TISSUE METABOLISM OF GUINEA PIGS DEFICIENT IN THE ANTI-STIFFNESS FACTOR

by

DONALD HOWARD SIMONSEN

A THESIS
submitted to the
OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of
MASTER OF ARTS

June 1945
APPROVED: Redacted for Privacy

Assistant Professor of Biochemistry
In Charge of Major
Redacted for Privacy

Head of Department of Chemistry
Redacted for Privacy

Chairman of School Graduate Committee

Chairman of State College Graduate Council
ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Dr. W. J. van Wagendonk for his valuable advice. The author also wishes to thank Miss Patricia Hackett for her assistance in making the determinations.
**TABLE OF CONTENTS**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Experimental Apparatus</td>
<td>5</td>
</tr>
<tr>
<td>Preparation of Tissues</td>
<td>11</td>
</tr>
<tr>
<td>Determination of $Q_{O_2}$</td>
<td>13</td>
</tr>
<tr>
<td>Determination of $Q_{N_2}$</td>
<td>15</td>
</tr>
<tr>
<td>Diet</td>
<td>17</td>
</tr>
<tr>
<td>Results</td>
<td>18</td>
</tr>
<tr>
<td>Table I</td>
<td>19</td>
</tr>
<tr>
<td>Table II</td>
<td>20</td>
</tr>
<tr>
<td>Table III</td>
<td>21</td>
</tr>
<tr>
<td>Table IV</td>
<td>22</td>
</tr>
<tr>
<td>Discussion</td>
<td>23</td>
</tr>
<tr>
<td>Summary</td>
<td>26</td>
</tr>
<tr>
<td>Protocol I</td>
<td>27</td>
</tr>
<tr>
<td>Bibliography</td>
<td>28</td>
</tr>
</tbody>
</table>
TISSUE METABOLISM OF GUINEA PIGS DEFICIENT IN THE ANTI-STIFFNESS FACTOR

INTRODUCTION

In the previous papers by van Wagtendonk and co-workers a study has been made of the physiology and biochemistry of a new dietary factor for guinea pigs. The occurrence of a characteristic syndrome was first noted by Wulzen and Bahrs (3, 44, 45), when guinea pigs were raised on a diet composed of skim milk, to which 10% of skim milk powder, adequate amounts of copper, iron, carotene, and orange juice had been added. One of the first outward signs of the deficiency is the appearance of a stiffness at the wrist joints of the experimental animals. In the advanced stages of the deficiency, a marked atrophy of the musculature is noted, and calcium deposits are found in many body tissues. Cures of deficient animals have been effected by the administration of a diet containing foods rich in the anti-stiffness factor. van Wagtendonk and Wulzen (42) reported the partial isolation of the factor from raw cream. The factor is also present in several plant materials (personal communication).

Marked changes occur in the phosphorous distribution in the liver and kidneys of the experimental
animals (37). The concentration of the easily hydro-
lyzable phosphorous is lowered considerably. This
fraction of the total phosphorous accounts for 67% of
the adenosine triphosphate and 50% of the adenosine
diphosphate. Paralleling these changes in the liver
and kidneys, a drop in the concentration of adenosine
triphosphate and creatine phosphate occurs in muscle
tissue (39). Adenosine triphosphate, adenosine diphos-
phate, and creatine phosphate play an important role
in the energy relationship during the fermentative
carbohydrate breakdown (21). Any significant changes
in the concentration of these energy-rich compounds
should inhibit the anaerobic breakdown and shift the
respiration over to the direct utilization of oxygen
and subsequent production of carbon dioxide.

Many workers have measured the changes in the
respiratory rates of animal tissues during the various
vitamin deficiencies. It has been noted that a muscular
dystrophy is generally accompanied by an increased
oxygen consumption in the muscle (1, 9, 22).
Kaunitz and Pappenheimer (17) observed changes in the
in vitro oxygen consumption of rat and chick muscles
during vitamin E deficiency. The oxygen consumption
of both the rat and chick muscles increased while
the oxygen uptake of the rat liver remained unchanged.
Houchin and Mattill (15, 16) have confirmed these results with experiments on the muscle tissue from hamsters and rabbits deficient in vitamin E.

