It should be noted that the heart rate was measured electronically only on Wednesdays. On the other two days of training, the subjects walked 30 seconds longer than the preceding time.

**Perceived Exertion Scale**

Subjective effect was measured with the aid of a rating scale constructed by Borg in 1962 (35). The scale was graded in 21 equal steps with the odd values from 3 to 19 being defined (Appendix C). The purpose for using such a scale was to give each student a basis for determining his perceived exertion and to determine the effects of training on the perception of exertion.

**IV. TESTING PROCEDURE**

A pre-test or observation period of one week preceded the actual testing. All subjects collected urine samples for the prescribed length of time on January 14, 1970. On January 16 and 19 the subjects walked on the treadmill using the Modified Balke Treadmill Test to a heart rate of 180 beats per minute. After a one-week rest period the subjects returned to the laboratory to begin the actual testing. Testing was conducted during the 1970 Winter Quarter.
The study began on Wednesday and subsequent meetings were Friday, Monday, and so on. The first meeting was a control day. Each subject urinated before coming to the laboratory and upon arriving at the laboratory each subject drank five milliliters (ml.) of water per kilogram (kg.) of body weight. Data forms were filled out. The remainder of this session consisted of instructions. There was no physical activity for any of the subjects this first meeting, and they collected their urine samples for the next three hours.

Subjects collected two urine samples totaling three hours each Wednesday after training. Sample one was up to one hour after training. Sample two was from one to three hours after training. Subjects were supplied with dark bottles containing 10 ml. of HCL. They collected their urine samples in these containers. All bottles were returned to the laboratory as soon as possible after collection.

Perceived exertion was measured when the subject had been on the treadmill six minutes and also when his heart rate was 150 beats per minute. This was accomplished by holding up a card (Appendix C) and asking the subject "how does it feel?". His numerical reply was then recorded.

On Friday the subjects completed the T180 treadmill walk. This marked the beginning of the actual testing.
Balke Treadmill Test

Before the control week, the subjects selected a time period during which they were able to report to the laboratory on Monday, Wednesday, and Friday. On these days the Modified Balke Treadmill Test was administered. This was carried out from February 2, 1970 to March 11, 1970.

Urine Collection and VMA Estimate

On Wednesday of each week, each subject was given two labeled containers for the collection of urine samples. The containers were for one- and two-hour samples. The subjects were told the importance of keeping the urine samples cool. They were also instructed to return the urine samples to the Human Performance Laboratory as soon as possible after the three-hour period. The urine samples were stored in a refrigerator until analysis was completed. The urine volume was measured in a 100 ml. graduated cylinder. Fifty ml. of urine were used from each sample and then subjected to the Sunderman method of analysis for VMA.

Training Procedure

Subjects were scheduled to train three times a week. Each subject reported to the laboratory at the predetermined
hour. They were wired for monitoring of heart rate and the Modified Balke Test was administered on Wednesdays. On Friday and Monday the subjects trained 30 seconds longer than the preceding time.
CHAPTER IV

RESULTS AND DISCUSSION

I. AVERAGE DAILY EXCRETION OF EPINEPHRINE AND RELATED COMPOUNDS

The hormones epinephrine and norepinephrine disappear very rapidly from the plasma. The amounts excreted in the urine, in relationship to the total amount secreted by the glands, are 0.5 to 2.0 per cent epinephrine and 3.0 to 6.0 per cent norepinephrine. A small portion of the hormones are excreted in conjugated form with a larger portion being excreted as VMA (69).

A 24-hour sample contains 25 to 50 mg. of free norepinephrine and 4 to 8 ug. of free epinephrine. This represents 1.5 and 3.0 per cent of the amounts of the free hormones respectively. The urine also contains VMA (0.7 to 6.4 mg./24 hr. or 29.6 to 2,666.6 ug./hr.) as well as very small amounts of dihydroxyphenylethylamine and dihydroxyphenylacetic acid (69).

The urinary level of VMA is influenced by the person's age (75), hypoglycemia, drugs, and stimulation of the sympathetic nervous system (69). Also, the time of the day affects the excretion of VMA (75). It has been shown that increased excretion occurs between the hours of 7 a.m. and

34
3 p.m. (75).

