Effects of organophosphate pesticides on lipogenesis in adipose tissue and skeletal muscle of steers

Michele Lenore Trankina

Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Agriculture Commons, and the Animal Sciences Commons

Recommended Citation
Trankina, Michele Lenore, "Effects of organophosphate pesticides on lipogenesis in adipose tissue and skeletal muscle of steers" (1982). Retrospective Theses and Dissertations. 8390.
https://lib.dr.iastate.edu/rtd/8390

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
INFORMATION TO USERS

This reproduction was made from a copy of a document sent to us for microfilming. While the most advanced technology has been used to photograph and reproduce this document, the quality of the reproduction is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help clarify markings or notations which may appear on this reproduction.

1. The sign or “target” for pages apparently lacking from the document photographed is “Missing Page(s)”. If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure complete continuity.

2. When an image on the film is obliterated with a round black mark, it is an indication of either blurred copy because of movement during exposure, duplicate copy, or copyrighted materials that should not have been filmed. For blurred pages, a good image of the page can be found in the adjacent frame. If copyrighted materials were deleted, a target note will appear listing the pages in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed, a definite method of “sectioning” the material has been followed. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For illustrations that cannot be satisfactorily reproduced by xerographic means, photographic prints can be purchased at additional cost and inserted into your xerographic copy. These prints are available upon request from the Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases the best available copy has been filmed.
Trankina, Michele Lenore

EFFECTS OF ORGANOPHOSPHATE PESTICIDES ON LIPOGENESIS IN ADIPOSE TISSUE AND SKELETAL MUSCLE OF STEERS

Iowa State University Ph.D. 1982

University Microfilms International 300 N. Zeeb Road, Ann Arbor, MI 48106
Effects of organophosphate pesticides on lipogenesis in adipose tissue and skeletal muscle of steers

by

Michele Lenore Trankina

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department: Animal Science
Major: Nutritional Physiology

Approved: Members of the Committee:
Signature was redacted for privacy.

In Charge of Major Work
Signature was redacted for privacy.

For the Major Department
Signature was redacted for privacy.

For the Graduate College
Iowa State University
Ames, Iowa

1982
## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INTRODUCTION</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>REVIEW OF LITERATURE</strong></td>
<td>3</td>
</tr>
<tr>
<td><strong>Growth Promotion in Food-Producing Animals</strong></td>
<td>3</td>
</tr>
<tr>
<td>Anabolic agents</td>
<td>3</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>13</td>
</tr>
<tr>
<td>Enhancers of rumen propionate production</td>
<td>15</td>
</tr>
<tr>
<td>Organic pesticides</td>
<td>19</td>
</tr>
<tr>
<td>Lipogenesis in Ruminant Adipose Tissue</td>
<td>23</td>
</tr>
<tr>
<td>Growth of Skeletal Muscle</td>
<td>30</td>
</tr>
<tr>
<td>Summary</td>
<td>34</td>
</tr>
<tr>
<td><strong>PART I. EFFECTS OF RONNEL ON METABOLIC ACTIVITY OF</strong></td>
<td>35</td>
</tr>
<tr>
<td>SUBCUTANEOUS ADIPOSE TISSUE AND SKELETAL MUSCLE FROM 6-, 12-, AND 18-MONTH-OLD STEERS</td>
<td></td>
</tr>
<tr>
<td><strong>INTRODUCTION</strong></td>
<td>36</td>
</tr>
<tr>
<td><strong>MATERIALS AND METHODS</strong></td>
<td>38</td>
</tr>
<tr>
<td>Experimental Animals</td>
<td>38</td>
</tr>
<tr>
<td>Tissue Sampling</td>
<td>38</td>
</tr>
<tr>
<td>Incubation Procedures</td>
<td>38</td>
</tr>
<tr>
<td>Analytical Procedures</td>
<td>39</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>40</td>
</tr>
<tr>
<td><strong>RESULTS</strong></td>
<td>41</td>
</tr>
<tr>
<td>Metabolic Activity in Subcutaneous Adipose Tissue</td>
<td>41</td>
</tr>
<tr>
<td>Metabolic Activity in Skeletal Muscle</td>
<td>43</td>
</tr>
<tr>
<td><strong>DISCUSSION</strong></td>
<td>69</td>
</tr>
<tr>
<td><strong>PART II. EFFECTS OF DURSBAN® ON METABOLIC ACTIVITY</strong></td>
<td>78</td>
</tr>
<tr>
<td>OF SUBCUTANEOUS ADIPOSE TISSUE AND SKELETAL MUSCLE FROM 12- AND 18-MONTH-OLD STEERS</td>
<td></td>
</tr>
<tr>
<td><strong>INTRODUCTION</strong></td>
<td>79</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td></td>
</tr>
<tr>
<td>Experimental Animals</td>
<td>81</td>
</tr>
<tr>
<td>Tissue Sampling</td>
<td>81</td>
</tr>
<tr>
<td>Incubation Procedures</td>
<td>81</td>
</tr>
<tr>
<td>Analytical Procedures</td>
<td>82</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>83</td>
</tr>
<tr>
<td>RESULTS</td>
<td>84</td>
</tr>
<tr>
<td>Metabolic Activity in Subcutaneous Adipose Tissue</td>
<td>84</td>
</tr>
<tr>
<td>Metabolic Activity in Skeletal Muscle</td>
<td>85</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>103</td>
</tr>
</tbody>
</table>

**PART III. EFFECTS OF THYROXINE, GROWTH HORMONE, AND ORGANOPHOSPHATES ON METABOLIC ACTIVITY OF SUBCUTANEOUS ADIPOSE TISSUE FROM STEERS** 110

INTRODUCTION

MATERIALS AND METHODS

<table>
<thead>
<tr>
<th>Subsection</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Sampling</td>
<td>113</td>
</tr>
<tr>
<td>Incubation Procedures</td>
<td>113</td>
</tr>
<tr>
<td>Analytical Procedures</td>
<td>114</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>115</td>
</tr>
</tbody>
</table>

RESULTS

<table>
<thead>
<tr>
<th>Subsection</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of T₄ and T₄ Plus Organophosphates</td>
<td>116</td>
</tr>
<tr>
<td>Effects of GH and GH Plus Organophosphates</td>
<td>117</td>
</tr>
<tr>
<td>Effects of T₄ and T₄ Plus Organophosphates Versus GH and GH Plus Organophosphates</td>
<td>118</td>
</tr>
</tbody>
</table>