A few reports in the literature mention the influence of a vitamin A deficiency upon the respiratory rates of rat tissues. Ruddy (26) reported no changes in the liver respiration, while Shibata (30) found a slight increase in the oxygen consumption of the liver and a decrease in the oxygen uptake of the kidney. Chevallier and Rour (4) investigated the effects of supplementing the normal substrate with varying concentrations of vitamin A. No correlation was found between the vitamin A concentration and the respiratory rate of rat and guinea pig livers.

Stotz, Harrer, Schultze, and King (32) observed a marked increase in the oxygen uptake of liver from scorbutic guinea pigs, as compared to that of normal animals. Shibata (30), however, reported no changes in the liver respiration, and only a slight inhibition of the respiratory rate for the kidney of scorbutic guinea pigs.

The effects of a thiamine deficiency upon the respiratory rate of rat tissues have been described by several investigators. Goldschmidt and Lewin (13)
reported a decrease in the oxygen uptake of rat liver slices. According to Muus, Weiss, and Hastings (24) the ventricular muscle tissue of the rat heart retains its normal rate of respiration whereas the rate of oxygen consumption of auricular tissue is double. The same investigators (25) reported the effects of deficiencies of other members of the B complex upon the respiratory rate of rat liver and diaphragm muscle. The diets studied were deficient in riboflavin, in the heat stable components of the B complex, or deficient in both. It was found that the diaphragm respiration was accelerated only during a riboflavin deficiency, while the diet supplemented with riboflavin but lacking the heat stable components of yeast produced a decrease in the liver oxygen consumption.

The present investigation was undertaken as a part of a project to determine the effect of the administration of a diet lacking the anti-stiffness factor, upon the in vitro respiratory mechanisms of guinea pig tissues.
EXPERIMENTAL

Apparatus: The manometric technique of studying the metabolism of tissues was originated by Warburg (43) in his studies on tumor tissues. From his earlier work on the metabolism of sea-urchin eggs, a technique was developed which was applicable to the studies of tissue metabolism. The present accepted techniques are well covered by Dixon (6) and in the new manual by Umbreit, Burris, and Stauffer (35). In this study, several relatively new techniques have been used to advantage.

The determination of the oxygen consumption ($Q_{O_2}$) and the anaerobic glycolysis ($Q_{N_2}/Q_{CO_2}$) was made with the simple Warburg apparatus. This apparatus consists of a water bath and fourteen manometers with conical flasks containing three compartments, i.e., a main compartment for the tissue, an inset (center cup), and an onset (side cup). One of the manometers was used as a thermobarometer. The manometers were mounted on a shaking mechanism which was set to operate at the rate of 120 complete cycles per minute with an

1) The symbols, $Q_{O_2}$ and $Q_{N_2}/Q_{CO_2}$ are used to denote the following entities:

- $Q_{O_2}$ oxygen uptake in mm.$^3$/mg. dry weight/hour
- $Q_{N_2}/Q_{CO_2}$ carbon dioxide in mm.$^3$/mg. dry weight/hour.
amplitude of 5 cm. This provided adequate equilibrium conditions between the gas and liquid phases. The temperature of the water bath was maintained at 37 ± 0.02° C. by means of a metastatic, mercury thermo-regulator.

The determinations are based on the following principle: In a constant volume of gas, kept at a constant temperature, changes in the amount of gas can be measured by changes in the pressure. A constant volume is maintained in the reaction system by adjusting the level of the manometer liquid in the closed arm to a fixed reference point. The manometer fluid is prepared according to Brodie (6) and consists of: 500 cc. water, 23 g. sodium chloride, 5 g. sodium tauroglycocholate, and a few drops of an alcoholic thymol solution. The solution was colored with methyl violet. Ten thousand mm. Brodie solution is equivalent to 760 mm. mercury.

For the purposes of calculation, the constants for the manometers have to be determined. Various chemical methods have been proposed for the calibration of the manometer vessels. These usually consist of the quantitative liberation of a gas from a known amount of reagent and measurement of the resulting pressure changes. The most accurate method is that recommended
by Summerson (33) in which the evolution of nitrogen resulting from the action of an excess of alkaline hydrazine solution on a standard solution of potassium iodate is measured manometrically. Calibration of the vessels with mercury is more accurate and was therefore selected. According to the method of Schales (28), the reaction flasks and manometer were filled with mercury to the 150 mm. mark on the closed arm of the manometer. The mercury was removed from the system and weighed on an analytical balance. Check calibrations were made in every case, by refilling the system and reweighing the mercury. Checks were considered satisfactory if two successive weighings agreed within 100 milligrams. The true volumes of the systems were then obtained by dividing the weight of the mercury in the vessel by the density of the mercury at the temperature of calibration.