In the present study the average control excretion of VMA was 433.4 ug./hr. The range was 51.2 to 1052.7 ug./hr. for the control week. Table 2 contains the individual averages for the seven-week period.

II. THE EFFECTS OF EXERCISE ON VMA RESPONSE

The results of the VMA analysis are found in Table 2 (also Appendix D). The five subjects' average excretion of VMA was 433.4 ug./hr. for the control period. After the first Wednesday of exercise the average rose to 2505.9 ug./hr. The greatest individual increase was 5184.4 ug./hr., while the smallest individual increase was 1362.4 ug./hr. One individual did not increase during this period but decreased.

The mean group excretion decreased by 350.4 ug./hr. after the second week. However, two subjects had an increase during this time period. From this point on, the group mean continued to fall until the final control, which was administered during final examination week of the 1970 Winter Quarter.

Figure 8 illustrates the group trend in the excretion of VMA. As illustrated, the group mean rose during the initial part of the training program, then fell during the latter portion of the training program.
Table 2

Individual VMA Excretion Throughout Study Period

<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>wk.1</th>
<th>wk.2</th>
<th>wk.3</th>
<th>wk.4</th>
<th>wk.5</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.S.</td>
<td>1052.7a</td>
<td>682.3</td>
<td>2288.0</td>
<td>3783.3</td>
<td>844.8</td>
<td>1382.4</td>
<td>1091.4</td>
</tr>
<tr>
<td>M.P.</td>
<td>438.4</td>
<td>3097.2</td>
<td>2337.0</td>
<td>576.0</td>
<td>2627.0</td>
<td>789.3</td>
<td>695.6</td>
</tr>
<tr>
<td>J.T.</td>
<td>51.2</td>
<td>5235.6</td>
<td>1948.1</td>
<td>906.0</td>
<td>2346.0</td>
<td>863.3</td>
<td>1967.6</td>
</tr>
<tr>
<td>R.F.</td>
<td>353.7</td>
<td>1881.0</td>
<td>1442.9</td>
<td>2127.3</td>
<td>2111.6</td>
<td>573.6</td>
<td>2013.6</td>
</tr>
<tr>
<td>R.V.</td>
<td>271.0</td>
<td>1633.4</td>
<td>2761.8</td>
<td>1263.2</td>
<td>922.3</td>
<td>649.3</td>
<td>1444.1</td>
</tr>
<tr>
<td>(\bar{Y})</td>
<td>433.4</td>
<td>2505.9</td>
<td>2155.5</td>
<td>1731.2</td>
<td>1770.3</td>
<td>851.6</td>
<td>1446.3</td>
</tr>
</tbody>
</table>

\(^a\text{ug./hr.}\)
Figure 8. Average VMA Excretion, Treadmill Time, and Perception Rating Per Week.
To test the significance of VMA excretion an analysis of variance procedure was utilized (83). The between-subject and within-subject measures were not significant. However, the individual treatments were significant at the .05 level with an F ratio of 2.651 (Table 3).

To determine where the significance for the treatment measures was located, two post mortem tests were conducted: the Newman-Keule's method (Appendix E) and Scheffé method. The former showed significance to occur between control one and the first week of exercise. The latter test showed no significant differences.

III. EFFECTS OF EXERCISE ON PERCEPTUAL RESPONSE

For the most part there was little change within the perceived exertion scores at heart rate of 150 and at six minutes (Table 4). The averages for the first exercise perceptual responses at six minutes and heart rate of 150 were 9.6 and 11.4 respectively. The range for the first exercise was 5.0 to 12.0 for the six-minute perceived exertion scores and 11.0 to 13.0 for the 150 perceived exertion scores. When comparing six minutes to the total T180 times, it can be seen that the interval was relatively short. Therefore, six-minute perceived exertion scores were not subjected to further evaluation.
Table 3
Measure of Variance Showing Significance