DISCUSSION

DISCUSSION AND SUMMARY

<table>
<thead>
<tr>
<th>Subsection</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of Organophosphates on Metabolic Activity in Adipose Tissue and Skeletal Muscle from Steers</td>
<td>127</td>
</tr>
<tr>
<td>Interactions of Organophosphates with Thyroxine and Growth Hormone</td>
<td>131</td>
</tr>
</tbody>
</table>
LITERATURE CITED

ACKNOWLEDGEMENTS

APPENDIX

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LITERATURE CITED</td>
<td>135</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>153</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>155</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 1. Effect of ronnel on oxidation of acetate to CO$_2$ in subcutaneous adipose tissue from 6-month-old steers 46
Figure 2. Effect of ronnel on oxidation of acetate to CO$_2$ in subcutaneous adipose tissue from 12-month-old steers 48
Figure 3. Effect of ronnel on oxidation of acetate to CO$_2$ in subcutaneous adipose tissue from 18-month-old steers 50
Figure 4. Effect of ronnel on fatty acid synthesis from acetate in subcutaneous adipose tissue from 6-month-old steers 52
Figure 5. Effect of ronnel on fatty acid synthesis from acetate in subcutaneous adipose tissue from 12-month-old steers 54
Figure 6. Effect of ronnel on fatty acid synthesis from acetate in subcutaneous adipose tissue from 18-month-old steers 56
Figure 7. Effect of ronnel on recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 6-month-old steers 58
Figure 8. Effect of ronnel on recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 12-month-old steers 60
Figure 9. Effect of ronnel on recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 18-month-old steers 62
Figure 10. Effect of ronnel on conversion of glucose to product in skeletal muscle from 6-month-old steers 64
Figure 11. Effect of ronnel on conversion of glucose to product in skeletal muscle from 12-month-old steers 66
Figure 12. Effect of ronnel on conversion of glucose to product in skeletal muscle from 18-month-old steers 68
| Figure 13. | Effect of Dursban® on oxidation of acetate to CO₂ in subcutaneous adipose tissue from 12-month-old steers | 88 |
| Figure 14. | Effect of Dursban® on oxidation of acetate to CO₂ in subcutaneous adipose tissue from 18-month-old steers | 90 |
| Figure 15. | Effect of Dursban® on fatty acid synthesis from acetate in subcutaneous adipose tissue from 12-month-old steers | 92 |
| Figure 16. | Effect of Dursban® on fatty acid synthesis from acetate in subcutaneous adipose tissue from 18-month-old steers | 94 |
| Figure 17. | Effect of Dursban® on recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 12-month-old steers | 96 |
| Figure 18. | Effect of Dursban® on recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 18-month-old steers | 98 |
| Figure 19. | Effect of Dursban® on conversion of glucose to product in skeletal muscle from 12-month-old steers | 100 |
| Figure 20. | Effect of Dursban® on conversion of glucose to product in skeletal muscle from 18-month-old steers | 102 |
LIST OF TABLES

Table 1. Interactions between thyroxine (T₄), growth hormone (GH), and organophosphates (ronnel and Dursban®) on oxidation of acetate to CO₂ and rates of fatty acid synthesis in subcutaneous adipose tissue from steers

Table A1. Rates of oxidation of acetate to CO₂, fatty acid synthesis, and recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 6-month-old steers as influenced by ronnel

Table A2. Rates of oxidation of acetate to CO₂, fatty acid synthesis, and recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 12-month-old steers as influenced by ronnel

Table A3. Rates of oxidation of acetate to CO₂, fatty acid synthesis, and recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 18-month-old steers as influenced by ronnel

Table A4. Rates of oxidation of glucose to CO₂, fatty acid synthesis, and glycerol production in skeletal muscle from 6-month-old steers as influenced by ronnel

Table A5. Rates of oxidation of glucose to CO₂, fatty acid synthesis, and glycerol production in skeletal muscle from 12-month-old steers as influenced by ronnel

Table A6. Rates of oxidation of glucose to CO₂, fatty acid synthesis, and glycerol production in skeletal muscle from 18-month-old steers as influenced by ronnel

Table A7. Rates of oxidation of acetate to CO₂, fatty acid synthesis, and recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 12-month-old steers as influenced by Dursban®

Table A8. Rates of oxidation of acetate to CO₂, fatty acid synthesis, and recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 18-month-old steers as influenced by Dursban®
| Table A9. | Rates of oxidation of glucose to $\text{CO}_2$, fatty acid synthesis, and glycerol production in skeletal muscle from 12-month-old steers as influenced by Dursban$^\circledR$ | 164 |
| Table A10. | Rates of oxidation of glucose to $\text{CO}_2$, fatty acid synthesis, and glycerol production in skeletal muscle from 18-month-old steers as influenced by Dursban$^\circledR$ | 165 |
| Table A11. | Effect of ronnel on rates of oxidation of acetate to $\text{CO}_2$ and rates of fatty acid synthesis expressed on a cellular basis in subcutaneous adipose tissue from 6-month-old steers | 166 |
| Table A12. | Effect of ronnel on rates of oxidation of acetate to $\text{CO}_2$ and rates of fatty acid synthesis expressed on a cellular basis in subcutaneous adipose tissue from 12-month-old steers | 166 |
| Table A13. | Effect of ronnel on rates of oxidation of acetate to $\text{CO}_2$ and rates of fatty acid synthesis expressed on a cellular basis in subcutaneous adipose tissue from 18-month-old steers | 167 |
| Table A14. | Effect of Dursban$^\circledR$ on rates of oxidation of acetate to $\text{CO}_2$ and rates of fatty acid synthesis expressed on a cellular basis in subcutaneous adipose tissue from 12-month-old steers | 168 |
| Table A15. | Effect of Dursban$^\circledR$ on rates of oxidation of acetate to $\text{CO}_2$ and rates of fatty acid synthesis expressed on a cellular basis in subcutaneous adipose tissue from 18-month-old steers | 168 |
INTRODUCTION

Perhaps the simplest explanation for why the agricultural industries in this country are dedicated to seeking improvements in production of animals used for human consumption is that increases in our population necessitate corresponding increases in the volume of food that must be produced. Improved efficiency of food production, specifically meat production, is, therefore, one obvious direction in which we must turn to deal with an ever-growing number of individuals in our own country as well as in the rest of the world.

When speaking of practices in the livestock industry that result in significant improvements in animal production, the reference is to improvements manifested primarily as greater rates of growth of animals used for human consumption and greater rates of conversion of feed an animal consumes to body weight it gains, i.e., greater feed efficiency. Supplementation of animal feeds with additives that promote growth and improve feed efficiency has played a major role in improving meat animal production as well as in decreasing costs of production.

