Levels of (3)H-diethylstilbestrol in tissues of C(3)H mice resulting from continuous hormone administration.

David J. Paterson
University of Windsor

Follow this and additional works at: https://scholar.uwindsor.ca/etd

Recommended Citation
https://scholar.uwindsor.ca/etd/6871
LEVELS OF $^{3}$H-DIETHYLSTILBESTROL IN TISSUES OF C$_{3}$H MICE RESULTING FROM CONTINUOUS HORMONE ADMINISTRATION

BY

DAVID J. PATERSON

A Thesis Submitted to the Faculty of Graduate Studies through the Department of Biology in Partial Fulfillment of the Requirements for the Degree of Master of Science at the University of Windsor

WINDSOR, ONTARIO, CANADA

1970
ABSTRACT

Diethylstilbestrol (DES), a synthetic estrogen, has the ability to induce mammary carcinoma in C3H mice. The results of many uptake studies of labelled estrogens have led to conflicting reports on the mechanism of estrogen action in the stimulation of mammary growth. The concentration of DES necessary to stimulate production of DNA by mammary epithelial cells has recently been calculated by Turkington and Hilf.

This study is concerned with a study of DES levels in tissues after continuous tritium-labelled DES administration. Levels of DES found in mammary glands are found to be far below that necessary to enhance DNA synthesis. High concentrations of DES in the pituitary gland seem to indicate that the effect of estrogens on mammary growth is not a direct action, but indirect via the hypophysis.

The effects of testosterone upon DES concentration in tissues is also discussed. Although these effects seem quite varied, it is hoped that they provide further insight into the mechanism of induction of mammary carcinoma.
ACKNOWLEDGEMENTS

I should like to extend my thanks to Dr. A. H. Warner and Dr. J. E. J. Habowsky, Department of Biology, and to Dr. R. J. Thibert, Department of Chemistry, at the University of Windsor, for their help on this thesis. I am especially thankful to Dr. A. B. Okey, Department of Biology, University of Windsor for his guidance during the course of my research and during the preparation of this thesis.

Financial assistance for an NRC bursary and an NRC of Canada Grant (No. A4670 to Dr. A. B. Okey) is gratefully acknowledged.

I would like to extend a special thanks to Mr. Denis Page and Miss Susan Renaud for their technical assistance throughout the course of my work.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>vii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>4</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION (TIME COURSE STUDY)</td>
<td>8</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION (COMPARISON STUDY)</td>
<td>14</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>28</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>52</td>
</tr>
<tr>
<td>VITA AUCTORIS</td>
<td>55</td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Time Course Study - Levels of $^3$H-DES (dpm/mg) in Various Tissues</td>
<td>9</td>
</tr>
<tr>
<td>2.</td>
<td>Comparison Study - Levels of $^3$H-DES (dpm/mg) in Various Tissues</td>
<td>15</td>
</tr>
<tr>
<td>3.</td>
<td>Comparison Study - Ratios of Uptake Values of Various Tissues to Uptake Values of 'Non-target' Tissues</td>
<td>16</td>
</tr>
<tr>
<td>4.</td>
<td>DES Batch Analysis Sheet</td>
<td>32</td>
</tr>
<tr>
<td>5.</td>
<td>Levels of $^3$H-DES (dpm/mg) in Various Tissues of Mice Receiving a 25 ppb DES Diet</td>
<td>45</td>
</tr>
<tr>
<td>6.</td>
<td>Total Radioactivity Recovered from Mouse 43 and Mouse 110</td>
<td>48</td>
</tr>
</tbody>
</table>
# List of Illustrations

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Time-Course Uptake of $^3$H-DES in tissues of C$_3$H mouse</td>
<td>10</td>
</tr>
<tr>
<td>2.</td>
<td>Time-Course Uptake of $^3$H-DES' in tissues of C$_3$H mouse (cont'd)</td>
<td>12</td>
</tr>
<tr>
<td>3.</td>
<td>Comparison Study - DES levels in Pituitary Gland (in ratio to cerebrum)</td>
<td>17</td>
</tr>
<tr>
<td>4.</td>
<td>Comparison Study - DES levels in Mammary Glands (in ratio to inguinal fat)</td>
<td>19</td>
</tr>
<tr>
<td>5.</td>
<td>Comparison Study - DES levels in Uterus (in ratio to gastrocnemius)</td>
<td>21</td>
</tr>
<tr>
<td>6.</td>
<td>Comparison Study - DES levels in Adrenal Glands (in ratio to gastrocnemius)</td>
<td>21</td>
</tr>
<tr>
<td>7.</td>
<td>Comparison Study - DES levels in Ovaries and Testes (in ratio to gastrocnemius)</td>
<td>23</td>
</tr>
<tr>
<td>8.</td>
<td>Comparison Study - DES levels in Blood (in ratio to perirenal fat)</td>
<td>23</td>
</tr>
<tr>
<td>9.</td>
<td>Comparison Study - Molar Concentration of DES in Inguinal Fat</td>
<td>24</td>
</tr>
<tr>
<td>10.</td>
<td>Comparison Study - Molar Concentration of DES in Perirenal Fat</td>
<td>24</td>
</tr>
<tr>
<td>11.</td>
<td>Comparison Study - Molar Concentration of DES in Seminal Vesicles</td>
<td>26</td>
</tr>
<tr>
<td>12.</td>
<td>Thin Layer Chromatography of DES</td>
<td>33</td>
</tr>
<tr>
<td>13.</td>
<td>Structure of Metabolism Cage</td>
<td>38</td>
</tr>
<tr>
<td>14.</td>
<td>Mammary Glands of C$_3$H Mouse</td>
<td>40</td>
</tr>
<tr>
<td>15.</td>
<td>Structure of DES</td>
<td>49</td>
</tr>
</tbody>
</table>
## LIST OF APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Preliminary Uptake Studies</td>
<td>29</td>
</tr>
<tr>
<td>II</td>
<td>Characterization of $^3$H-Diethylstilbestrol</td>
<td>31</td>
</tr>
<tr>
<td>III</td>
<td>Preparation of Pellets for Time-Course and Comparison Studies</td>
<td>35</td>
</tr>
<tr>
<td>IV</td>
<td>Description of Cages Used in Time-Course and Comparison Studies</td>
<td>38</td>
</tr>
<tr>
<td>V</td>
<td>Tissues Used in Time-Course and Comparison Studies</td>
<td>40</td>
</tr>
<tr>
<td>VI</td>
<td>Pilot Study with 25 Parts per Billion Diethylstilbestrol</td>
<td>44</td>
</tr>
<tr>
<td>VII</td>
<td>The Use of Testosterone</td>
<td>46</td>
</tr>
<tr>
<td>VIII</td>
<td>Recovery of Radioactivity</td>
<td>47</td>
</tr>
<tr>
<td>IX</td>
<td>Diethylstilbestrol</td>
<td>49</td>
</tr>
<tr>
<td>X</td>
<td>Conversion of Disintegration per Minute to Molar Concentration</td>
<td>51</td>
</tr>
</tbody>
</table>
INTRODUCTION

The purpose of this research project was to clarify the mechanism by which estrogen induces mammary cancer in the C₃H mouse. Diethylstilbestrol (DES) induction of mammary cancer in mice was found to occur when mice were fed continually a diet containing DES (Okey and Gass, 1968). Conflicting opinions exist on the actual induction mechanism. It is felt that either

a) estrogens act directly on the mammary gland

or b) that they act on the pituitary gland and enhance the production of pituitary mammotrophic hormone(s) which stimulates mammary growth and eventually causes tumour formation.

It was shown by Turkington and Hilf (1968) that the rate of DNA synthesis in mammary cells is dependent on estrogen concentration. This information has raised the first question which I hope to answer in this paper:

1) What are the mole concentrations of DES in the mammary and pituitary glands of mice which have been on a continuous diet containing a carcinogenic dose of tritium-labelled DES?

It is also known that castrated male C₃H mice have a higher breast tumour incidence than intact males receiving a similar amount of DES (Okey and Gass, 1968) and that the testes (or testosterone) inhibits mammary carcinoma in mice (Nathanson and Andervont, 1939; Jones, 1941;
Huseby, 1953). This has set up the second question which I hope to answer in this paper:

2) What is the effect of testosterone in the concentration of DES present in the mammary and pituitary glands?

There has been much work done on the uptake of radioactively labelled estrogens in rats and mice. However, these uptake data are based on administration of one "pulse" of estrogen by injection (subcutaneous, intraperitoneal, intravenous) or by stomach tube administration (Green, 1969; Terenius, 1965, 1966; King, 1965; Tivombly, 1951; Sander, 1968).