The constants of each vessel were then calculated according to the following method: (6)

\[ x = \text{the amount of gas evolved in mm.}^3 \text{ at normal temperature and pressure} \]
\[ h = \text{the reading of the manometer} \]
\[ v_G = \text{the volume of the gas space in the vessel} \]
\[ v_F = \text{the volume of liquid in the vessel} \]
\[ T = \text{the absolute temperature of the water bath} \]
\[ P = \text{the initial pressure in the vessel} \]
equal to the barometric pressure)

\[ P_0 = \text{the normal pressure (760 mm. Hg) in mm. of manometric fluid.} \]

If \( D \) is the density of the fluid,

\[ P_0 = 760 \frac{13.60}{D} \]

\( P \) = the vapor pressure of water at temperature \( T \)

\( \alpha \) = the solubility of the evolved gas in the liquid in the vessel (in mm.\(^3\) of liquid when in equilibrium with a partial pressure of the gas equal to \( P_0 \))

The initial amount of gas in gas space =

\[ v_G \left[ \frac{273}{T} \right] \left[ \frac{P - P_0}{P_o} \right] \]

The initial amount of gas dissolved =

\[ v_F \alpha \left[ \frac{P - P_0}{P_o} \right] \]

The final amount of gas in the gas space =

\[ v_G \left[ \frac{273}{T} \right] \left[ \frac{P - P_0 + h}{P_o} \right] \]

The final amount of dissolved gas =

\[ v_F \alpha \left[ \frac{P - P_0 + h}{P_o} \right] \]

The initial amount of gas plus \( x \), the amount produced during the experimental period, must equal the amount finally present. From this relationship, the following expression is derived.
It will be noted that the expression in the brackets remains constant during the experiment. This is known as the constant of the apparatus, \( k \). Thus, the basic equation for calculating changes occurring in the apparatus reduces to

\[
x = \left( \frac{\nu_G \frac{273}{T} + \nu_F}{P_0} \right) h
\]

The tissues for the experiments reported here (liver, kidney, and muscle) were prepared in a slicing chamber maintained at a temperature of 0 - 5°C. As has been noted by Fuhrman and Field (11) maintenance of the tissues at a low environmental temperature during their preparation for use in the Warburg apparatus, insures a much greater degree of constancy in the respiratory rates. A cold chamber similar to that described by these investigators was used. The walls were made of three-quarter inch plywood. The box was 18 inches high, 26 inches deep, and 36 inches wide, insulated inside with three-quarter inch insulating board, and lined with galvanized iron. A 10 x 32 inch glass window was hinged at the top of the box on a 45° angle with the front of the box. The front consisted of a rubber panel equipped with a 36 inch zipper.
Figure I

Cold Chamber during Slicing Operation
so that the hands could be placed within the chamber during the slicing operations. This insured the proper cold conditions for the preparation of the tissues. A fluorescent light was installed in the rear panel of the box behind a sheet of diffusion glass. This provided an ideal lighting arrangement for the box. The cooling mechanism consisted of a large household refrigerator coil and compressor unit. The box was finished in white enamel.

Preparation of Tissues: The experimental animals were killed by decapitation or a blow on the head. The organs were removed and placed in the cold box immediately after the death of the animal. The necessity of obtaining slices of uniform thickness with a minimum of damage to the tissues was pointed out by Warburg (43). Various methods were used, depending upon the tissues chosen for the experiments. The liver slices were prepared with the instrument described by Martin (23). With this instrument, slices of uniform thickness were readily obtained. The kidney was sliced by means of a modified Terry slicer (34). The muscle strips were teased out of the hind legs of

2) The slicers were obtained from Mr. F.D. Banham, Corporation Yard, Stanford University, California.
Figure II

View through Window of Cold Chamber during Slicing Operation
the animals with forceps by means of the method of Shorr (31).