A variety of agents has been shown to promote growth and to improve feed efficiency when administered to meat-producing animals, resulting in reduction in quantities of feed necessary to bring livestock to market weight as well as in decreasing time on feed, thereby lowering total maintenance costs. An illustration is that in 1964, the production of meat, milk, and eggs required 30 million tons less of feed than would have been necessary at 1950 feed efficiency levels (Prather, 1965).
Because adipose tissue is the major site of lipogenesis in ruminants, changes in metabolic activity of adipose tissue should be an indication of capacity to deposit fat as a component of animal growth. Furthermore, observing changes in metabolic activity in skeletal muscle that occur in conjunction with possible alterations in adipose tissue metabolism should provide an approach, at the level of body tissues, to studying effects of growth-promoting compounds on partitioning of dietary energy sources between adipose tissue and skeletal muscle. Additionally, by combining studies of effects of growth-promoting compounds on metabolic activity of adipose tissue and skeletal muscle with experiments to determine if such compounds act by way of interactions with hormones, information about the mechanism of action of growth-promoting compounds may be obtained. With these ideas in mind, the work described hereinafter was designed with two objectives: 1) to examine the effects of two organophosphate, growth-promoting compounds, ronnel and Dursban®, on substrate oxidation and lipogenesis in adipose tissue and skeletal muscle of 6-, 12-, and 18-month-old steers and 2) to consider possible interactions of ronnel and Dursban® with thyroxine or growth hormone on lipogenesis in adipose tissue from steers.
REVIEW OF LITERATURE

Growth Promotion in Food-Producing Animals

The intention of the first section of this review is to discuss growth-promoting agents and the mechanisms of action whereby they improve animal growth. This review is, however, not meant to be an exhaustive discussion of the literature dealing with growth-promoting agents but is, instead, a brief examination of some of the more well-known growth-promotants. Furthermore, this discourse will be limited to the use of growth-promoting agents in ruminant animals with a major emphasis on effects in cattle. The three classes of growth-promoting agents as well as their known or proposed mechanisms of action that will be dealt with here are 1) anabolic agents, 2) antibiotics including the enhancers of rumen propionate production, and 3) organic pesticides.

Anabolic agents

Commonly used anabolic agents or anaboles are androgens, estrogens, or progestational compounds that increase nitrogen retention and increase protein deposition in ruminants and swine so as to increase average daily body weight gains and improve feed conversion efficiency (Heitzman, 1980). Related effects attributable to administration of anabolic agents include increases in muscle mass, alterations in deposition of triacylglycerol in adipose tissue, leaner carcasses, and enhanced appetite (Fowler et al., 1978; Heitzman, 1980). These effects are not necessarily concomitant and depend on species, sex, and age of the treated animal.

Anaboles are administered as feed additives, oil-based injections, or
subcutaneous implants. The most common method of administration is through ear implants. The first anabolic compounds were introduced in the late 1940s and early 1950s at which time the synthetic estrogen, diethylstilbestrol (DES), was found to be an effective growth promotant for cattle when administered either subcutaneously or orally (Dinusson et al., 1948, 1950; Burroughs et al., 1954; Clegg and Carroll, 1956). Further discussion of DES is found in a later section of this review.

That administration of both natural and synthetic androgens to farm animals produces anabolic effects has been known for more than 30 years. One of the first accounts of effects of androgenic compounds in livestock is from Andrews et al. (1949) who observed improved growth performance and carcass quality in lambs that had been implanted with testosterone. Steers and heifers had leaner carcasses after weekly injections of testosterone (Burris et al., 1953). Administration of the synthetic androgen, trenbolone acetate (trienbolone acetate), stimulated growth in dairy and beef cows and resulted in a reduction in carcass fat deposition (Beranger and Malterre, 1968; Galbraith, 1980). When administered in combination with an estrogenic compound, trenbolone acetate increased growth rate and carcass weight of ruminants to a greater extent than when this synthetic androgen was given alone (Van der Wal, 1976; Galbraith and Watson, 1978; Roche et al., 1978).

The mechanism of action whereby androgenic compounds produce anabolic effects is not clearly delineated. There are two primary considerations; the androgen acts either directly or indirectly at the site of the skeletal muscle cell. A direct action is supported by the fact that
skeletal muscle cells possess receptors specific for structurally different androgens (Jung and Baulieu, 1972; Michel and Baulieu, 1974 cited in Heitzman, 1980).¹ Mayer and Rosen (1975) suggested that androgens increase protein deposition by displacing catabolic hormones, i.e., corticosteroids, from muscle cells, thereby decreasing rates of protein degradation in the muscle cells. Furthermore, Vernon and Buttery (1976, 1978a, 1978b) have found that treatment of female rats with trenbolone acetate produced significantly decreased rates of protein degradation and decreased rates of protein synthesis. The decreases in rates of degradation, however, were greater than the decrease in protein synthesis rates. The ultimate result was an increase in deposition of synthesized protein and less wasting of protein by degradation.

The anabolic actions of androgens also have been described as indirect effects that occur by way of mediation of effects of other hormones. Specifically, androgens may effect changes in concentrations of other hormones such as growth hormone, thyroxine, or corticosteroids.

As was the case for androgenic anabolic agents, it has been known for over 30 years that estrogenic compounds, when administered at low concentrations for a continuous period of time, are anabolic, because they increase growth rates and improve efficiency of feed conversion to body weight gain. The estrogenic compounds that produce these effects are either naturally occurring plant estrogens or are of synthetic origin.

A wide distribution of estrogenic compounds occurs in plant tissues

¹Data from Michel and Baulieu, 1974 are cited in Heitzman, 1980.
with the greatest concentrations present in leaves (Francis and Millington, 1965). These estrogens are the phytoestrogens and have been studied mainly for their ability to decrease fertility of grazing animals (Bennetts et al., 1946). The significance of plant estrogens is not fully understood with respect to stimulation of growth of foraging animals (Story et al., 1957; Stob et al., 1963; Oldfield et al., 1966). The phytoestrogens, however, are metabolized extensively in the rumen; parent compounds as well as their metabolites are absorbed from this site (Braden et al., 1967; Shutt et al., 1970). Possible improvements in growth of grazing animals, therefore, may be a result of either the plant estrogen or its metabolites.

A substance isolated from corn infected with the fungus Gibberella zeae was found to have a uterotrophic effect in mice (Stob et al., 1962). This compound was identified as a resorcylic acid lactone and was termed zearalenone (Urry et al., 1966), a name denoting its source in nature as well as its ketone properties. Zeranol, or zearalanol, as it was first named, is produced by reduction of zearalenone. The commercial name of zeranol is Ralgro®. While zeranol has definite estrogenic activity, the compound is a much less potent estrogen when administered either orally or parenterally than are the synthetic estrogens. Furthermore, the synthetic estrogen, DES, has 300 to 400 times the estrogenic activity of zeranol (Trenkle and Burroughs, 1978).