My research consisted of a "time-course" uptake study to examine the DES concentration in tissues at specific times following 12 to 96 hours of continuous $^3$H-DES administration, and a "comparison uptake" study to examine DES concentrations in the tissues of various groups of mice having different hormonal status. It is hoped that answers to the questions stated above will be provided by the time-course and the comparison studies, respectively.

It was shown by Gass in 1964 that continuous administration of DES at a concentration of 250 parts per billion (ppb) in food is very effective in inducing mammary tumours in C57H mice. Thus $^3$H-DES was mixed with food to provide a "labelled diet" of pellets containing DES at a 250 ppb concentration. After administration of $^3$H-DES, the mice were sacrificed and various tissues were removed. From the specific activity of labelled hormone administered and the concentration of the hormone in the food, the levels of DES in "target" tissues resulting from continuous dietary hormone administration could be determined.
Target tissues, such as the uterus and vagina which take up and retain estrogen for a prolonged period of time (Jensen, 1962; APPENDIX I) were compared to the cerebrum, gastrocnemius muscle, and diaphragm, i.e., "non-target" tissues as control (Paton, 1969). Although data from these experiments are used for the purpose of answering the two aforementioned questions, uptake values of other tissues are also discussed.
MATERIALS AND METHODS

All mice used in these experiments were of the C\textsubscript{3}H strain (an inbred strain with a high mammary tumour incidence) and were obtained from Cumberland View Farms (Clinton, Tennessee).

All uptake studies were done using \textsuperscript{3}H-DES from the Amersham-Searle Corp. (Des Plaines, Illinois). Preliminary studies (APPENDIX I) involved the use of \textsuperscript{3}H-DES (S.A. - 400 mCi/mM, 1.49 mCi/mg). Time-course and comparison studies involved the use of \textsuperscript{3}H-DES (S.A. - 6.2 Ci/mM, 23.2 mCi/mg). The labelled DES used for time-course and comparison studies was examined qualitatively and quantitatively (APPENDICES II and III) before use in the uptake experiments.

Labelled DES was mixed with Purina Laboratory Chow and other components (APPENDIX III) to form pellets used in the time-course and comparison studies. The pellets were designed for use in specially-made metabolism cages (APPENDIX IV).

The "time-course" uptake study followed the scheme indicated below:

<table>
<thead>
<tr>
<th>Number of Mice</th>
<th>Average Age (days)</th>
<th>Time on \textsuperscript{3}H-DES Diet (hrs.)</th>
<th>Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>180</td>
<td>12</td>
<td>prefed control pellets</td>
</tr>
<tr>
<td>10</td>
<td>185</td>
<td>24</td>
<td>prefed control pellets</td>
</tr>
<tr>
<td>10</td>
<td>190</td>
<td>48</td>
<td>prefed control pellets</td>
</tr>
<tr>
<td>10</td>
<td>195</td>
<td>72</td>
<td>prefed control pellets</td>
</tr>
<tr>
<td>9</td>
<td>200</td>
<td>96</td>
<td>prefed control pellets</td>
</tr>
</tbody>
</table>
In addition, all mice used in these experiments were non-ovariectomized virgin females.

The "comparison" uptake studies involved ten groups, each containing 5 mice, which were given unlimited access to the labelled diet for 24 hours. The groups consisted of the following:

<table>
<thead>
<tr>
<th>Group Code</th>
<th>Average Age (days)</th>
<th>Sex</th>
<th>Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFCD</td>
<td>55-60</td>
<td>intact</td>
<td>prefed control diet</td>
</tr>
<tr>
<td>PFCD</td>
<td>55-60</td>
<td>castrate</td>
<td>prefed control diet</td>
</tr>
<tr>
<td>PFH</td>
<td>60-65</td>
<td>intact</td>
<td>prefed unlabelled 250 ppb DES (2 weeks)</td>
</tr>
<tr>
<td>PFH</td>
<td>60-65</td>
<td>castrate</td>
<td>prefed unlabelled 250 ppb DES (2 weeks)</td>
</tr>
<tr>
<td>PFH</td>
<td>55-60</td>
<td>intact</td>
<td>prefed unlabelled 250 ppb DES (2 weeks)</td>
</tr>
<tr>
<td>PFH</td>
<td>55-60</td>
<td>ovariectomized</td>
<td>prefed unlabelled 250 ppb DES (2 weeks)</td>
</tr>
<tr>
<td>PFCD</td>
<td>180</td>
<td>intact</td>
<td>prefed control diet</td>
</tr>
<tr>
<td>PFCD</td>
<td>60-65</td>
<td>ovariectomized</td>
<td>prefed control diet</td>
</tr>
<tr>
<td>PFCD + Test</td>
<td>60-65</td>
<td>intact</td>
<td>prefed control diet. Given injection (ip) of 0.3 mg testosterone ½ hr. before commence-ment of labelled diet (see APPENDIX VII)</td>
</tr>
<tr>
<td>PFCD + Test</td>
<td>60-65</td>
<td>ovariectomized</td>
<td>prefed control diet. Given testosterone as above</td>
</tr>
</tbody>
</table>

Ovariectomies and castrations were performed on mice in the comparison study two weeks before being given the labelled diet. All
other mice in this study were sham-operated at the same time. For the
time-course and comparison studies, mouse body weights were recorded
at the start and end of the labelled diets. Food consumption was
measured and DES intake for each mouse was calculated.

The following procedures were used for all mice in the time-course
and comparison studies.

Removal and Preparation of Tissues

Upon completion of time on labelled diet, each mouse was
anaesthetized in an ether jar and killed by cervical transection. The
animal was then pinned on its back in a dissecting pan and an incision
was made through the skin from the urogenital opening to the base of
the neck. Lateral incisions up to and along each of the four limbs
were made from the central incision. The skin was peeled back on both
sides and the mammary glands were removed. The left brachial artery
was then severed and a 25-μl sample of blood was taken. After opening
the peritoneal cavity, the uterus ♀ (seminal vesicles ♂),
oviducts ♀ (testes ♂), adrenal glands, inguinal and perirenal
fat, liver, gallbladder, diaphragm, gastrointestinal tract, and
gastrocnemius muscle were removed. The skull was then opened and
the pituitary gland and part of the cerebrum were removed (see APPENDIX
V for details).

Where possible, all tissues were cleaned of fat and blood before
being weighed. Tissues were weighed on a Roller-Smith Precision balance
(readability 0.2 mg). The carcass and GI tract were weighed on a triple-
beam balance (to the nearest 0.1 gram).
Tissue Digestion

The weighed tissues were put into scintillation vials containing 1.0 ml of Soluene-100, a tissue solubilizer (Packard Inst.), and the vials were then placed in a water bath for five hours at 65°C to ensure solubilization.

Counting of Radioactivity

After tissue solubilization, 10 mls of counting cocktail (4 g PPO and 50 mg POPOP per litre of toluene) were added to each vial. Each vial was wiped thoroughly and counted in a liquid scintillation system (Nuclear Chicago Mark II). All samples were allowed to dark-adapt for at least ½ hour before counting.

Efficiency was calculated by the channels ratio of external standard method and all samples were corrected to dpm.

Six quenched tritium standards were counted with every group of vials to ensure proper efficiency calculation.

The accuracy of this method of counting activity in tissues is described and compared to other methods by Herberg (1960).

Calculations

The disintegrations per minute per milligram of tissue were calculated for all tissues. The mean value for each tissue was calculated for each group of animals. Levels were also expressed as moles of DES or the number of moles (gram molecular weights) of DES per mg of tissue. It should be noted that molar concentration also refers to moles of DES per mg of tissue.
RESULTS AND DISCUSSION

A  Time Course Study

The purpose of the time course experiments was to determine the DES concentration in the mammary and pituitary glands and to compare this level with that which stimulates DNA synthesis in mammary epithelial cells.