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>S.S.</th>
<th>d.f.</th>
<th>M.S.</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.S. Between</td>
<td>1,440,865.0</td>
<td>4</td>
<td>360,216.2</td>
<td>.368</td>
</tr>
<tr>
<td>S.S. Within</td>
<td>36,970,452.4</td>
<td>30</td>
<td>1,299,015.0</td>
<td>1.33</td>
</tr>
<tr>
<td>S.S. Treatments</td>
<td>15,534,126.9</td>
<td>6</td>
<td>2,589,021.1</td>
<td>2.651</td>
</tr>
<tr>
<td>S.S. Residual</td>
<td>23,438,325.5</td>
<td>24</td>
<td>976,513.5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>41,317.4</td>
<td>34</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant at .05 level*
Table 4
Perceived Exertion at 150 H.R. and Six Minutes

<table>
<thead>
<tr>
<th>Subjects</th>
<th>C1</th>
<th>wk.1 min. 150</th>
<th>wk.2 min. 150</th>
<th>wk.3 min. 150</th>
<th>wk.4 min. 150</th>
<th>wk.5 min. 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.S.</td>
<td>12</td>
<td>13</td>
<td>7</td>
<td>12</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>M.P.</td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>11</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>JJ.T.</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>R.F.</td>
<td>9</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>R.V.</td>
<td>5</td>
<td>11</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>( \bar{x} )</td>
<td>9.6</td>
<td>11.4</td>
<td>8.6</td>
<td>10.4</td>
<td>9.4</td>
<td>11.0</td>
</tr>
</tbody>
</table>

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A $t$ test was conducted on the pre- and post-150 heart rate times. This test was significant at the .05 level with a $t$ score of 3.717 (Table 5 and Appendix F). It can thus be seen that a greater amount of work was accomplished at the end of the study to reach a heart rate of 150 than at the beginning. Since the perceived exertion scores remained approximately the same (10.6 at heart rate of 150) it is obvious that they were reported at a higher work load. Thus it can be said that training alters the perceptual response to exercise. However, the training did not seem to influence the heart rate-perceived exertion relationship.

IV. EFFECT OF TRAINING ON TREADMILL TIMES

The group mean during the control was 749 seconds, with a range of 630 to 945 seconds. The group mean continued to increase throughout the study. This can be seen in Figure 8. Although the group mean continued to increase, there were isolated incidences where individuals did not increase their T180 times (Table 6). Some of the isolated incidences of decline in treadmill times can be accounted for by subjects developing winter colds and sore throats.

V. DISCUSSION

In this study the amount of physical exercise was adjusted upward during the training period. The VMA level
Table 5
Heart Rate 150 Times--Pre- and Post-Training

<table>
<thead>
<tr>
<th>Subject</th>
<th>Pre</th>
<th>Post</th>
<th>D</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.S.</td>
<td>480a</td>
<td>590</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>M.P.</td>
<td>480</td>
<td>570</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>J.T.</td>
<td>735</td>
<td>765</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>R.F.</td>
<td>570</td>
<td>750</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>R.V.</td>
<td>570</td>
<td>610</td>
<td>40</td>
<td>3.717b</td>
</tr>
</tbody>
</table>

a seconds  

b significant at .05 level

Table 6
Individual T180 Treadmill Times

<table>
<thead>
<tr>
<th>Subject</th>
<th>Cl</th>
<th>wk.1</th>
<th>wk.2</th>
<th>wk.3</th>
<th>wk.4</th>
<th>wk.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.S.</td>
<td>790a</td>
<td>800</td>
<td>890</td>
<td>920</td>
<td>930</td>
<td>960</td>
</tr>
<tr>
<td>M.P.</td>
<td>630</td>
<td>720</td>
<td>810</td>
<td>870</td>
<td>810</td>
<td>860</td>
</tr>
<tr>
<td>J.T.</td>
<td>640</td>
<td>1030</td>
<td>1110</td>
<td>1080</td>
<td>1185</td>
<td>1185</td>
</tr>
<tr>
<td>R.F.</td>
<td>740</td>
<td>840</td>
<td>855</td>
<td>915</td>
<td>1005</td>
<td>975</td>
</tr>
<tr>
<td>R.V.</td>
<td>945</td>
<td>1050</td>
<td>910</td>
<td>930</td>
<td>960</td>
<td>935</td>
</tr>
<tr>
<td>X</td>
<td>749</td>
<td>888</td>
<td>915</td>
<td>943</td>
<td>978</td>
<td>983</td>
</tr>
</tbody>
</table>

10 minutes equals 600 seconds  
12 minutes equals 720 seconds  
14 minutes equals 840 seconds  
16 minutes equals 960 seconds  
18 minutes equals 1080 seconds

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increased during the early part of the training session, but started a decline toward the control levels in the later stages of training. The rise and fall in the level of VMA did not reflect the fact that the exercise was increased as the training program progressed.