Zeranol is administered to cattle and sheep in the form of subcutaneous ear implants. Its metabolism is similar to that of other implanted estrogens because absorption is from the implantation site into the circulation and clearance and conjugation occur in the liver. A major
percentage of the metabolized estrogen is eliminated by way of bile through the large intestine into feces (Sharp and Dyer, 1972; Aschbacher and Thacker, 1974). Zeranol was attractive as a new anabolic estrogen because, unlike some synthetic estrogens, accumulation in body organs such as liver, kidney, heart, pancreas, and brain did not occur (Sharp and Dyer, 1972). When implanted in cattle and sheep, zeranol increases body weight gains and improves feed efficiency (Sharp and Dyer, 1971; Wilson et al., 1972; Borger et al., 1973; Roche and Davis, 1977). Furthermore, zeranol implants significantly increase growth rate when amounts of dietary crude protein are varied (Sharp and Dyer, 1971). Greatest growth response was observed at a ration concentrate to roughage ratio of 70 to 30 versus 80 to 20 or 60 to 40 (Sharp and Dyer, 1971). Zeranol-treated ruminants tend to have greater protein, water, and mineral contents and less fat in their bodies as revealed by body composition studies than do untreated ruminants (Fowler et al., 1970; Sharp and Dyer, 1971). Furthermore, Borger et al. (1973) suggested that zeranol-treated steers had greater water and decreased fat content and similar amounts of protein in the longissimus muscle as did untreated steers. In contrast, Wilson et al. (1972) found no significant effects of zeranol on carcass composition in lambs. Sharp and Dyer (1971) found that cattle and sheep implanted with zeranol retained more dietary nitrogen than did controls. They suggested that zeranol treatment results in partitioning of dietary energy sources toward more efficient use of dietary nitrogen to favor deposition of lean versus adipose tissue. The increase in growth rate induced by zeranol treatment is a result of increased efficiency of nutrient utilization rather than enhanced
absorption of nutrients or increases in feed consumption. Indeed, as will be discussed in the section immediately following, the explanation for anabolic effects of estrogens, in general, comes in increased retention of dietary nitrogen. Further, Tyrrell et al. (1975) present evidence indicating that the less efficient use of dietary energy by animals fed synthetic estrogens is a result of increased basal metabolism. This suggestion may be applicable to zeranol-treated animals as well.

So far, two classes of estrogenic compounds have been considered, the phytoestrogens and estrogens of mycological origin, specifically, zeranol. The third group of estrogens, the synthetic estrogens, includes the nonsteroidal compound DES and its stilbene derivatives, hexestrol and dienestrol. Diethylstilbestrol is the compound most well-known among all growth-promoting agents used in the livestock industry. Relative to beef cattle, adding small quantities of DES (10 mg per head daily) to their feed or implanting DES pellets subcutaneously produced significant increases in daily body weight gains and only slight increases in feed consumption. The net result, therefore, was a decrease in the amount of feed required per unit of body weight gain (Burroughs et al., 1955; Hale et al., 1955). More specifically, feeding DES to beef cattle increased body weight gains by 12 to 15% and improved feed efficiency by about 10% (Burroughs and Zmolek, 1963). A more recent study by Rumsey et al. (1981b) reported that feeding DES to steers increased body weight gains by 17% and improved feed efficiency by 12% when compared with steers fed no DES. Furthermore, body composition studies showed that body weight gain in DES-fed steers consisted of more protein, water, and minerals and less fat than did gain in control steers (Rumsey et al., 1981b). Earlier reports in the
literature clearly indicate that DES increases nitrogen retention (Clegg and Carroll, 1956; Wilson et al., 1963; Oltjen and Lehmann, 1968; Preston, 1975; Byers, 1979), and this finding was used as indirect evidence to suggest that DES-fed steers deposit more lean and less adipose tissue than do untreated steers. Body composition studies by Rumsey et al. (1981b) that have been described above provided direct evidence that DES increases body weight gain by increasing skeletal muscle growth.

The mechanism of action of estrogenic anabolic agents is not fully understood. There is a dearth of evidence for a direct action of estrogens with respect to anabolism at the level of body tissues. This possibility is, however, worth investigating and more experiments will undoubtedly be undertaken to test the tissue site hypothesis. The more efficient use of dietary nitrogen by DES-, hexestrol-, and zeranol-treated cattle is well-established (Burgess and Lamming, 1960; Trenkle, 1969; Fowler et al., 1970; Sharp and Dyer, 1971). Furthermore, the digestibility of dietary nitrogen or other components of the diet is not changed significantly, suggesting that estrogens do not act by way of altered rumen function (Adeyanju et al., 1969). Much evidence exists for increased utilization of dietary nitrogen in estrogen-treated ruminants. Plasma concentrations of urea (Preston, 1968; Davis et al., 1970b) and amino acids (Oltjen et al., 1973) have been shown to decrease after estrogen administration. Furthermore, urinary excretion of urea decreased after estrogen treatment (Huber, 1970).

Most of the experimental evidence regarding the mechanism of action whereby estrogens are anabolic favors the explanation that estrogen administration results in increased plasma growth hormone concentrations
that subsequently result in increased growth rates in ruminants. An interesting observation is that estrogen administration results in increases in plasma growth hormone concentrations, even in species (rats and humans) in which estrogens are not anabolic (Frantz and Rabkin, 1965; Lloyd et al., 1971). Support for increases in plasma growth hormone concentrations comes from the fact that pituitary glands from estrogen-treated ruminants are heavier relative to body weight than are pituitary glands of untreated control animals (Clegg and Cole, 1954; Struempler and Burroughs, 1959; Preston and Burroughs, 1960; Trenkle, 1970). Because pituitary glands from estrogen-treated ruminants are heavier relative to body weights than are pituitary glands from untreated animals, their growth hormone contents are greater than those of untreated ruminants, while pituitary contents of adrenocorticotrophic hormone (ACTH) and thyroid-stimulating hormone (TSH) are similar between estrogen-treated and untreated ruminants (Clegg and Cole, 1954; Shroder and Hansard, 1958; Burgess and Lamming, 1960).

Additional evidence that estrogens act in ruminants by way of growth hormone is that administration of either estrogen or growth hormone produces the same effects on growth including increased nitrogen retention (Struempler and Burroughs, 1959; Davis et al., 1970a), increased calcium and phosphorus retention (Whitehair et al., 1953; Bell et al., 1957; Braithwaite, 1975), decreased plasma urea and amino acid concentrations (Davis et al., 1970a), increased plasma glucose and insulin concentrations (Wallace and Bassett, 1966; Davis et al., 1970a), and increased metabolic rate (Yousef and Johnson, 1966).