It can be seen in FIGURE 1 that DES concentration on mg wet weight basis is approximately 30 times higher in the pituitary than in the mammary glands. The levels of DES in the pituitary gland remain fairly constant throughout the experiment, but DES levels in the mammary glands show a slow but steady increase with time. However, even after 96 hours, levels of DES found in mammary glands do not exceed $0.3 \times 10^{-13}$ M which is a dose having no effect on DNA synthesis in mammary epithelium (DNA synthesis is shown by Turkington and Hilt in 1969 to be enhanced by concentrations of DES around $8.0 \times 10^{-8}$ M). Perhaps the DES level in mammary tissue does reach the "DNA-synthesis enhancing" level with time, or perhaps "estrogen receptor sites" in mammary tissue are saturated with endogenous estrogenic hormones present in the intact $\varphi$ mice used for this study. This is suspected because of the low levels of DES found in the uterus which is a highly estrogen-responsive tissue. If estrogen receptor sites are saturated, then exchange of estrogen molecules with labelled DES may occur only over long periods of time but further experimentation is needed for support. This possibility is not entirely so, however, since it will be shown in the comparison study that uteri of ovariectomized mice show similarly low DES concen-
TABLE 1

Time Course Study - Levels of $^3\text{H}$-DES (dpm/mg) in Various Tissues

The average disintegrations per minute per milligram (dpm/mg) wet weight of tissues are shown. Each is the mean value of ten mice. Standard deviations are indicated. The code letters used to represent the various tissues are explained in APPENDIX II.

The dpm values for gastrointestinal tract (GI) and carcass (R) are expressed as disintegrations per gram. These were later converted to disintegrations per milligram as seen in FIGURES 1 and 2.

Values for feces extract (FE) are representative of total DES.
<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Mam</th>
<th>U</th>
<th>O</th>
<th>A</th>
<th>IF</th>
<th>PF</th>
<th>L</th>
<th>D</th>
<th>G</th>
<th>C</th>
<th>H</th>
<th>B-T</th>
<th>GI</th>
<th>CR</th>
<th>FE</th>
<th>(total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>1.53</td>
<td>1.83*</td>
<td>3.57</td>
<td>17.3</td>
<td>2.01</td>
<td>3.38</td>
<td>6.42</td>
<td>2.01</td>
<td>1.87</td>
<td>2.19</td>
<td>33.7</td>
<td>27.85</td>
<td>12,000</td>
<td>1400</td>
<td>20,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>±.29</td>
<td>±.51</td>
<td>±.50</td>
<td>±.37</td>
<td>±.42</td>
<td>±.91</td>
<td>±.14</td>
<td>±.27</td>
<td>±.28</td>
<td>±.55</td>
<td>±.17</td>
<td>±4,300</td>
<td>±180</td>
<td>±6,800</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>1.58</td>
<td>1.84</td>
<td>3.98</td>
<td>16.13</td>
<td>1.99</td>
<td>3.33</td>
<td>6.04</td>
<td>1.85</td>
<td>1.95</td>
<td>2.63</td>
<td>36.65</td>
<td>19.72</td>
<td>12,000</td>
<td>3000</td>
<td>35,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>±.21</td>
<td>±.15</td>
<td>±.55</td>
<td>±.23</td>
<td>±.42</td>
<td>±.40</td>
<td>±.22</td>
<td>±.31</td>
<td>±.39</td>
<td>±.55</td>
<td>±.02</td>
<td>±4,800</td>
<td>±360</td>
<td>±9,800</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>1.99</td>
<td>1.75</td>
<td>4.42</td>
<td>14.37</td>
<td>2.42</td>
<td>4.60</td>
<td>4.20</td>
<td>1.94</td>
<td>1.73</td>
<td>2.53</td>
<td>37.68</td>
<td>12.93</td>
<td>14,000</td>
<td>4000</td>
<td>100,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>±.32</td>
<td>±.32</td>
<td>±.44</td>
<td>±.22</td>
<td>±.25</td>
<td>±.50</td>
<td>±.39</td>
<td>±.27</td>
<td>±.36</td>
<td>±.59</td>
<td>±.92</td>
<td>±8,000</td>
<td>±370</td>
<td>±2,100</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>2.60</td>
<td>1.74</td>
<td>4.73</td>
<td>13.72</td>
<td>3.32</td>
<td>4.64</td>
<td>4.62</td>
<td>2.14</td>
<td>1.74</td>
<td>1.83</td>
<td>39.6</td>
<td>8.63</td>
<td>25,000</td>
<td>3500</td>
<td>109,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>±.27</td>
<td>±.20</td>
<td>±.30</td>
<td>±.32</td>
<td>±.30</td>
<td>±.58</td>
<td>±.19</td>
<td>±.26</td>
<td>±.23</td>
<td>±1.2</td>
<td>±.45</td>
<td>±8,000</td>
<td>±270</td>
<td>±1,800</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>2.86</td>
<td>2.63</td>
<td>4.78</td>
<td>13.83</td>
<td>3.42</td>
<td>4.66</td>
<td>5.93</td>
<td>2.67</td>
<td>1.72</td>
<td>1.61</td>
<td>38.34</td>
<td>9.72</td>
<td>30,000</td>
<td>3900</td>
<td>250,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>±.27</td>
<td>±.18</td>
<td>±.67</td>
<td>±.21</td>
<td>±.44</td>
<td>±.55</td>
<td>±.32</td>
<td>±.32</td>
<td>±.22</td>
<td>±1.2</td>
<td>±.34</td>
<td>±2,600</td>
<td>±350</td>
<td>±8,700</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± Standard Deviation of Mean
FIGURE 1

Time Course Uptake of $^3$H-DES in the C$_3$H Mouse

All values are expressed as disintegrations per minute (dpm) per milligram wet weight of tissues, except feces extract (FE) which represents total dpm.


Vertical axes are divided into 3 scales to accommodate the plots of as many tissues as possible.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
trations.

The data indicate that the pituitary gland is a target site for DES because of the high concentrations found there and it was therefore surmised that DES acts (see introduction page 5) primarily on the pituitary gland. However, much more experimental evidence is needed to support this conclusion.

**DES Concentration in Other Tissues**

Most of the $^3$H-DES is excreted as shown in FIGURE 1. This is indicated by high dpm level in fecal extract and in the cage wash samples. Values for cage wash (representative of urine excretion) are not included because of extremely wide variation in values. Twombly (1951) found that 75-80% of all activity is excreted through the bile into the feces and 15-30% appears in the urine. The results of my work are in agreement with these findings.

The adrenal glands showed a remarkable uptake of DES. This phenomenon has been mentioned by Bengtsson (1963) who has indicated that DES localizes in the adrenal cortex. Ullberg (1963) showed that activity found in the adrenal cortex is the unchanged administered DES indicating that uptake of estrogens by the adrenal cortex is not related to the synthesis of adrenal steroids.

Levels of DES in blood taken after death showed a slight drop with time although activity never fell below $8 \times 10^{-12}$M. High initial levels of labelled estrogen in blood and a dropping-off with time have been reported in all previous uptake experiments although these were "pulse" experiments. Recent evidence (Barlow, 1969) indicates the
FIGURE 2

Time Course Uptake of $^3$H-DES in the C$_3$H Mouse (cont'd)

A different scale from FIGURE 1 is used to show more clearly the uptake patterns of those tissues whose plots fall close together.

L - liver, O - ovaries, PF - perirenal fat, IF - inguinal fat, Mam - mammary glands, U - uterus, C - cerebrum.
existence of proteins with high binding affinities for estrogens and testosterone in blood serum. The possible existence of these "receptors" in blood help account for the levelling off of DES concentration in blood between 48 and 96 hours. It is suggested that initially the blood becomes saturated with DES absorbed from the intestine and carries it to various organs of the body. As blood loses its DES to tissues, it absorbs more from the steady supply in the gastrointestinal tract. As all tissues become saturated, DES concentration in blood reaches a steady level.

Levels of radioactivity in the liver remain at a fairly high level but these values are not constant with time. Excess DES in blood undergoes degradative metabolism in the liver as was shown by King (1965) who indicated that the only activity shown in organs termed "estrogen target tissues" (pituitary, uterus) is due to labelled estrogens, whereas activity in the liver is attributed to at least 6 compounds.

Both inguinal and perirenal fat showed levels higher than that found in uterus and mammary glands. The apparent uptake of DES by adipose tissue is probably due to the high levels of DES in the system and the fact that DES is soluble in fats. Excess levels of DES could be considered "stored" in inguinal and perirenal fat. Bengtsson (1963) suggests that endometrium, adrenal cortex, follicular epithelium in Graafian follicles, corpora lutea, and the interstitial cells of the testes serve as a depot for DES which would slowly be released and then accumulate in target organs. That fat tissue may be an "estrogen" depot is further supported by the fact that DES levels in ovaries and perirenal fat are very similar and as mentioned above certain parts of ovaries are considered by Bengtsson to be "depot" tissue.
As was expected the gastrocnemius, cerebrum and diaphragm all show low concentrations of DES. The slightly higher concentration of DES in diaphragm is likely due to inadequate removal of blood from this tissue.