Slices of the tissues were weighed in small drying tubes and placed in a 105° C. drying oven until a constant weight was attained. The tissues to be used in the experiments were weighed rapidly on glass weighing pans and placed in the reaction flasks.3

**Determination of the $Q_{O_2}$**: For the determination of the $Q_{O_2}$ values, 0.3 cc. of 20% potassium hydroxide was pipetted into the inset, and a small roll of filter paper (Whatman #40) of such size that it barely extended above the lip of the cup was inserted.4 This served to insure the rapid absorption of the carbon dioxide produced by providing a large surface for the uptake of the gas. Following this, two cubic centimeters of a Dickens and Greville (5) phosphate Ringer solution of the following composition was added to the main chamber of the flask:

| sodium chloride | 0.120 M |

3) Although a microtorsion balance is to be recommended for this type of work, the balance used (a Chainomatic balance equipped with a magnetic damper) was found to be satisfactory for the rapid determination of the weights of the tissue used.

4) It is found advisable to place the potassium hydroxide in the inset before adding the reaction media, as any spilling into the outer chamber is more readily noticed in the dry flask.
The solution was buffered to a pH of 7.4 and saturated with oxygen before using.

As soon as the tissues were weighed and placed in the medium, the flasks were fastened to the manometers. Anhydrous lanolin was found to be satisfactory for greasing the joints. Purified oxygen (soda lime) was then passed through the system at an over pressure of approximately 100 mm. of brodie fluid, for about ten minutes. The manometers were transferred to the water bath, and a period of 15 minutes was allowed for the thermal equilibration. At the end of this period, the manometer fluid was adjusted to the 150 mm. mark on both arms of the instrument, by momentarily opening the stopcocks. After recording the zero reading, the shaking was started. Readings were recorded at ten minute intervals during a reaction period of ninety minutes. For all of the experiments, at

5) The phosphate buffer consisted of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (0.005 M) and $\text{Na}_2\text{HPO}_4$ (0.005 M). The phosphates were dissolved in water. This solution was added to the other components, also dissolved in water, to insure complete solution of the phosphate and prevent the precipitation of calcium phosphate.
least one duplicate determination was made on the same tissue and in a number of cases, triplicate determinations were carried out. The results were then calculated as shown in the protocol of an actual experiment (Protocol I).

**Determination of the** \( \frac{N_2}{CO_2} \): The determination of the anaerobic glycolysis was carried out in the same manner as the determination of the \( Q_{O_2} \), with the exception that a different substrate and gas mixture were used, and the alkali was omitted from the center inset. A bicarbonate Ringer solution was prepared according to Krebs and Henseleit (19).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Percentage</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>sodium chloride</td>
<td>0.90%</td>
<td>100 cc.</td>
</tr>
<tr>
<td>potassium chloride</td>
<td>1.15%</td>
<td>4 cc.</td>
</tr>
<tr>
<td>calcium chloride</td>
<td>1.22%</td>
<td>3 cc.</td>
</tr>
<tr>
<td>( KH_2PO_4 )</td>
<td>2.11%</td>
<td>1 cc.</td>
</tr>
<tr>
<td>( MgSO_4 \cdot 7 H_2O )</td>
<td>3.32%</td>
<td>1 cc.</td>
</tr>
<tr>
<td>sodium bicarbonate( _6 )</td>
<td>1.30%</td>
<td>21 cc.</td>
</tr>
<tr>
<td>glucose</td>
<td></td>
<td>0.26 g.</td>
</tr>
</tbody>
</table>

This mixture was thoroughly saturated with the gas.

6) To maintain the proper conditions of pH, it is necessary to saturate the solution of bicarbonate with carbon dioxide before mixing the other components of the solution.
mixture and stored for use in a well-stoppered bottle.