Estrogen implants in cattle and sheep produced significant increases in plasma growth hormone concentrations (Trenkle, 1976; Donaldson, 1977).
that were associated with increases in plasma insulin concentrations (Trenkle 1969, 1970, 1976). Increased growth hormone concentrations may result in increased plasma glucose concentrations (Preston and Burroughs, 1960; Harter and Vetter, 1967; Davis et al., 1970b; Riis et al., 1974). Increases in plasma glucose concentrations then stimulate release of insulin with subsequent increases in plasma insulin concentrations (Trenkle and Burroughs, 1978). Insulin is an anabolic hormone in that it causes increased transport of amino acids into cells as well as increases in protein synthesis. Both growth hormone and insulin are essential for growth to occur, and neither hormone alone results in significant growth rates (Guyton, 1976). Because estrogen administration results in rises in plasma growth hormone and insulin concentrations, the conclusion is both logical and also supported by much experimental data that estrogens produce anabolic effects in ruminants indirectly by way of growth hormone and insulin.

Trenkle and Burroughs (1978) put forth the logical question that if estrogens are anabolic in cattle and sheep and, in light of the intact female's endogenous source of estrogen, why is the growth response seen in females of these species not as great as the growth response observed in males? The answers to this pertinent question are equally logical. The cyclic nature of estrogen secretion in the female, with peak secretion rates occurring over a period of days, is not as effective in stimulating growth as is continuous administration of estrogen over a period of weeks. The net effect, therefore, is a diminished growth response in the female ruminant relative to the intact or castrated male (Trenkle and Burroughs, 1978). Clearance rates of growth hormone from plasma may be greater in female cattle than in males (Trenkle, 1971) and could result in decreases
in plasma growth hormone concentrations in females, even though pituitary secretion of growth hormone may have increased (Trenkle and Burroughs, 1978).

Before leaving the discussion of anabolic agents and going on to the use of antibiotics in animal feeds, a brief report on the use of combinations of anabolic agents to promote growth will be given. Characteristically, the growth response to a combination of anabolic agents, e.g., androgen plus estrogen, is greater than the response to a single agent (Heitzman et al., 1977; Roche and Davis, 1977; Stollard et al., 1977). Diethylstilbestrol has been used with hexestrol to improve growth of steers (Alder et al., 1964; Everitt and Duganzich, 1965; Macdearmid and Preston, 1969).

So far in this review, no discussion of progesterone or synthetic progestins possessing anabolic activity in ruminants has been given although, in the definition of anabolic agents stated earlier, progestational compounds were included among androgens and estrogens as being anabolic. Most available experimental evidence indicates that when administered alone, neither progesterone nor synthetic progestin is anabolic in ruminants (Bloss et al., 1966; Hafs et al., 1971; Purchas et al., 1971; Grigsby et al., 1972). In combination with estrogen, however, progesterone must contribute somehow to anabolism in steers (Heinnemann and Van Keuren, 1962; Prior, 1978; Rumsey, 1978). Synovex-S is one such combination, estrogen- and progesterone-containing implant. Currently used in the beef cattle industry. Increased growth rates occur when steers are implanted with Synovex-S (Dinius et al., 1976; Embry and Gates, 1976; Kahl et al., 1978). In an effort to study the mechanism of action whereby Synovex-S increases growth rate, Kahl et al. (1978) measured
plasma triiodothyronine \((T_3)\) and thyroxine \((T_4)\) concentrations in growing and finishing steers. They found that plasma \(T_4\) concentrations and binding capacity for \(T_4\) in plasma were greater in implanted steers than in non-implanted control steers. Reports from other laboratories indicate no differences in protein-bound iodine between estrogen-treated and control steers (Clegg and Carroll, 1956; Trenkle, 1969). Kahl et al. (1978) conservatively suggest, therefore, that changes in plasma \(T_4\) concentrations may contribute to the mechanism of action whereby estrogens exert anabolic effects in cattle. The role of progesterone in these anabolic effects was not dealt with in the report by Kahl et al. (1978).

**Antibiotics**

The addition of antibiotics to animal feeds began in the late 1940s when investigators found that chlortetracycline promoted growth of chickens (Stokstad et al., 1949; Couch and Atkinson, 1950; Whitehill et al., 1950). Since then, supplementation of animal feeds with antibiotics has been shown to be of great economic value in animal production, especially in the poultry and swine industries (Braude and Johnson, 1953; Combs and Bossard, 1963; Teague et al., 1966; Jukes, 1971). General claims for the beneficial effects of antibiotic addition to feeds for poultry, swine, cattle, and sheep are growth promotion and improved feed efficiency (Jukes, 1971). Specific benefits with respect to treatment of diseases of domestic animals varies with species (Jukes, 1971).

Between 1955 and 1958, perhaps because of the impact DES was having on beef cattle production, an increased interest in supplementing cattle feeds with antibiotics came about. Earlier, Bartley et al. (1950) observed
that growth rates of dairy calves increased markedly when chlortetracycline
was added to feed. Similar reports of improvements in daily body weight
gains when antibiotics were added to cattle feeds appeared later (Beeson
et al., 1966; Embry et al., 1969; Preston and Vance, 1970 cited in Jukes,
1971).

Three theories have been outlined that offer possible explanations for
the mechanisms responsible for improved growth that occurs in animals fed
antibiotics along with their feed (Wallace, 1970). The first of these is
termed the "metabolic effect" and states that antibiotics act on enzyme
systems of the microbial flora occupying an animal's digestive tract such
that some benefit occurs to the host animal. A less likely possibility is
that a direct effect on the host animal's metabolism occurs because
absorption of antibiotics from the digestive tract into the animal's
circulation is limited (Wallace, 1970). The second theory consists of two
seemingly contradictory ideas with respect to the microorganisms involved.
The net result, however, is a greater availability of nutrients to the host
animal. This second theory, therefore, has been termed the "nutrient-
sparing effect" and states that feeding of antibiotics may destroy a
proportion of the microorganisms occupying the gut and thereby decrease
competition between microorganisms and host for dietary nutrients (Kellogg
et al., 1964, 1966). On the other side of the coin, antibiotic additives
may stimulate microbial synthesis of an essential nutrient, which is then
available for utilization by the animal (Anderson et al., 1952). While
both of these first two ideas are attractive and logical, perhaps the most

\footnote{Data from Beeson et al., 1966, Embry et al., 1969, and Preston and
Vance, 1970 are cited in Jukes, 1971.}
practical theory of how antibiotics improve growth performance is the third theory called the "disease effect". Simply stated, when antibiotics are added to feed, the main effect of the antibiotics is in treatment of an existing subclinical disease and growth performance increases because the animal's symptoms are alleviated or cured (Wallace, 1970). To put this in even more elementary terms, a healthy animal will eat more feed per day and will grow faster than a sick animal. All three of these theories may be interrelated as is most often the case when dealing with physiological mechanisms of action.