Levels of DES per milligram of carcass are higher than expected for non-target tissue. This may be due to inaccurate extraction technique.

B Comparison Uptake Study

Tissues in this study are compared only to equivalent tissues in animals having different hormonal status to examine the effect of testosterone on DES concentrations. Tissues relevant to this thesis as well as several other tissues are discussed.

The time-course study showed that DES concentrations have reached fairly steady levels within 24 hours. For this reason and also that this was a convenient time, all mice in the comparison study remained on the diet for 24 hours.

The mammary glands (Mam), pituitary gland (H), adrenal glands (A), and blood (B-T) were chosen as possible "target" tissues. DES concentrations in these tissues were divided by DES concentrations in inguinal fat (IF), cerebrum (C), perirenal fat (PF), and gastrocnemius muscle (G) respectively, which were considered to be "non-target" tissues. The possibility exists that one group of mice may take in more labelled DES than another group. Comparison of the above ratios nullifies this effect to some extent.

The DES concentrations found in IF, PF and seminal vesicles (SV)
TABLE 2

Comparison Study - Levels of $^3$H-DES (dpm/mg) in Various Tissues

The average dpm per mg wet weight of tissues are shown. Values are the average of five mice used in each group. Standard deviations are indicated. The code letters representing mice groups and tissues are found in "Materials and Methods" in APPENDIX II respectively.
<table>
<thead>
<tr>
<th>Group</th>
<th>Mam</th>
<th>U</th>
<th>O</th>
<th>A</th>
<th>IF</th>
<th>PF</th>
<th>L</th>
<th>D</th>
<th>G</th>
<th>C</th>
<th>H</th>
<th>B-T</th>
<th>GI</th>
<th>CR</th>
<th>FE</th>
<th>T</th>
<th>SV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFCD 3.1</td>
<td>-</td>
<td>-</td>
<td>2.84*</td>
<td>4.6</td>
<td>7.74</td>
<td>12.57</td>
<td>3.75</td>
<td>2.41</td>
<td>5.52</td>
<td>119.8</td>
<td>3506</td>
<td>35325</td>
<td>1187</td>
<td>80895</td>
<td>1.59</td>
<td>1.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+.48</td>
<td>+.48</td>
<td>+1.24</td>
<td>+5.46</td>
<td>+.41</td>
<td>+.47</td>
<td>+1.6</td>
<td>+22.6</td>
<td>+2000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFCD 1.24</td>
<td>-</td>
<td>-</td>
<td>9.00</td>
<td>1.84</td>
<td>2.65</td>
<td>6.82</td>
<td>1.39</td>
<td>1.31</td>
<td>1.03</td>
<td>9.84</td>
<td>357</td>
<td>29949</td>
<td>1118</td>
<td>53540</td>
<td>5.12</td>
<td>+1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+.66</td>
<td>+.25</td>
<td>+.814</td>
<td>+1.09</td>
<td>+.25</td>
<td>+.25</td>
<td>+.20</td>
<td>+2.16</td>
<td>+90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFH 0.67</td>
<td>-</td>
<td>-</td>
<td>7.77</td>
<td>0.88</td>
<td>1.40</td>
<td>8.98</td>
<td>0.75</td>
<td>0.82</td>
<td>1.37</td>
<td>9.10</td>
<td>26</td>
<td>37191</td>
<td>954</td>
<td>35860</td>
<td>1.13</td>
<td>2.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+.77</td>
<td>+.23</td>
<td>+.65</td>
<td>+.67</td>
<td>+.32</td>
<td>+.33</td>
<td>+.40</td>
<td>+2.11</td>
<td>+13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFH 1.47</td>
<td>-</td>
<td>-</td>
<td>8.16</td>
<td>2.97</td>
<td>5.46</td>
<td>7.76</td>
<td>3.65</td>
<td>2.24</td>
<td>1.83</td>
<td>8.88</td>
<td>607</td>
<td>35163</td>
<td>1614</td>
<td>7210</td>
<td>8.3</td>
<td>+.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+.74</td>
<td>+.82</td>
<td>+1.02</td>
<td>+3.3</td>
<td>+6.5</td>
<td>+1.7</td>
<td>+4.2</td>
<td>+1.0</td>
<td>+128</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFH 1.29</td>
<td>-</td>
<td>3.19</td>
<td>3.19</td>
<td>2.96</td>
<td>1.23</td>
<td>1.91</td>
<td>13.43</td>
<td>2.52</td>
<td>2.01</td>
<td>1.71</td>
<td>7.11</td>
<td>30</td>
<td>35228</td>
<td>2740</td>
<td>24410</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+.40</td>
<td>+.13</td>
<td>+.66</td>
<td>+.09</td>
<td>+.21</td>
<td>+2.4</td>
<td>+.30</td>
<td>+.34</td>
<td>+.31</td>
<td>+.89</td>
<td>+2.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFH 1.55</td>
<td>-</td>
<td>2.57</td>
<td>20.4</td>
<td>3.68</td>
<td>6.47</td>
<td>9.90</td>
<td>2.69</td>
<td>1.90</td>
<td>1.17</td>
<td>30.89</td>
<td>91</td>
<td>31898</td>
<td>3709</td>
<td>51800</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+.33</td>
<td>+1.55</td>
<td>+.25</td>
<td>+.58</td>
<td>+1.24</td>
<td>+.31</td>
<td>+.46</td>
<td>+.18</td>
<td>+3.44</td>
<td>+5.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFCD -</td>
<td>1.84</td>
<td>3.98</td>
<td>16.13</td>
<td>1.99</td>
<td>3.33</td>
<td>6.04</td>
<td>1.85</td>
<td>1.95</td>
<td>2.63</td>
<td>36.7</td>
<td>20</td>
<td>12000</td>
<td>3000</td>
<td>35000</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+.21</td>
<td>+.15</td>
<td>+.55</td>
<td>+.22</td>
<td>+.42</td>
<td>+.40</td>
<td>+.22</td>
<td>+.31</td>
<td>+.39</td>
<td>+.55</td>
<td>+1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFCD 0.73</td>
<td>2.79</td>
<td>5.58</td>
<td>1.63</td>
<td>1.59</td>
<td>6.82</td>
<td>1.25</td>
<td>1.20</td>
<td>1.02</td>
<td>7.13</td>
<td>46</td>
<td>28048</td>
<td>1400</td>
<td>13440</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+.29</td>
<td>+.62</td>
<td>+.21</td>
<td>+.30</td>
<td>+.49</td>
<td>+.16</td>
<td>+.23</td>
<td>+.19</td>
<td>+1.01</td>
<td>+80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFCD 0.63</td>
<td>1.17</td>
<td>1.77</td>
<td>5.13</td>
<td>0.77</td>
<td>1.20</td>
<td>5.23</td>
<td>0.82</td>
<td>0.53</td>
<td>0.54</td>
<td>13.0</td>
<td>220</td>
<td>32459</td>
<td>1665</td>
<td>43100</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+.26</td>
<td>+.30</td>
<td>+1.2</td>
<td>+.20</td>
<td>+.28</td>
<td>+.44</td>
<td>+.16</td>
<td>+.05</td>
<td>+0.07</td>
<td>+1.67</td>
<td>+28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFCD 0.85</td>
<td>2.67</td>
<td>7.65</td>
<td>1.73</td>
<td>2.55</td>
<td>6.44</td>
<td>1.68</td>
<td>1.35</td>
<td>1.32</td>
<td>4.46</td>
<td>84</td>
<td>27096</td>
<td>1350</td>
<td>33750</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+.29</td>
<td>+.50</td>
<td>+.14</td>
<td>+.22</td>
<td>+.39</td>
<td>+.17</td>
<td>+.18</td>
<td>+.14</td>
<td>+.38</td>
<td>+10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± Standard Deviation of Mean
TABLE 3
Comparison Study - Ratios of Uptake Values of Possible 'Target' Tissues to Uptake Values of 'Non-target' Tissues

Values of $^3$H-DES uptake for possible target tissues are divided by values of $^3$H-DES uptake in non-target tissues from the same animal. The ratios are used to compare DES uptake levels in animals of different hormonal status.
### TABLE 3

Comparison Study - Ratios of Uptake Values of Possible 'Target' Tissues to Uptake Values of 'Non-target' Tissues