Several studies (8, 29, 35, 40, 65) have suggested that a change in adrenal medulla activity may be due to emotional activity. If emotional activity was the factor which caused the increase in VMA, then an exercise program on the treadmill should have a greater amount of emotional activity at the beginning than at the end.

If one accepts a statement by Whiddon (81) that prolonged exercise program on the treadmill should have a greater amount of emotional stress at the beginning of training, when subjects are apprehensive, than at the end when they become familiar with the program...,

than one might agree with a statement by Euler (29), that epinephrine secretion is increased as a result of the emotional state of apprehension. On this basis it might be safe to assume that the increase in VMA was a result of epinephrine secretion. However, a one-week pre-test was conducted in which subjects were exposed to the entire exercise test. They trained on the treadmill, collected urine samples, and followed the general outline as described in Appendix A. Therefore, it might be suggested that the subjects were familiar with the testing situation and should
not have been apprehensive. Thus, it is interesting to look at statements by Ganong (38) and Pineius (65) in which they state that norepinephrine is increased by emotional stress with which the individual is familiar, and that epinephrine is increased by emotional stress in which the individual faces an unfamiliar situation.

From this it can be seen that two different conclusions about the same phenomenon can be reached. To clarify the situation, Euler (29) reports that a fair correlation between VMA and epinephrine exists, whereas VMA and norepinephrine have no correlation. With this information and that stated above, it would seem more reasonable to suggest that epinephrine caused the increased VMA excretion rather than norepinephrine.

The results of the Frankenhauser (35) study on subjective reactions to different physical activity indicated that an increase in adrenal-medullary secretion occurs during muscular work when the work grows so heavy that a feeling of unpleasantness is evoked. At a heart rate of 150 most subjects in this study perceived the work to be 11 or neither light or difficult (Appendix C). If it was assumed that a rating of 11 was equal to a heart rate of 150, a rating of 13 being equal to a heart rate of 160 and so forth, it can be seen that when a heart rate of 180 was reached a subjective feeling of extremely difficult would also be reached. It
would seem that a heart rate of 180 is unpleasant, especially if it is preceded by a period of increasing work. Therefore, it is probable that the increased VMA excretion during the early part of this study might have been due to the unpleasantness of vigorous exercise.

A significant difference occurred between the pre-150 heart rate time and the post-150 heart rate time, whereas perception did not change significantly. Thus more work was accomplished to reach a heart rate of 150 at the end of the study than at the beginning. Therefore, it would seem that perception is not related to the degree of work but to heart rate. This was in accordance with data reported by Frankenhauser (35). She found that catecholamine excretion was close to the control level at lower workloads (lower heart rate; lower perception) and increased at the higher workloads (higher heart rate; higher perception). Thus an almost linear relationship occurred between heart rate and perception.

Another interesting phenomenon is that Euler (29) has shown that norepinephrine was increased in the blood during heavy muscular work while Zuberk (85) has shown that complete removal of external stimulus increases norepinephrine. The increased norepinephrine during heavy muscular work is in accord with what might be expected. That is norepinephrine is increased when a strain is placed on the
blood pressure homeostatic system (4, 29, 38).

It was noticed that the average VMA excretion per hour had increased between the beginning control and final control and, for that matter, between week five and the final control (Table 2). Supposedly both control situations were identical. Subjects reported to the laboratory at predetermined times and then collected appropriate urine samples. There was no exercising. The final control was taken during the final examination week of the 1970 Winter Quarter. Therefore, the final control increase can probably be attributed to the emotional factors associated with examinations. This data agrees with those reported by other authors and supports the contention that the increased VMA excretion responds to an emotional rather than strictly physical stimulus. It is of interest to note that even after a five-week training period physical activity may not have caused a decrease in emotional response to an apprehensive situation. This may be in direct conflict with what some authors believe regarding possible adaption to non-exercise stress situations.