Since anaerobic conditions had to be maintained, a 95% nitrogen-5% carbon dioxide gas mixture was used. This mixture was purified by passing the gas over a hot copper screen. The purification unit was modelled after that described by Savage and Ordal (27), and consisted of a 50 inch Calrod over heating unit around which was wrapped a medium mesh copper screen. This screen was wrapped as tightly as possible, and packed into a Pyrex tubing (35 mm. diameter). Side-arms were sealed onto the tube for the inlet and outlet of the gas. The ends of the tube were packed with asbestos and tightly corked. The wire leads were run through the corks and gas-tight seals were made with picene. The outflowing gas was passed through a copper cooling coil, and saturated with water vapor at 37.0°C. It was then allowed to flow through the manometer flasks which had been placed in position in the thermostat. It is important that this should be done while the flasks are in the thermostat, as the solubility of O₂ is considerably less at 37.0°C than at room temperature.
Diet: The animals were fed ad libitum a diet of the following composition: skim milk powder 16 g., copper sulfate 0.25 mg., ferric chloride 0.25 mg., and water 34 gm. Food was prepared twice a day. To the morning diet was added a solution of the water-soluble vitamins in such a concentration that the average daily vitamin intake was as follows: thiamine hydrochloride 0.2 mg., riboflavin 0.5 mg., pyridoxine hydrochloride 0.1 mg., nicotinic acid 1 mg., Ca pantothenate 0.1 mg., inositol 10 mg., p-aminobenzoic acid 2 mg., choline 50 mg., and biotin (S.M.A. Concentrate S-200) 0.01 mg. A solution of the fat-soluble vitamins in cottonseed oil was added to the evening diet. The average daily intake of these vitamins was \( \beta \)-carotene 150 I.U., viosterol 40 I.U., \( \alpha \)-tocopherol 0.1 mg., 2-methyl-1,4-naphthoquinone 0.1 mg. Once a week 50 mg. of crystalline l-ascorbic acid, dissolved in water immediately before use, were administered by mouth. Water and iodized salt were provided ad libitum. The control animals received a stock diet composed of rolled barley, greens, water, and iodized salt ad libitum.
RESULTS

In Table I are represented the mean values of the \( Q_{O_2} \) and \( Q_{N_2} \) of liver tissue. The \( Q_{O_2} \) increased almost immediately after the animals were given the deficient diet and remained on this high level during the investigated period of the deficiency. This was accompanied by a decrease of the anaerobic glycolysis. No significant differences were noted between normal and deficient muscle tissue until the advanced stages of the deficiency (70 weeks) (Table II), when a significant lowering of the \( Q_{O_2} \) was noticed. No change was observed in the rate of anaerobic glycolysis.

Similar changes were evident in the kidney cortex (Table III). Marked decreases in the kidney \( Q_{O_2} \) were apparent during the thirteenth week of the deficiency. The anaerobic glycolysis, however, showed no change after 70 weeks on the deficient diet.

The water content of the tissues did not change significantly during the time limit of the experiment.
TABLE I

Respiration and Anaerobic Glycolysis of the Liver of Guinea Pigs on a Stock and a Deficient Diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. Obs.</th>
<th>( Q_{O_2} )</th>
<th>No. Obs.</th>
<th>( Q_{N_2}^{CO_2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>11</td>
<td>2.83 ± 0.18</td>
<td>8</td>
<td>3.04 ± 0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.83 - 3.98)</td>
<td></td>
<td>(1.95 - 4.10)</td>
</tr>
<tr>
<td>Deficient</td>
<td>11</td>
<td>4.08 ± 0.20</td>
<td>12</td>
<td>1.69 ± 0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.08 - 5.07)</td>
<td></td>
<td>(0.69 - 2.80)</td>
</tr>
</tbody>
</table>

The values in parentheses indicate the range.
TABLE II

Respiration and Anaerobic Glycolysis of the Kidney of Guinea Pigs on a Stock and a Deficient Diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Time on Diet wks.</th>
<th>No. Obs.</th>
<th>( Q_{O_2} ) Mean and Standard Error</th>
<th>No. Obs.</th>
<th>( Q_{CO_2} ) Mean and Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>12</td>
<td>10.9 ± 0.36 (8.00 - 14.7)</td>
<td>9</td>
<td>4.94 ± 0.44 (2.52 - 7.28)</td>
</tr>
<tr>
<td>Deficient</td>
<td>9-10</td>
<td>3</td>
<td>11.4 ± 0.53 (10.9 - 12.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>13-14</td>
<td>3</td>
<td>8.3 ± 0.36 (7.6 - 9.10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>20-24</td>
<td></td>
<td></td>
<td>8</td>
<td>5.40 ± 0.52 (3.08 - 6.55)</td>
</tr>
<tr>
<td>Deficient</td>
<td>66-70</td>
<td>4</td>
<td>6.7 ± 0.55 (5.1 - 7.90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>70-77</td>
<td>3</td>
<td></td>
<td></td>
<td>5.11 ± 0.25 (4.48 - 5.58)</td>
</tr>
</tbody>
</table>