Enhancers of rumen propionate production

Monensin and lasalocid are biologically active, polyether, ionophore compounds. Both monensin and lasalocid are effective anticoccidial agents when added to poultry diets (Shumgard and Callendar, 1967) or ruminant diets (Fitzgerald, 1973) and, therefore, are classified rightly as antibiotics. The successful use of monensin and lasalocid as growth-promoting agents in cattle, however, is not related to the riddance of pathogens from the animals' bodies (Fitzgerald, 1973; Potter et al., 1976) as is the case with the antibiotic compounds discussed above. Rather, monensin and lasalocid are better described as enhancers of propionate production by rumen microorganisms (Raun et al., 1974; Richardson et al., 1974; Perry et al., 1976; Utley et al., 1976; Bartley et al., 1979; Nagaraja et al., 1982). While propionate is produced more efficiently by rumen microflora than is either acetate or butyrate (Wolin, 1960; Hungate, 1966), the mechanism of action whereby rumen microflora increase propionate output is poorly understood. The benefit of increased availability of
propionate to the ruminant, however, is well-established (Leng et al., 1967; Eskeland et al., 1974; Davis and Erhart, 1976; Raun et al., 1976).

The name monensin originates from the fact that this Food and Drug Administration-approved polyether is produced by the microorganism, *Streptomyces cinnamoneus*. Some of the well-documented effects of feeding monensin to ruminants include increased daily body weight gains in either grain- or forage-fed cattle (Potter et al., 1976; Raun et al., 1976; Utley et al., 1976) or no change in daily gain (Embry and Swan, 1974; Anthony et al., 1975; Perry et al., 1976; Joyner et al., 1979), decreased daily feed consumption (Brown et al., 1974; Embry and Swan, 1974; Perry et al., 1976; Raun et al., 1976; Joyner et al., 1979), and increased efficiency of conversion of feed energy to body weight gain (Eskeland et al., 1974; Davis and Erhart, 1976; Potter et al., 1976; Raun et al., 1976; Joyner et al., 1979). The most consistently found result of monensin supplementation to ruminant diets is alteration in the molar proportions of volatile fatty acids produced by rumen microflora. Characteristically, monensin feeding results in greater proportions of propionate to either acetate or butyrate produced intraruminally with no concomitant increase in total concentration of volatile fatty acids (Eskeland et al., 1974; Raun et al., 1974; Richardson et al., 1974; Perry et al., 1976; Raun et al., 1976; Utley et al., 1976; Lemenager et al., 1978). The improvements in feed efficiencies observed in monensin-fed ruminants are attributed to benefits of increased availability of propionate, an important precursor for glucose synthesis. Leng et al. (1967) state that 32% of the propionate produced in the rumen is used for glucose synthesis. Furthermore, between 20 and 50% of glucose in ruminants is synthesized from propionate.
(Lindsay, 1970; Bergman, 1973). With more propionate available for glucose synthesis, amino acids are in less demand for gluconeogenesis, thereby producing a sparing of protein (Leng et al. 1967). More evidence for a protein-sparing effect as a result of increased glucose synthesis from propionate comes from Eskeland et al. (1974). They found that propionate increased nitrogen retention in ruminants to a greater degree than did acetate or butyrate. Supportive evidence for more efficient utilization of dietary nitrogen is that in a study where energy metabolism in monensin-fed lambs was measured by indirect calorimetry, energy losses in methane, feces, and urine were decreased, suggesting more efficient use of amino acids for protein synthesis rather than for gluconeogenesis (Joyner et al., 1979). Another contribution of increased rumen propionate production to improved feed efficiency in monensin-fed ruminants may be by way of a resultant decrease in methanogenesis (Thornton et al., 1976; Van Nevel and Demeyer, 1977; Prange et al., 1978; Joyner et al., 1979). Monensin inhibited the four major rumen microbial species that produce formate and hydrogen (methane precursors). Inhibition of methane-generating microorganisms may explain the decreased methane production that occurs in monensin-fed ruminants (Chen and Wolin, 1979; Dennis et al., 1981). Greater loss of feed energy through methane formation occurs with acetate and butyrate production than occurs with propionate production (Wolin, 1960; Hungate, 1966). With an increase in propionate production and decreases in acetate and butyrate production, as occurs in monensin-fed ruminants, less methane is generated and more dietary energy is available to the animal, resulting in increased efficiency of utilization of feed (Eskeland et al., 1974). This capacity to retain more dietary energy is
the basis for explaining why feed consumption decreases with monensin-feeding. With an increase in propionate production and a decrease in methanogenesis, the animal can eat less and obtain the same amount of energy as an animal whose diet has not been supplemented with monensin because of less wasting of energy by methane production. Along this line, the decrease in feed consumption that occurs in monensin-fed ruminants may be the result of a chemostatic satiety mechanism that is triggered when rumen or blood propionate concentrations increase (Baile and Mayer, 1970; Theurer et al., 1974; Raun et al., 1976).

Effects on daily body weight gain and improvements in feed to gain ratios as a result of supplementation of ruminant diets with lasalocid are similar to effects observed in monensin-fed ruminants (Bartley et al., 1979; Thonney et al., 1981). Unlike monensin, however, supplementation of ruminant diets with lasalocid is in the experimental stages as lasalocid has not been approved by the Food and Drug Administration as a feed additive.

More recently, both monensin and lasalocid have been tested for their efficacy in preventing lactic acidosis in cattle (Dennis et al., 1981; Nagaraja et al., 1981, 1982). An abrupt change in a ruminant's diet, whereby the animal is suddenly given a high-grain diet without a proper period of acclimation, may result in the development of lactic acidosis (Hungate et al., 1952; Krogh, 1961; Dunlop and Hammond, 1965; Williams and MacKenzie, 1965; Mann, 1970; Allison et al., 1975; Slyter, 1976). A high-grain diet increases the availability of carbohydrates for rumen fermentation, resulting in the proliferation of Streptococcus bovis, which ferments carbohydrates to lactate. Increased rumen lactate concentrations
and lowered rumen pH then result and are conditions highly conducive to proliferation of a second species of lactate-producing bacteria, *Lactobacillus* spp. (Elam, 1976). Intraruminal administration of monensin or lasalocid inhibits these two major lactate-producing bacterial species (Dennis et al., 1981; Nagaraja et al., 1981, 1982). Furthermore, increased numbers of lactate-utilizing bacteria occurred with intraruminal administration of either monensin or lasalocid (Nagaraja et al., 1982). The increased molar proportion of propionate associated with monensin- or lasalocid-feeding may be a result of selection for rumen bacteria to favor succinate-producers and lactate-fermenters, thereby decreasing the incidence of lactic acidosis in ruminants that are changed from roughage to high-grain diets.