<table>
<thead>
<tr>
<th>Group</th>
<th>Mam</th>
<th>H</th>
<th>U</th>
<th>A</th>
<th>O</th>
<th>B-T</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFCD</td>
<td>.674</td>
<td>21.7</td>
<td>11.8</td>
<td></td>
<td>453</td>
<td>.66</td>
<td></td>
</tr>
<tr>
<td>PFCD</td>
<td>.674</td>
<td>9.6</td>
<td>6.9</td>
<td></td>
<td>135</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFH</td>
<td>.761</td>
<td>6.6</td>
<td>9.5</td>
<td></td>
<td>19</td>
<td>1.38</td>
<td></td>
</tr>
<tr>
<td>PFH</td>
<td>.495</td>
<td>4.9</td>
<td>3.6</td>
<td></td>
<td>111</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFH</td>
<td>1.05</td>
<td>4.2</td>
<td>1.59</td>
<td>1.5</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFH</td>
<td>.421</td>
<td>3.3</td>
<td>1.35</td>
<td>10.7</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFCD</td>
<td>.794</td>
<td>13.9</td>
<td>.944</td>
<td>8.3</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFCD</td>
<td>.448</td>
<td>7.0</td>
<td>4.7</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFCD + test</td>
<td>.818</td>
<td>11.1</td>
<td>10.0</td>
<td>3.3</td>
<td>183</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFCD + Test</td>
<td>.491</td>
<td>1.7</td>
<td>5.7</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Levels of DES in pituitary glands are expressed in ratio to DES levels in the cerebrum (C). The ratios are used to show the difference in DES levels between groups having different hormonal status. Group codes found in FIGURES 3 to 11 are explained in "Materials and Methods". (i.e., PFCD = intact males prefed control diet)
Ratio
Moles of DES in H/

Moles DES in C

Figure 3

Pituitary Gland

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
are found on FIGURES 9, 10 and 11. No ratios are calculated in these instances as these tissues are not of primary interest in this thesis.

In cases of DES levels in the pituitary gland, male mice show higher DES concentrations than females similarly treated. This suggests that the pituitary gland of male mice has unsaturated receptor sites for estrogens. The high pituitary uptake of DES in \( \sigma \) PFCD is suggestive of testosterone stimulation of DES uptake. The low uptake of \( \sigma \) PFH is likely due to a saturation of the pituitary gland with "cold" DES from the pretreatment diet.

Testosterone appears to inhibit uptake of DES by pituitary in mice and may be an indication of protection of mammary cancer by testosterone. That male pituitaries take up such high levels is in disagreement with this statement.

It would seem that the pituitary gland of an ovariectomized mouse should show high concentrations of DES but this is not shown in my results. Perhaps due to the absence of the ovarian hormones, the pituitary is producing a large amount of follicle stimulating hormone (FSH) and low uptake is due to FSH production, although this is just speculation. This is supported by the high levels found in \( \sigma \) PFH.

The pituitary glands are compared to cerebrum as both tissues are at least, in part, of nerve tissue origin.

Comparison of DES concentration in mammary tissue is hard to understand. Levels of DES are higher in mammary tissue of intact females than intact males. Conversely, levels of DES are higher in mammary tissues of castrate males than in ovariectomized females. This
FIGURE 4

Comparison Study - DES Levels in the Mammary Glands (Mam) (in ratio to inguinal fat)

Levels of DES in mammary glands are expressed in ratio to DES levels in the inguinal fat (IF). The ratios are used to show the difference in DES levels between groups having different hormonal status.
Ratio

\[
\frac{\text{Moles of DES in Mam}}{\text{Moles DES in IF}}
\]

Figure 4

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
would suggest testosterone inhibits DES uptake by mammary tissue as dpm due to \(^3\)H-DES are all lower in mammary tissue to mice with testosterone than those without. The levels of DES found in females treated with testosterone are similar to females prefed control diet indicating little or no effect is exerted by testosterone on uptake of DES in mammary glands (24 hours after injection).

The mammary glands are compared to fat tissue since a large portion of mammary tissue is fat.

The testosterone effect on DES concentrations in pituitary and mammary glands of female mice may be due to the fact that these mice have been exposed to this hormone for only \(24\frac{1}{2}\) hours. Perhaps if these mice were given access to labelled diets for longer periods of time greater differences would be observed. In all cases studied, the pituitary shows higher levels of DES than found in the mammary glands. These findings are in agreement with those of the time-course study.

Other Tissues

Blood (FIGURE 8) - The concentration of DES found in blood is higher in males than in females. The highest blood concentration is found in \(\sigma\) PFCD suggesting testosterone stimulation of absorption of DES by the blood from the gastrointestinal tract. This is supported by the higher levels of blood DES found in females treated with testosterone than females without testosterone. Ovariectomized females pretreated with control diet showed higher DES uptake in blood than intact females.

Uterus (FIGURE 5) - Values for DES concentrations in the uterus are low for all groups studied, as was found in the time-course study. The
FIGURE 5

Comparison Study - DES Levels in the Uterus (U)
(in ratio to gastrocnemius)

Levels of DES in the uterus are expressed in ratio to DES levels in the gastrocnemius muscle (G). The ratios are used to show the difference in DES levels between groups having different hormonal status.

FIGURE 6

Comparison Study - DES Levels in the Adrenal Glands (A)
(in ratio to gastrocnemius)

Levels of DES in the adrenal glands are expressed in ratio to DES levels in the gastrocnemius muscle (G). The ratios are used to show the difference in DES levels between groups having different hormonal status.
Figure 5
Uterus

Figure 6
Adrenal Glands

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
higher DES levels shown by ♀ PFH and ♂ PFH may indicate replacement of natural estrogens with DES. If these results do reflect replacement, then this process is relatively slow. Another possibility for the higher DES levels in these two groups is that specific estrogen receptors in the uterus are already saturated and the two week pretreatment with unlabelled hormone results in the saturation or binding of DES to non-specific receptors. The existence of specific and non-specific receptors for estrogens was mentioned by Jensen and Jacobsen (1962).

Testosterone appears to have slightly stimulated uptake of DES by the uterus. This is supported by Lerner (1969).

Adrenal Glands (FIGURE 6) - The uptake of DES by the adrenal glands (adrenal cortex) has been shown in the uptake study to be quite extensive. The presence of testosterone appears to enhance uptake of DES by the adrenal cortex. Due to saturation, mice pretreated with "cold" 250 DES, show lower DES levels than mice pretreated with control diet. Castrated and ovariectomized mice show lower uptake than intact mice. The only exception to these observations is the much higher uptake in ♂ PFH than in ♀ PFH. No explanation is offered for this phenomenon.

Ovaries (FIGURE 7) - Testosterone seems to show a stimulatory effect on uptake of DES by ovaries. Low DES concentrations found in ♀ PFH is probably due to saturation.

Testes (FIGURE 7) - The testes (interstitial cells) have been termed DES depots by Bengtsson (1963). In this work, DES concentration of ♂ PFCD is quite low whereas ♂ PFH show higher levels. This
FIGURE 7

Comparison Study - DES Levels in the Ovaries (O) and Testes (T)
(in ratio to gastrocnemius)

Levels of DES in the ovaries and testes are expressed in ratio to DES levels in the gastrocnemius muscle (G). The ratios are used to show the difference in DES levels between groups having different hormonal status.

FIGURE 8

Comparison Study - DES Levels in the Blood (B-T)
(in ratio to perirenal fat)

Levels of DES in the blood are expressed in ratio to DES levels in the perirenal fat (PF). The ratios are used to show the difference in DES levels between groups having different hormonal status.
Figure 7

Ovaries

Testes

Figure 8

Blood

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
FIGURE 9
Comparison Study - Molar Concentration of DES in Inguinal Fat (IF)

The molar concentrations of DES found in inguinal fat tissue are derived from values of dpm of $^3$H-DES per mg wet weight of inguinal fat tissue. Standard deviations from the mean are indicated.

FIGURE 10
Comparison Study - Molar Concentration of DES in Perirenal Fat (PF)

The molar concentrations of DES found in perirenal fat tissue are derived from values of dpm of $^3$H-DES per mg wet weight of perirenal fat tissue. Standard deviations from the mean are indicated.
supports the DES depot idea in that there is no specific uptake but DES can be found in interstitial cells when high levels of DES are found in the system.

Fat (FIGURES 9 and 10) - Values for uptake of DES in inguinal and perirenal fat vary considerably but in most cases are quite high. Generally the perirenal fat seemed to have higher uptake values than inguinal fat. This was also observed in the time-course study. The presence of testosterone appears to enhance the storage of DES in fat except in those cases where mice are pretreated with "cold" 250 DES in which case, the fat tissue may already be saturated.

Fat tissue in males appears to have a higher concentration than fat in females.