The values in parentheses indicate the range.
### TABLE III

Respiration and Anaerobic Glycolysis of the Muscle of Guinea Pigs on a Stock and a Deficient Diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Time on Diet wks.</th>
<th>Mean and Standard Error</th>
<th>No. Obs.</th>
<th>$Q_{O_2}$</th>
<th>Mean and Standard Error</th>
<th>No. Obs.</th>
<th>$Q_{N_2}/O_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td></td>
<td>2.73 ± 0.15</td>
<td>(1.77 - 3.49)</td>
<td>9</td>
<td>4.70 ± 0.38</td>
</tr>
<tr>
<td>Deficient</td>
<td>9-10</td>
<td>3</td>
<td></td>
<td>2.52 ± 0.90</td>
<td>(2.31 - 2.70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>13-14</td>
<td>4</td>
<td></td>
<td>2.60 ± 0.09</td>
<td>(2.39 - 2.84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>20-24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>4.73 ± 0.40</td>
</tr>
<tr>
<td>Deficient</td>
<td>66-70</td>
<td>3</td>
<td></td>
<td>1.95 ± 0.25</td>
<td>(1.54 - 2.54)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>70-77</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>4.25 ± 0.47</td>
</tr>
</tbody>
</table>

The values in parentheses indicate the range.
### TABLE IV

Water Content of Liver, Muscle, and Kidney Tissues of Guinea Pigs Raised on a Normal Diet and a Deficient Diet

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Diet</th>
<th>No. of Det'ns</th>
<th>Wet Dry Ratio</th>
<th>Stand. Deviation</th>
<th>Stand. Error</th>
<th>Mean % Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Normal</td>
<td>17</td>
<td>3.77</td>
<td>0.23</td>
<td>0.055</td>
<td>73.50</td>
</tr>
<tr>
<td></td>
<td>Def.</td>
<td>23</td>
<td>3.64</td>
<td>0.23</td>
<td>0.058</td>
<td>72.15</td>
</tr>
<tr>
<td>Muscle</td>
<td>Normal</td>
<td>16</td>
<td>4.80</td>
<td>0.49</td>
<td>0.123</td>
<td>79.20</td>
</tr>
<tr>
<td></td>
<td>Def.</td>
<td>24</td>
<td>4.51</td>
<td>0.49</td>
<td>0.099</td>
<td>77.90</td>
</tr>
<tr>
<td>Kidney</td>
<td>Normal</td>
<td>15</td>
<td>4.70</td>
<td>0.34</td>
<td>0.088</td>
<td>78.80</td>
</tr>
<tr>
<td></td>
<td>Def.</td>
<td>21</td>
<td>4.73</td>
<td>0.35</td>
<td>0.076</td>
<td>79.00</td>
</tr>
</tbody>
</table>
DISCUSSION

The results presented in this paper indicate that a derangement of the glycolytic function of the liver and kidneys of guinea pigs results from a deficiency of the anti-stiffness factor. The anti-stiffness factor apparently plays a role in the normal series of energy transfers associated with the breakdown of carbohydrates. This could be expected since the activity of most vitamin factors may be attributed to their ability to couple with proteins to form enzymes which act as catalysts in the breakdown of the carbohydrate. Under the circumstances, it would be expected that an impaired anaerobic glycolysis would cause a shift towards a direct utilization of oxygen, resulting in an increase in the oxygen consumption. This was actually observed in liver tissue. A similar reaction was not observed in the kidney cortex tissue. However, during the advanced stages of the deficiency, the kidneys show a marked proliferation. Consequently, the effective metabolic tissue per gram of kidney tissue may have decreased, so that the balance between the \( \frac{N_2}{C_0_2} \) and the \( Q_0_2 \) may actually show a similar change as that of the liver if the \( Q \) notation were expressed
on a different basis than the dry weight basis. The extent of proliferation might be taken into account in the evaluation of the Q value by weighing the whole organ and comparing this with the normal organ. This discussion could also apply to the muscle metabolism.