It can be suggested that the early increase in VMA was caused by epinephrine evoked by the unpleasant aspects of the task. Also, it can be suggested that part of the increase in VMA each week would be caused by norepinephrine because of its relationship to the cardiovascular response to exercise.

Exercise has the possibility of affecting the
adaptive mechanism of the adrenal glands without itself increasing the glands activity (50). Therefore, an advantage of exercise lies in the fact that it may stimulate the defense mechanism of stress, not that it is similar to stress (50). If so, then it is possible for exercise to reduce the factors associated with high incidences of coronary artery disease. These factors, as speculated by Byers (11), may be increased VMA excretion, increased serum cholesterol and hastened blood clotting time.

In this study, the results suggest that exercise itself does not cause increased VMA excretion, but it is increased by the psychological factors that accompany the exercise. This is in accord with the results obtained by Frankenhauser (35). She attributed increased VMA excretion to unpleasant exercise.

The literature is vague and inconsistent in regard to the level of serum cholesterol during exercise whereas blood clotting is definitely enhanced by exercise. Whiddon (81) has shown that blood clotting is significantly faster at the beginning of a training program than at the end. Therefore, caution must be observed when exercise is used as a therapy for people who have coronary problems or for those who tend toward such problems.
CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

I. SUMMARY

The purpose of this study was to determine the effect of exercise and training on VMA excretion. Subjects did not perform any physical exercise during the control week. At this time subjects collected a one- and two-hour urine sample. After the control week the Modified Balke Treadmill Test was administered every Monday, Wednesday, and Friday for a period of 5 weeks. On Wednesday of each training week, subjects collected one and two-hour urine samples.

The control VMA excretion was within the normal excretion values for normal individuals. During the first week a sharp rise was noted with some of the values being outside the normal limits. From the second week of training until the conclusion of training a downward trend was observed.

Although the evidence of this study suggests that emotional activity played a role in causing the increased VMA excretion, it is not possible to specify which of the catecholamines caused the increase. Also, it is probable
that outside emotional factors contributed to the VMA excretion.

II. CONCLUSIONS

On the basis of the results found in this study the following conclusions can be made:

1. The increased excretion of VMA occurs as a result of psychological factors associated with exercise rather than the physical activity itself.

2. An individual's perceived exertion seems more related to his fitness and heart rate than to the actual work load.

III. RECOMMENDATIONS

In view of the findings of this study the following recommendations have been made. Further study should be done:

1. to determine if different training heart rates (intensities) are related to catecholamine secretion;

2. to determine if age and training are related to catecholamine secretion;

3. to determine the relationship of competitive, exhaustive activity to catecholamine secretion; and
4. to determine if exercise training reduces the psychic reactivity to non-exercise stressors.
SELECTED BIBLIOGRAPHY


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Gentlemen:

We appreciate your cooperation in this experiment. To insure that our combined efforts are not wasted we ask you to do your best to comply with a few necessary regulations. Since this is an evaluation of training effects it is imperative that you avoid strenuous exercise, especially that which would prompt rapid breathing and rapid heart rates. We further ask that you be as accurate as possible when completing the short daily reports, particularly the items referring to prior exercise.

The following is a list of guidelines and requirements for you to observe during the next eight weeks. Please observe this list.

1. You will need tennis shoes.

2. When exercising on the treadmill you will have the best results by walking erect. Place the heel of the foot first on each step. (Exercising will be every Monday, Wednesday, Friday).

3. You are responsible for filling out your data sheet each time you report to Lab (M-W-F). This should be done before exercising.

4. For the next eight weeks you are asked to refrain from the following things from TUESDAY THROUGH THURSDAY.
   A. No smoking.
   B. No alcoholic beverages.
   C. No foods containing vanilla (candy, cake or cookies).

5. Please do not engage in any type of vigorous physical activity outside of your laboratory exercising.
6. Follow your normal living habits with the above exceptions from Tuesday through Thursday.