Seminal Vesicles (SV) (FIGURE 11) - It should be stated that when the seminal vesicles were removed from mice, the fluid was not drained. This oversight may misconstrue results of DES concentrations expressed per mg wt. basis. Results are such that the seminal vesicles and contents are treated as an entire tissue.

The lowest DES concentration and the highest SV weights were shown by the intact males. The SV's of \( \text{\&} \) PFH had higher weights and higher DES levels than those of \( \text{\&} \) PFCD.

Secretory epithelial cells in seminal vesicles are maintained by androgens and may have testosterone receptors (Turner, 1967. P. 434). That seminal vesicles of castrate males show higher DES concentrations than intact males suggest that seminal vesicle hormone receptors have an affinity for DES when no androgens are present. It has been shown that the administration of testosterone to castrated males with degenerated
FIGURE 11

Comparison Study - Molar Concentration of DES in Seminal Vesicles (SV)

The molar concentrations of DES found in seminal vesicles are derived from values of dpm of $^3$H-DES per mg wet weight of seminal vesicles. Standard deviations from the mean are indicated.
Figure 11

Seminal Vesicles

Dpm $^3$H-DES/Mg

Moles of DES x 10$^{-14}$

PFCD  PFCD  PFH  PFH

$^\circ$ $^\circ$ $^\circ$ $^\circ$

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
seminal vesicles returns the seminal vesicles to the "normal" state (Turner, 1967). The results of this work (DES levels and weight differences in seminal vesicles) seem to indicate that estrogens may have the ability to do the same.
SUMMARY

Concentrations of DES in mammary tissue resulting from continuous dietary administration are not at levels high enough to stimulate DNA synthesis in mammary epithelial cells. The level of DES in mammary tissue is quite similar to that found in fat tissue. The DES concentrations found in the pituitary gland, however, are quite high. Although it is known that mammary development is dependent on the presence of estrogens, it appears from this study that a main controlling influence of mammary tissue growth is the pituitary gland (see INTRODUCTION).

The effects of testosterone on the concentration of DES in tissues are quite varied. It appears to stimulate uptake of DES by the pituitary in \( \sigma \) mice, but inhibits (at 0.3 mg dose) this response in \( \varphi \) mice. To fully ascertain the effects of testosterone on the concentration of DES in mammary tissue, more experiments must be done.

The questions presented in this thesis are not answered satisfactorily but the techniques used in this research could be the basis for further study in this area. It is suggested that the comparison study groups described in this paper be placed on a time-course study. Examination of DES concentration in tissues could be done at various times between 1 hour and 4 weeks of continuous \(^{3}\)H-DES dietary administration. Data from this type of experiment could be quite revealing as to the mechanism of breast tumour induction by DES as well as clarify the effects of testosterone, the interaction of hormone systems, and the nature of DES "depots".

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Preliminary Uptake Studies

Preliminary studies involved the stomach tube administration of one pulse of $^3$H-DES in 0.5 ml saline solution to 100 day old intact mice. At times up to 20 hours after administration, the liver, adrenals, pituitary gland, ovaries, uterus, mammary glands, gastrocnemius muscle and cerebrum were removed, weighed, digested, and counted in a liquid scintillation counter.

Uptake in "non-target" organs such as the gastrocnemius muscle was much lower than in target organs such as the uterus. Although uptake patterns differ slightly according to the method of administration used, it is apparent that there are basic differences in uptake among organs such that some may be referred to as "target organs" and others as "non-target organs". Target organs show the possibility of having a definite receptor mechanism(s) for estrogens (Jensen and Jacobsen, 1962).

Sander in 1965 reported the uptake of estradiol 17-β in various tissues of the ♀ rat after intramuscular injection of a labelled estrogen. His experiments showed maximum uptake in all tissues ½ to 1 hr. after injection. The breast tissue continued to accumulate radioactivity for one hour before a decrease in concentration was shown. The uptake of radioactivity by the uterus followed a pattern similar to that in the breast tissue, but the uptake level in the uterus was distinctly higher. The skeletal muscle and fat showed a lower concentration of activity. This work, as well as that by Bengtsson (1963),...
Terenius (1965, 1966), Jensen and Jacobsen (1962), and Stone (1966), provided the background for the subject of this thesis.
APPENDIX II

Characterization of $^{3}$H-DES

The counting of stock dilutions revealed that batch 9 contained 75.2 mg/ml, or almost twice the amount indicated in the data sheet below.

As shown below, the purity of batch 9 was found to be greater than 99% pure on May 19, 1969. The purity was rechecked using an Eastman Chromagram Developing Apparatus (Rochester, N.Y.). Thin-layer chromatograms were composed to 100μ thick layer of silica gel on 200μ thick polyethylene, with a polyvinyl alcoholic binder. A fluorescent indicator was incorporated in the active layer. Unlabelled and labelled DES were spotted 3/4" from the bottom of the chromatogram and the chromatogram was developed for 1-1½ hours (3/4 length of chromatogram) in chloroform: acetone (17:3) (Ponder, 1966). After air-drying, visualization was accomplished using a shortwave U.V. lamp, or using a fine spray of a mixture of equal volumes of 1% ferric chloride and 1% potassium ferricyanide (Ingram, 1952).

Visualization by U.V. light allowed the chromatograms to be divided into sections which were scraped, extracted with 100% ethanol, and checked for activity. (U.V. does not spread activity as is possible with a spray.)

As seen in FIGURE 12, labelled DES was compared with standard DES. For each, two spots appeared with Rf's of 0.68 and 0.59 representing the trans and cis forms of DES, respectively. Visualization with spray as well as work by Ramaut in 1967 supported my results.
TABLE 4

DES Batch Analysis Sheet

The data sheet received with $^3$H-DES used in these experiments is included in APPENDIX II.
Diethylstilboestrol generally labelled with tritium is prepared by an exchange procedure and is purified by chromatography over alumina in benzene:ether followed by crystallization from benzene.

<table>
<thead>
<tr>
<th>TECHNICAL DATA</th>
<th>TRA.50 Batch 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific activity</td>
<td>6.2 Ci/mM</td>
</tr>
<tr>
<td>(using TRR.1 n-hexadecane-1,2-T as reference material)</td>
<td>23.2 mCi/mg</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>268</td>
</tr>
<tr>
<td>Radioactive concentration</td>
<td>1 mCi/ml</td>
</tr>
<tr>
<td>Radiochemical purity</td>
<td>99%</td>
</tr>
<tr>
<td>by thin-layer chromatography in (a) di-isopropyl ether:ethanol (90:10)</td>
<td>99%</td>
</tr>
<tr>
<td>(b) chloroform:acetone (60:40)</td>
<td>99%</td>
</tr>
<tr>
<td>by paper chromatography in Bush '81'</td>
<td>99%</td>
</tr>
</tbody>
</table>

Analysed 19th May 1969
FIGURE 12

Thin Layer Chromatography of DES

A sketch of a thin layer chromatogram used to examine purity of $^3$H-DES is shown. The radioactive counts recovered from the divided areas are indicated in their appropriate squares. In each of the cases, 0.075 µg of DES in 10 µl of benzene was spotted to the chromatogram. In the case of labelled DES, this is equivalent to $3.9 \times 10^6$ dpm. Standard DES samples were spotted at positions 1 and 3. Labelled DES samples were spotted at positions 2 and 4.
FIGURE 12

Thin Layer Chromatography of DES

Solvent Front

Trans Form

Cis Form

Starting Point

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
These results indicate that $^3$H-DES stock solution (batch 9) is above 90% pure. The stock solution used for the time-course and uptake studies had a specific activity of 23.2 mCi/mg and contained 75.2 µg/ml (5 mls total volume).
APPENDIX III

Preparation of Pellets Used for Time-Course and Comparison Studies

Purina Laboratory Chow was mixed in 100-gm portions with 25.2 µg of DES resulting in a final concentration of 251.88 ppb. A combination of both "cold" and "hot" DES in the food was prepared such that enough labelled DES was present per gram of food to result in satisfactory activities in tissues for uptake studies. It was found that food in powdered form was inadequate for use in the uptake studies as mice scattered the food throughout the cage making it impossible to determine the exact amount of DES taken by the mouse during his period on the diet. Pellets were therefore made from the powdered food for use in a specially constructed cage (APPENDIX IV) such that all radioactivity involved could be accounted for.