A critical analysis of the value of using the dry weight of the tissue for expressing the respiratory rates has been made by Laser (20). There are two methods commonly used to convert the wet weights to a dry basis. Most investigators have used the method of removing the tissues from the Ringer solution at the end of the experimental period, washing, drying, and weighing these tissue slices to obtain a value for the dry weight. A significant error might be introduced in this procedure due to the difficulty of removing the entire tissue slice from the reaction vessel at the end of the metabolic period. Also, as has been pointed out by Bach (2), an appreciable imbibition of water by the metabolizing tissues may occur. The errors introduced by these factors can be a magnitude of 300-400%. It is suggested that these errors may be controlled by using very nearly the same amounts of tissue for a series of comparative experiments, or by limiting the incubation period to less than thirty minutes. Field
and Fuhrman (11) have also discussed this problem and conclude that a more accurate method is the determination of the wet/dry ratio on fresh tissue. The $Q_{O_2}$ values of normal guinea pig tissues as reported previously (4, 14, 18, 22, 30, 32) have shown considerable variation and in general have been higher than those reported in this study. This may be due to the fact that the methods used previously for determining the dry weights have not been adequately controlled. From the studies which have been made of other nutritional deficiencies it has been observed that there was an increase in the $Q_{O_2}$ of liver tissue only during a vitamin C deficiency. Stotz and co-workers (32) reported an increased oxygen consumption of the liver and no change in the kidneys of scorbutic guinea pigs. The anaerobic glycolysis for both of these tissues remained unchanged. It is interesting to note that the diet used to induce the vitamin C deficiency was the Sherman basal ration (29). This diet contains 30% heated skimmed milk powder. It is possible that their investigations were complicated by the fact that their animals were receiving a sub-minimal ration of the anti-stiffness factor.
SUMMARY

1) The $Q_O^2$ of normal and deficient guinea pig tissues was determined manometrically with a Warburg respirometer apparatus. A 45% increase in the respiratory rate was observed in the liver during deficiency. The kidney showed a drop in the respiratory rate of 16% after the animal had been on the deficient diet for 60 days, and a 38% decrease after 16 months. The muscle $Q_O^2$ did not change until the advanced stages of the deficiency (16 months), when a 28% decrease was noticed.

2) Anaerobic glycolysis determinations were made upon the same tissues. A 44% decrease in the glycolytic capacity was noticed for liver tissue. There was no apparent change in the kidney and muscle.

3) The water content of the tissues was determined and no significant changes were noted in the water balance during the deficiency.
PROTOCOL I

Experiment: #25

Date: March 19, 1945

Normal Guinea Pig, Female
Weight: 700 g.
Liver Tissue: 153 mg.

Manometer #2
$k_{O_2} = 1.494$

<table>
<thead>
<tr>
<th>Time</th>
<th>Th.Bar.</th>
<th>$\Delta'$</th>
<th>$h$</th>
<th>$h'$</th>
<th>$\Delta''$</th>
<th>$\Delta'''$</th>
<th>$x$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:00</td>
<td>153.0</td>
<td>0.0</td>
<td>150.8</td>
<td>150.8</td>
<td>-17.3</td>
<td>-25.9</td>
<td>-25.9</td>
</tr>
<tr>
<td>4:10</td>
<td>153.0</td>
<td>0.0</td>
<td>133.5</td>
<td>133.5</td>
<td>-15.0</td>
<td>-22.4</td>
<td>-48.3</td>
</tr>
<tr>
<td>4:20</td>
<td>152.0</td>
<td>-1.0</td>
<td>117.5</td>
<td>113.5</td>
<td>-17.5</td>
<td>-26.2</td>
<td>-74.5</td>
</tr>
<tr>
<td>4:30</td>
<td>153.8</td>
<td>+0.8</td>
<td>101.8</td>
<td>101.0</td>
<td>-13.6</td>
<td>-20.3</td>
<td>-94.8</td>
</tr>
<tr>
<td>4:40</td>
<td>153.8</td>
<td>+0.8</td>
<td>88.2</td>
<td>87.4</td>
<td>-14.4</td>
<td>-21.5</td>
<td>-116.3</td>
</tr>
<tr>
<td>4:50</td>
<td>156.0</td>
<td>+3.0</td>
<td>76.0</td>
<td>73.0</td>
<td>-13.8</td>
<td>-20.6</td>
<td>-136.9</td>
</tr>
<tr>
<td>5:00</td>
<td>156.0</td>
<td>+3.0</td>
<td>62.2</td>
<td>59.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CALCULATION:

$\Delta' = \text{change in thermobarometer during experiment}$
$\Delta'' = \text{change during 10 minute interval}$
$\Delta''' = \text{actual amount of gas absorbed during 10 minute interval}$
$x = \text{actual amount of gas absorbed}$

$h' = h - \Delta$

$Q_{O_2}$ (wet weight) = 0.87
$Q_{O_2}$ (dry weight) = 0.87 x 3.63 = 3.15
BIBLIOGRAPHY

42. van Wagтендонк, W.J., and Wulzen, R., Arch. Biochem. 1, 373 (1943).