A relatively new experimental compound, Polyether A, a carboxylic acid ionophore with antibiotic properties, produces improvements in feed efficiency similar to those associated with monensin- or lasalocid-feeding. Effects of Polyether A on rumen fermentation of dietary nutrients to volatile fatty acids are, however, unlike effects produced by either monensin or lasalocid (Westley, 1977; Bartley et al., 1979). The prediction that other biologically active ionophore compounds will be introduced into the agricultural industry in the near future seems rather logical.

**Organic pesticides**

A fourth class of compounds studied in relation to growth promotion in cattle is the organic pesticides. Bris (1968) showed that, when Dichlorvos, an organophosphate, was added to diets of steers, significant
increases in feed efficiency occurred. Significant increases in body weight gains occurred in cows fed silage containing residues of the organophosphate compound, Phosvel® (Johnson et al., 1971; Johnson et al., 1974).

Ronnel [0,0-dimethyl O-(2,4,5-trichlorophenyl) phosphorothioate] is a systemic and topical organophosphate pesticide with the following structural formula:

![Structural formula of Ronnel](image)

The compound was introduced in 1954 by Dow Chemical, U.S.A. under the name of Dow ET-57. Ronnel is available commercially as Korlan® (spray form), Trolene®, and Nankor®. Other names that have been used over the years include Dow ET-14, Dow ET-57, and Fenchlorphos (Eto, 1974; Wissnesser, 1976). Ronnel is approved by the Food and Drug Administration as an insecticide and as an anthelmintic for controlling several ectoparasites of cattle and sheep. The organophosphate is versatile because it has been used successfully to rid livestock of screw worms (Graham et al., 1959), flies (Roberts, 1959; Eddy, 1961; Johnson and Turner, 1964), lice (Hoffman, 1961), and mites (Foulk and Matthysse, 1963). A more extensive review of the chemical properties, metabolites of, and toxicology of ronnel as well as its anticholinesterase properties is available (Trankina, 1979).
The use of ronnel as a systemic insecticide is of unquestionable value. More recently, ronnel has been studied from a different standpoint, namely for its role in growth promotion in cattle. The dosages employed in these experiments are considerably lower than doses used previously, thus creating potential for a more physiological rather than a toxicological response. Several researchers in the last five to six years have shown that adding ronnel to cattle diets results in increased average daily body weight gains and improvements in feed efficiency (Rumsey et al., 1975; Rumsey, 1976; Riley and Ware, 1977; Thomas and Ware, 1978; Rumsey, 1979; Rumsey et al., 1981a).

Unlike the anabolic agents, antimicrobial compounds, and propionate enhancers considered so far in this discussion, little information is available regarding the physiological mechanism whereby ronnel promotes growth. Rumsey (1979) has found that ronnel has a positive effect on growth that is independent of its benefits as a systemic pesticide. Plapp and Casida (1958) have shown that metabolism of ronnel is similar in the cow and in the rat, a nonruminant, thereby suggesting that ronnel's effects occur postruminally. Furthermore, Rumsey (1979) has shown that improved growth performance in ronnel-fed cattle is not the result of changes in molar proportions of rumen volatile fatty acid concentrations or in rumen microbe populations. Additionally, Trankina et al. (1981, 1982) have observed that ronnel-fed laboratory rats exhibit tendencies toward increased growth and improvements in feed efficiency that are similar to effects seen in ruminants, thus lending support to the conception that ronnel acts by way of a postruminal mechanism. While improvements in growth performance of ruminants produced by ronnel are not the result of either riddance of
ectoparasites or of intraruminal effects of ronnel, as are the cases with antibiotic feed additives and propionate enhancers, respectively, there is little comparison in the literature of ronnel and its mechanism of action to anabolic agents and their modes of action. As stated previously in this review, anabolic agents, especially the estrogens, seem to act primarily by way of alterations in endogenous hormone secretion. In the case of estrogen administration to cattle, much evidence is available to suggest that the anabolic nature of estrogens results indirectly by way of the two anabolic hormones, growth hormone and insulin. With this knowledge in mind, a reasonable prediction is that organophosphate pesticides may be anabolic in nature and that they may act indirectly by way of changes in endocrine function. Currently, a plausible theory is that organophosphate pesticides alter thyroid function, thereby producing metabolic changes that subsequently could be manifested in increased body weight gains and greater feed conversion efficiency (Beck, 1976; Rumsey et al., 1981a). Support comes in that several metabolites of the organophosphate, ronnel, are structurally similar to the "outer" halogenated phenolic ring of thyroxine, the chemical moiety of thyroxine that is most responsible for its effects on metabolism (Tapley and Basso, 1959). Furthermore, physiological doses of T4 result in protein anabolism (Cantarow and Schepartz, 1962). Other researchers have found a positive relationship between growth and plasma T4 concentrations (Kahl et al., 1977, 1978; Wrenn et al., 1980). Rumsey et al. (1981a) studied the possible effects that ronnel, when fed to steers, might have on thyroid and adrenal function. Results of their study were that ronnel-fed steers had greater plasma T4 concentrations than did steers not fed ronnel; no significant differences were observed in plasma concentrations
of triiodothyronine (T₃), cortisol, or aldosterone between ronnel-fed and control steers. While the findings of Rumsey et al. (1981a) are initially quite supportive of the contention that ronnel may be an anabolic agent acting by way of an influence on thyroid function, whether the increase in plasma T₄ concentration is a direct result of ronnel administration or whether the increased plasma T₄ concentration is a result of increased growth rate is not clear (Rumsey et al., 1981a). Future experiments to determine whether elevated plasma T₄ concentrations are a direct result of ronnel administration undoubtedly will be performed. Additionally, experiments similar to those undertaken to determine the effects of estrogenic anabolic agents on pituitary secretion of growth hormone seem highly appropriate.

Another explanation for the mechanism of action of ronnel is that positive effects on growth and feed efficiency may be related to alterations in proportions of fat accretion and muscle protein deposition. Ronnel may alter substrate utilization and metabolic activity in adipose tissue and skeletal muscle and, thereby, contribute to partitioning of dietary nutrients between fat and lean tissue (Trankina and Beitz, 1982). Once again, the proposal is that, like the hormonal anabolic agents, ronnel may act to increase utilization of dietary nitrogen to favor protein anabolism and, subsequently, deposition of lean tissue.