7. On Wednesdays:
   A. Urinate before coming to lab.
   B. Fill out Lab. report.
   C. Before exercising in Lab. drink 5 ml. of water/kg. body weight.
   D. Collect urine samples for the next three hours following exercise on Wednesday. The three hours will be broken down into two periods of one hour and two hours. That means you should urinate at the end of one hour, and then after three hours from the time you exercised and then return the samples to the Lab. as soon as possible.
   E. You will be supplied with dark bottles to collect the urine in. They will contain ten ml. of HCL so be careful!!
   F. Store your urine bottles in a cool place and/or return them to the Lab. as soon as possible.
   G. The hours that someone will be in the Lab. will be posted on the outside door. You may return your urine samples during these periods or call 542-0183.

Directions under number 7 must be observed every Wednesday for the eight-week period. Please do your best to follow them.

Try to keep training appointments and we will do our best to keep them brief. If for reason of illness, accident, etc. you cannot come, do let us know.

I do hereby volunteer my services for this experiment. I understand what is expected and will fulfill the regulations which are required of me.

Signature of Subject __________________________
Local Address __________________________________
Local Phone _________________________________
Date _________________________________________
APPENDIX B
CHEMICAL PROCEDURE

1. Collection of Urine
   A. 10 m. of 6N HCL was placed in sample containers
   B. Stored in refrigerator
   C. Volume measured

2. Adsorption of Interfering Constituents
   A. 50 ml. of urine was placed in a flask
      1. Add 5 ml. of conc. HCL
      2. Add 5 g. of Forisil
      3. Swirl for 10 minutes and filter

3. Extraction of Vanilmandelic Acid
   A. Set up the following tubes
      1. 5 ml. of urinary filtrate
      2. 5 ml. of urinary filtrate
      3. .5ml of vanilmandelic acid working standard plus 5 ml. of conc. HCL
      4. .5 ml. of conc. HCL plus 5 ml. of water
   B. 22.5 ml. ethyl acetate to each tube
      1. shake
      2. centrifuge
      3. discard infranatant layer
   C. 2.5 ml. of Potassium Carbonate to each tube
1. shake
2. centrifuge
3. **discard supernatant layer**

D. 1.25ml. of conc. HCL is added immediately to extract
1. shake
2. let stand for 5 minutes

4. Oxidation of Vanilmandelic Acid

A. .5 ml. of Potassium Ferrous Cyanide Complex
   1. none (urine blank)
   2. add here
   3. add here (standard)
   4. add here (reagent blank)

B. .5 ml. of Zinc Sulphate to **all tubes**
   1. shake
   2. place in water bath at 37°C for 2 hours

5. Extraction of Vanillin

A. 22.5 ml. of Toluene to **each tube**
   1. shake
   2. centrifuge
   3. remove infranatant layer and discard
   4. recentrifuge

B. **Each Toluene extract was decanted into another tube**
   1. add 1.7 ml. of Potassium Carbonate
   2. shake
   3. remove 1.5 ml. of infranatant layer
      place in 8 ml. test tube

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a. blow gently to remove Toluene
b. place in ice bath

6. Indole Color Reaction

A. Add .25 ml. of conc. Sulfuric to each tube in ice bath

B. Add 2.0 ml. of Indole-Phosphoric to each tube

1. invert once
2. replace in ice
3. let stand for 5 minutes

7. Measure Optical Density at 495 millimicrons
APPENDIX C

PERCEPTION RATING

1.
2.
3. Extremely Light
4.
5. Very Light
6.
7. Light
8.
9. Fairly Light
10.
11. Neither Light Nor Difficult
12.
13. Fairly Difficult
14.
15. Difficult
16.
17. Very Difficult
18.
19. Extremely Difficult
20.
21.
## APPENDIX E

### Table 7

**Individual UV-A Excretion by Sample**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Cl</th>
<th>wk.1</th>
<th>wk.2</th>
<th>wk.3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 hr.</td>
<td>2 hr.</td>
<td>1 hr.</td>
</tr>
<tr>
<td>R.S.</td>
<td></td>
<td>1425.2</td>
<td>1743.4</td>
<td>731.0</td>
</tr>
<tr>
<td>M.P.</td>
<td></td>
<td>192.0</td>
<td>1123.2</td>
<td>--</td>
</tr>
<tr>
<td>J.T.</td>
<td></td>
<td>61.8</td>
<td>91.8</td>
<td>13870.0</td>
</tr>
<tr>
<td>R.F.</td>
<td></td>
<td>588.0</td>
<td>473.6</td>
<td>3366.0</td>
</tr>
<tr>
<td>R.V.</td>
<td></td>
<td>72.0</td>
<td>759.0</td>
<td>2677.2</td>
</tr>
<tr>
<td><strong>X</strong></td>
<td></td>
<td>465.8</td>
<td>838.2</td>
<td>5161.1</td>
</tr>
</tbody>
</table>
### Table 7 (Cont'd.)