Labelled DES in Food

The stock solution of $^3$H-DES had a specific activity of 23.2 mCi/mg (APPENDIX II). Therefore 0.188 µg of $^3$H-DES is equivalent to $0.68 \times 10^6$ dpm. If this were mixed with 100 g of food, 1 g of food has approximately $9.68 \times 10^4$ dpm of activity. The average mouse consumes 3.0 g of food per day (Okey, 1968) and therefore has daily intake of roughly $2.90 \times 10^5$ dpm of activity. Considering the weight of the average mouse to be 30 g and assuming equal distribution of activity throughout the mouse's body, this amount of activity allows for $9.68$ dpm/mg of tissue.

It has been shown by Jensen and Jacobsen in 1962 that only 0.1 to 0.2% of the administered dose of a hormone actually enters a growth-responsive organ. Pilot uptake studies showed that 0.188 µg of $^3$H-DES...
plus 25 µg unlabelled DES was satisfactory for showing concentrations of DES such that significant uptake differences in various tissues could be shown.

**Pellet Preparation**

Pellets were formed from the following components:

- 100 gms laboratory chow
- 10.0 gms powdered Acacia (Fisher Scientific)
- 70.0 mls sugar solution (0.05 gms Dextrose/ml distilled H₂O)
- 4.0 mls stock ³H-DES dilution (0.047 µg ³H-DES/ml EtOH)
- 25.0 gms standard DES (from 25.0 µg DES/ml 95% EtOH solution)
- 10.0 mls 95% EtOH

"Hot" and "cold" DES (in 95% EtOH) were mixed in a vial (total 5 mls) and added to 100 gms of chow using a 2-ml syringe. The mixing vial was rinsed with two 5-ml portions of 95% EtOH and added to the food with the same syringe. The food was mixed thoroughly and the alcohol was allowed to evaporate by air-drying the mixture.

Acacia was then added and mixed with the food. A pliable dough was then formed by adding the sugar solution and working the mixture manually. The dough was rolled and pellets (approximately 4 gms each) were cut off. Pellets were then dried in an oven for 2 hrs. at 100°C and then left at room temperature for 72 hours.

Ten batches of food were mixed in this way and each batch was checked for accuracy.
Pellet Characterization

Approximately four 100-mg samples of pellet mixture were taken at random from each food batch. Each sample was weighed and placed in a test tube. Five mls of 100% EtOH were added to each tube and the contents were mixed thoroughly. The tubes were then covered for 12 hrs. after which they were centrifuged (500 rpm for 2 minutes) and the supernatant was removed. The food portions were then re-extracted in the same way using another 5-ml portion of 100% EtOH. The two 5-ml supernatants were combined and a 1-ml portion was removed and placed in a scintillation vial. Cocktail was added, the vials were counted and amount of $^3$H-DES was calculated per mg of food. An average recovery of 85% activity was found using this procedure.

From the remaining supernatant of each of the samples, 25 μl were removed and spotted on a chromatogram. The chromatographic procedure previously described (APPENDIX II) was used. Food extracts (containing labelled and unlabelled DES) were compared to samples from stock $^3$H-DES solution and standard DES solution.

It was seen that up to 5 spots appeared on the chromatogram when visualized with the spray, whereas only 3 spots appeared when visualized with U.V. light. As in the stock dilution checks (APPENDIX II) most of the activity remained with the spots representing the trans and cis forms of DES. Other spots on the chromatogram had activity, but greater than 85% of all activity recovered was accounted for by the cis and trans spots.

It was concluded that the food batches were properly mixed, and contained the correct amount of DES. If any breakdown products did exist, they would account for a very small percentage of activity.
FIGURE 13

Structure of Metabolism Cage

Diagramatic sketches of the metabolism cage used in this study are shown.
APPENDIX IV

Description of Cages Used in Time-Course and Comparison Studies

Specially constructed metabolism cages were used such that the "fate" of $^3$H-DES could be demonstrated. The intake of radioactivity (in food) by the mouse as well as the excretion of activity in the urine and feces was able to be easily calculated.

Clear plastic cages (11" x 6" x 5") were obtained from Maryland Plastics (Scientific Division, New York, N.Y.). Each cage had a hole melted into the bottom at one end such that the screw-on top of a photographic film can (1½" diameter; 1" deep) fit firmly into the cage. The tops of each can had a hole 15 mms in diameter drilled into its centre before being set into the cage proper. This hole size enabled a mouse to put his head into the food can, but not his front paws, thus enabling the mouse to eat but not to drag food back into the cage.

This set-up simplified the weighing of food at the beginning and the end of the time the mouse was on labelled diet, and enabled the calculation of food and $^3$H-DES consumed.

FIGURE 13 METABOLISM CAGE

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
The method of determination of DES excretion is described in APPENDIX V.

The cages could be easily cleaned and were therefore re-usable as well as being cheap and easily made. For any mice in the cage longer than 24 hours (i.e., Time Course study), food and water could be checked every day. Additional food was easily weighed and placed in the food can.

Each cage had a "cage data" sheet describing mouse treatment, mouse weights (at beginning and end of diet), time started on diet, time finished, age of mouse, and amount of food consumed.
FIGURE 14

Mammary Glands of the C₃H Mouse

The five pairs of mammary glands of the C₃H mouse are shown.
APPENDIX V

Tissues Studied in Time-Course and Comparison Studies

Mammary Glands (Mam) - The mouse has 5 pair of mammary glands as shown in FIGURE 14. Mammary tissue areas 1,2,3, and 4 were removed and treated as separate entities. Final dpm/mg values were averaged and used to represent total mammary tissue. It should be noted that all tissue including underlying fat pads were removed.

![Figure 14: Mammary Glands of the C3H Mouse](image)

Cage Wash (CW) - After feces had been removed, the cage was rinsed with three 10-ml portions of 100% EtOH. The rinses were combined and a 1.0-ml portion was removed and placed in a scintillation vial. Values for CW represent the activity excreted by the mouse through urine.
Uterus (U) - The entire uterus (from end of horns to the cervix) was removed. Outermost connective tissue and fat were removed prior to weighing.

Ovaries (O) - Both ovaries, cleaned of surrounding fat, were removed and treated as one tissue.

Adrenal Glands (A) - Both adrenal glands, cleaned of fat and blood, were removed and treated as one tissue.

Inguinal Fat (IF) - A small piece of fat (30-60 mg) was removed from the inguinal area of the mouse.

Perirenal Fat (PF) - The fat lying around kidney is usually quite sparse. Perirenal fat was taken from area around both kidneys and combined.

Liver (L) - A small piece of liver (80 mg) was removed from the central lobe.

Diaphragm (D) - The entire diaphragm was removed and thoroughly cleaned of connective tissue and blood before being weighed.

Gastrocnemius Muscle (G) - A major portion of this muscle (50 mg) was removed from either hindlimb.

Cerebrum (C) - A piece of cerebrum (40 mg) was removed and weighed.

Pituitary Gland (H) - The entire pituitary gland was removed. Care must be exercised in the removal of this gland.
Blood (B.T.)
- After removal of the mammary tissue, 25 µl of blood were taken from the left brachial artery and transferred to a scintillation vial.

Gastrointestinal Tract (GI)
- The entire tract, from and including stomach to the rectum was cleaned of fatty and connective tissue, then removed and weighed to the nearest 0.1 gm. It was then placed in a Waring Blender containing 70 mls 100% EtOH and blended for 5 minutes. The mixture was poured through two layers of cheesecloth, then allowed to settle for 10 minutes. A 1-ml portion was removed and placed in a scintillation vial.

Carcass (CR)
- After removal of desired tissues, the carcass was weighed to the nearest 0.1 g, cut into 3 or 4 pieces and placed in a Waring Blender containing 100 mls 100% EtOH and then blended for 5 minutes. The mixture was poured through two layers of cheesecloth, then allowed to settle for 10 minutes. A 1.0-ml portion was removed and placed in a scintillation vial.

Feces Extract (FE)
- After mouse was removed from metabolism cage, all feces were collected and extracted with two 5-ml portions of 100% EtOH. The two extractions
were combined, and a 1.0-ml portion was
removed and placed in a scintillation vial.
APPENDIX VI

Uptake Studies Using $^3$H-DES at a 25 Parts Per Billion (ppb) Level

It was thought that since the physiological levels of estrogen in a mouse are so small, the failure to demonstrate high concentration in the uterus and mammary glands was due to flooding the rest of the animal with DES. Therefore, pellets for this study were made as previously described, but contained only 1/10 the amount of DES (labelled and unlabelled).