<table>
<thead>
<tr>
<th>Subject</th>
<th>wk.4</th>
<th></th>
<th>wk.5</th>
<th></th>
<th>C2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hr.</td>
<td>2 hr.</td>
<td>1 hr.</td>
<td>2 hr.</td>
<td>1 hr.</td>
<td>2 hr.</td>
</tr>
<tr>
<td>R.S.</td>
<td>--</td>
<td>2534.4</td>
<td>--</td>
<td>4147.2</td>
<td>1176.6</td>
<td>2097.6</td>
</tr>
<tr>
<td>M.P.</td>
<td>--</td>
<td>7881.0</td>
<td>--</td>
<td>2368.0</td>
<td>--</td>
<td>2086.8</td>
</tr>
<tr>
<td>J.T.</td>
<td>--</td>
<td>7038.0</td>
<td>--</td>
<td>2590.0</td>
<td>2072.8</td>
<td>3754.8</td>
</tr>
<tr>
<td>R.F.</td>
<td>3131.0</td>
<td>3203.8</td>
<td>347.2</td>
<td>1373.6</td>
<td>2612.8</td>
<td>3427.2</td>
</tr>
<tr>
<td>R.V.</td>
<td>1221.2</td>
<td>1545.6</td>
<td>1200.0</td>
<td>748.0</td>
<td>855.6</td>
<td>3476.8</td>
</tr>
<tr>
<td>X</td>
<td>2176.1</td>
<td>4440.6</td>
<td>773.6</td>
<td>2245.4</td>
<td>1713.1</td>
<td>2968.6</td>
</tr>
</tbody>
</table>
APPENDIX E

FORMULAS FOR MEASURE OF VARIANCE AND
NEWMAN-KEULS TEST

1. Measure of Variance.
   A. \[ \frac{G^2}{n} \]
   where:
   \( G = \) sum of each \( P_i \) or each \( T_j \)
   \( n = \) number of subjects

   B. \[ \sum \sum X^2 \]
   where:
   \( \sum X = \) each subject score

   C. \[ \frac{\sum T_j^2}{n} \]
   where:
   \( \sum T_j = \) sum of each treatment
   \( n = \) number of subjects

   D. \[ \frac{\sum P_i^2}{k} \]
   where:
   \( \sum P_i = \) sum of each subject's score each week
   \( k = \) number of treatments

A. \[ q_{1-\alpha} (r \sqrt{f/nMS_{\text{error}}}) \]

where:
- \( q_{1-\alpha} \) = level of confidence
- \( r \) = distance between two treatments
- \( f \) = degrees of freedom for error of mean square
- \( n \) = number of subjects
- \( MS_{\text{error}} \) = error of the Mean Square
APPENDIX F

FORMULA FOR TESTING THE DIFFERENCE BETWEEN MEANS-CORRELATED DATA FOR PRE-POST TEST SCORES

1. \[ \sum d^2 = \sum D^2 - \left( \frac{\sum D^2}{N} \right) \]
   
   where: \( D \) = difference from pre to post score
   \( \sum D^2 \) = sum of differences squares
   \( N \) = total number of subjects
   \( \sum d^2 \) = sum of squares for the differences

2. \[ S_D = \sqrt{\frac{\sum d^2}{N}} \]

   where: \( S_D \) = standard deviation of the differences

3. \[ S_D^- = \frac{S_D}{\sqrt{N-1}} \]

   where: \( S_D^- \) = standard error of mean differences

4. \[ t = \frac{D}{S_D} \]

   where:
   \( \overline{D} \) = mean difference

   The \( t \) value was located in a table of Distribution of \( t \) probability with appropriate degrees of freedom.