Eight intact female mice were used in this study. Four mice were given access to the diet for 24 hours and others for 48 hours. Tissues were removed, digested, and counted as was done in the time-course and comparison studies. The results are shown below. Differences in uptake of DES in tissues between mice on 25 ppb and 250 ppb diets were noted, but in most instances, these were quite small. It appears that a 250 ppb level does not flood tissues, but this may not be the case. Further study with a 25-ppb diet should be carried out.
TABLE 5

Levels of $^3$H-DES (dpm/mg) in Various Tissues of Mice Receiving a 25 ppb DES Diet

The average dpm per mg wet weight of tissues are shown. Values are the average of 4 mice used in each group. Standard deviations are indicated. The code letters representing tissues are found in APPENDIX II.
### TABLE 5
Levels of $^3$H-DES (dpm/mg) in Various Tissues of Mice Receiving a 25 ppb DES Diet

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>Group</th>
<th>Mam</th>
<th>U</th>
<th>O</th>
<th>A</th>
<th>IF</th>
<th>PF</th>
<th>L</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs.</td>
<td>-</td>
<td>1.27*</td>
<td>2.06</td>
<td>3.54</td>
<td>1.12</td>
<td>2.11</td>
<td>4.61</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+.20</td>
<td>+.05</td>
<td>+.61</td>
<td>+.10</td>
<td>+.10</td>
<td>+.61</td>
<td>+.26</td>
</tr>
<tr>
<td></td>
<td>48 hrs.</td>
<td>-</td>
<td>1.06</td>
<td>2.22</td>
<td>6.65</td>
<td>1.16</td>
<td>2.27</td>
<td>3.97</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+.20</td>
<td>+.49</td>
<td>+.59</td>
<td>+.26</td>
<td>+.30</td>
<td>+.43</td>
<td>+.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24 hrs.</td>
<td>1.53</td>
<td>1.08</td>
<td>8.98</td>
<td>11.72</td>
<td>34,364</td>
<td>318</td>
<td>34,513</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+.13</td>
<td>+.12</td>
<td>+.76</td>
<td>+.72</td>
<td>+.9</td>
<td>+.9</td>
<td>+.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48 hrs.</td>
<td>1.0</td>
<td>0.57</td>
<td>5.22</td>
<td>47.64</td>
<td>20,193</td>
<td>550</td>
<td>38,070</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+.23</td>
<td>+.19</td>
<td>+.92</td>
<td>+.92</td>
<td>+.92</td>
<td>+.92</td>
<td>+.92</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± Standard Deviation of Mean
APPENDIX VII

The Use of Testosterone

One milligram of testosterone is uterotrophic in mice, or is a dose which stimulates the uptake of estrogens in both the uterus and vagina (Lerner, 1964). Much lower dose levels (i.e., $5 \times 10^{-12}\mu g$) have been shown to inhibit estrogen uptake. For the comparison studies described in this thesis, a dose of 0.3 mg (0.3 ccs corn oil) was chosen for its uterotrophic effects at this level.

Mice in the comparison study were given intraperitoneal testosterone injections $\frac{1}{2}$ hour prior to starting the labelled diet. No vehicle injections were given to the other mice in the comparison study. This omission was not noticed until well near the completion of this thesis and is a likely source of error in studying the effects of testosterone.
Recovery of Radioactivity

To demonstrate how activity is accounted for in each mouse, examples were taken from both the time-course and comparison studies. These mice were chosen randomly.

Time Course Study

Mouse 43 - intact
- pretreated with control diet
- consumes 1.10 g of labelled food (12 hrs.)

Comparison Study

Mouse 110 - castrate
- pretreated with control diet
- consumed 3.1 g of labelled food (24 hrs.)

Since food contains 96,800 dpm per gram, mouse 43 had ingested 106,480 dpm and mouse 110 had ingested 300,080 dpm. The total activity recovered from mouse 43 and mouse 110 (see TABLE 6) were 91,992 and 268,680 dpm respectively. This represents 86.0 and 89.5% recovery.

Per cent recovery ranged from 79 to 93 (mean 87%) for all mice tested. Unaccounted for activity may be due to inaccurate cage wash, feces, carcass and gastro-intestine extracts.
TABLE 6

Sample Recoveries of Total Activity (Mouse 43 and Mouse 110)

Total activity recovered from mice 43 and 110 is shown. These totals are compared with total administered activity such that percent recovery can be calculated.
TABLE 6
Total Radioactivity Recovered from Mouse 43 and Mouse 110

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Total Activity (dpm)</th>
<th>Tissue</th>
<th>Total Activity (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mouse 43</td>
<td>Mouse 110</td>
<td>Mouse 43</td>
</tr>
<tr>
<td>M-1</td>
<td>75</td>
<td>95</td>
<td>D</td>
</tr>
<tr>
<td>M-2</td>
<td>115</td>
<td>104</td>
<td>G</td>
</tr>
<tr>
<td>M-3</td>
<td>86</td>
<td>108</td>
<td>C</td>
</tr>
<tr>
<td>M-4</td>
<td>107</td>
<td>103</td>
<td>H</td>
</tr>
<tr>
<td>U</td>
<td>133</td>
<td>-</td>
<td>B-T</td>
</tr>
<tr>
<td>O</td>
<td>92</td>
<td>-</td>
<td>GI</td>
</tr>
<tr>
<td>A</td>
<td>204</td>
<td>89</td>
<td>CR</td>
</tr>
<tr>
<td>IF</td>
<td>96</td>
<td>87</td>
<td>CW</td>
</tr>
<tr>
<td>PF</td>
<td>92</td>
<td>90</td>
<td>FE</td>
</tr>
<tr>
<td>L</td>
<td>331</td>
<td>452</td>
<td>SV</td>
</tr>
<tr>
<td>GB</td>
<td>279</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
The chemical structure of diethylstilbestrol (DES) is shown.
Diethylstilbestrol (DES)

DES is a synthetic estrogen that exists primarily in the trans form. The cis form is inactive and tends to revert back to the trans form. DES is insoluble in \( H_2O \), soluble in alcohol, ether, chloroform, fatty oils and has a molecular weight of 268.34.

DES (\( \alpha, \alpha' \)-diethylstilbenediol)

DES is highly active when administered orally and is rapidly eliminated from the body. It is capable of inducing cancer of the breast in rats and mice, fibromyomas of the uterus and abdominal cavity of guinea pigs, and testicular cells of Leydig tumours in mice, as well as causing an increase in incidence of lymphoid tumours in mice. DES can cause breast cancer in human males as well as stimulate rudimentary mammary apparatus. It can cause regression and healing of women with breast cancer as well as increase the survival chances of a victim of cancer of the prostate (Stecher, 1968).

The distribution pattern of DES has been shown to be identical with natural estrogens except for penetration into and distribution within the fetus.

* Merck Index

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
The fact that DES is cheap and easily obtainable, as well as being an important compound in cancer research, are the basic reasons for the use of this compound in my study.

It has been shown by Turkington and Hilf (1968) that the inhibitory or stimulatory effect of estradiol on the rate of insulin-stimulated synthesis of DNA in mammary epithelial cells is highly dependent on estradiol concentration. For estradiol concentrations that inhibit, it is indicated that DES has \( \frac{1}{6} \) the potency of estradiol. This would mean that at \( 8 \times 10^{-8} \text{M} \), DES inhibits synthesis of DNA in mammary epithelial cells.

This information must be considered when examining the concentrations of DES in mammary tissue.
Appendix X

Conversion of Dpm to Molar Concentration

The knowledge of specific activity of the administered DES allows the transformation of radioactivity to the chemical amount of DES present in tissue.

One hundred grams of food contain 25.188 μg of "cold" and "hot" DES. This is equivalent to 96,800 dpm/g of food.

\[
1 \text{ mole DES} = 268.34 \text{ g} = 268.34 \times 10^6 \text{ μg}
\]

Since there are 25.188 μg of DES in 100 g of food then 100 g of food contain 0.94 x 10^-7 mole with respect to DES.

Therefore 1 gm of food contain 0.94 x 10^-9 mole with respect to DES and has 96,800 dpm activity.

Therefore 1 dpm is equivalent to

\[
\frac{9.4 \times 10^{-10}}{9.7 \times 10^4} = .971 \times 10^{-14} \text{ mole.}
\]

DES levels expressed in moles are representative of total administered DES ("cold" and "hot") per mg of tissue.
LITERATURE CITED


VITA AUCTORIS

Born:

Son of Mr. and Mrs. James M. Paterson

Elementary Education:

London, Ontario  
Tecumseh, Ontario

Secondary Education:


University Education:

B.Sc. in Biology.

Financial Assistance:

Teaching Assistantship in Biology at University of Windsor, 1968-1969.  
National Research Council of Canada Grant (No. A4670 to Dr. A. B. Okey).

Societies:

Biology Graduate Students Society, University of Windsor.