Lipogenesis in Ruminant Adipose Tissue

Postnatal growth can be thought of as development of body tissues with increasing age. Two of the major components of postnatal somatic growth are accretion of adipose tissue and of skeletal muscle. The purpose of
the following section of this review, therefore, is to discuss briefly some of the basic concepts of metabolic activity in adipose tissue and skeletal muscle. Most of this section will be devoted to a discussion of lipogenesis in ruminant adipose tissue. The term, lipogenesis, will be used interchangeably with fatty acid synthesis.

Unlike monogastric animals, ruminants are unique in their ability to digest, by way of microbial fermentation, vegetation that is high in cellulose content. This ability was of obvious adaptive advantage in undomesticated ruminant animals that spent their lives grazing in natural environments. With domestication of grazing animals, the practice of feeding energy-dense grain diets began and, unfortunately, brought along the undesirable effect that excess dietary energy was being deposited as trimmable carcass fat, an inefficient and expensive consequence (Allen, 1976; Hood, 1982). Our country is particularly plagued with the problem, because 50 to 100% of an annual Iowa corn crop is required to deposit the fat that is trimmed from carcasses of beef cattle each year in the United States (Allen et al., 1976). The obvious inequity is that, by feeding high-grain diets to domestic animals, we simultaneously are depleting the supply of a human food source. In time, we will need to reevaluate our priorities in terms of whether humans or domestic livestock are more important to receive feed grains. Any attempt at trying to choose between the survival of humans or domestic livestock, however, is rather absurd. We must, instead, find ways to cope with the dilemma. Fortunately, the animal production industries are not without hope. There are a number of possibilities for improvements in current management practices. One idea that already is in practice is the search for alternative food energy
sources for livestock to avoid depletion of human food sources. Another area of ongoing research already discussed in this review is the search for agents that promote growth and improve the efficiency with which feed energy is converted to body weight gain. And, to go one step further, from an economic and practical standpoint, the most desirable solution would be to find agents that selectively partition dietary nutrients toward growth of more lean and less adipose tissue such that proliferation of trimmable fat is reduced markedly.

Accretion of adipose tissue in ruminants and nonruminants occurs by two processes. Early in development, hyperplasia, increase in number of adipocytes, predominates. With increasing maturity, hyperplasia decreases and accumulation of lipid within adipocytes occurs. This accumulation of lipid, or hypertrophy, results in larger adipocytes and is the mechanism whereby fattening occurs (Hood, 1977). In the steer, internal fat (perirenal and omental) is deposited early in the course of development, and subcutaneous, intermuscular, and intramuscular fat deposition occur later (Callow, 1948; Pothoven and Beitz, 1973; Hood, 1977).

The primary sites of lipogenesis in the ruminant are adipose tissue and the lactating mammary gland (Hanson and Ballard, 1967). Liver, the major site of gluconeogenesis in ruminants, has a limited capacity for fatty acid synthesis (Hanson and Ballard, 1968). Possessing different tissue sites for lipogenesis and gluconeogenesis is advantageous to the ruminant because competition within tissues for substrates and energy is effectively eliminated (Tepperman and Tepperman, 1970). Furthermore, different adipose tissue sites differ in their lipogenic capacities (Ingle et al., 1972).
Previously, the thought was that the sole function of adipose tissue was to store triacylglycerol, thus classifying adipose tissue as a relatively inactive tissue with respect to metabolic activity. On the contrary, adipose tissue is one of the most dynamic tissues in the animal body. A major portion of the metabolizable energy consumed by a ruminant passes through adipose tissue (Emery, 1979). The two primary metabolic processes occurring in adipose tissue are triacylglycerol synthesis and lipolysis. This discussion will deal with triacylglycerol synthesis-how it occurs in adipose tissue and the limitations involved.

The adipocyte is supplied with four sources of fatty acids for triacylglycerol synthesis. Uptake of preformed fatty acids by adipocytes may occur and is largely the result of 1) hydrolysis by lipoprotein lipase of triacylglycerols of plasma lipoproteins and 2) transfer of free fatty acids in plasma into intercellular spaces of adipose tissue. Release of free fatty acids after hydrolysis of triacylglycerols within adipocytes is a result of the action of hormone-sensitive lipase, thus providing a third source of fatty acids (Lehninger, 1975). Because ruminant diets are low in fat content (2 to 5%) relative to diets of some nonruminants, de novo synthesis is the major source of fatty acids in ruminants (Bauman, 1976; Emery, 1979).

The two basic requirements for de novo fatty acid synthesis in ruminants or nonruminants are cytosolic acetyl coenzyme A (acetyl CoA) and reduced nicotinamide adenine dinucleotide phosphate (NADPH). The primary precursor for fatty acid synthesis in ruminant adipose tissue is acetate (Hanson and Ballard, 1967, 1968; Hood et al., 1972). Fetal and neonatal ruminants are able to use glucose for fatty acid synthesis in both adipose
tissue and liver (Hanson and Ballard, 1967, 1968; Bartos and Skarda, 1970). But, when microbial fermentation begins and the rumen of a young animal is functional, glucose is no longer absorbed to a significant extent and, instead, acetate, one of the major endproducts of rumen microbial fermentation, becomes the major substrate absorbed. In nonruminants, glucose, a major fatty acid precursor, undergoes glycolysis to yield pyruvate, which passes into the mitochondria to form citrate. Citrate then can move out of the mitochondria into the cytosol. The enzyme adenosine triphosphate (ATP)-citrate lyase (EC 4.1.3.8) is present in cytosol, the site of fatty acid synthesis, and catalyzes the splitting of citrate into acetyl CoA and (OAA). Because of the action of ATP-citrate lyase, glucose is the major source of acetyl CoA which, as previously stated, is necessary for fatty acid synthesis.

A second requirement for fatty acid synthesis is an adequate supply of reducing equivalents in the form of NADPH. Nonruminants have at their disposal three sources of reducing equivalents. First, generation of NADPH occurs by way of two enzymes in the pentose phosphate pathway: glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44; Flatt and Ball, 1964; Leveille, 1966; Ingle et al., 1973). Second, the isocitrate pathway provides NADPH because isocitrate can be transported out of mitochondria to the cytosol where conversion by oxidative decarboxylation to α-ketoglutarate occurs through the action of cytosolic NADP-isocitrate dehydrogenase (EC 1.1.1.42; Ingle et al., 1972). Because of the isocitrate pathway, mitochondrial acetyl CoA can be used to generate NADPH for fatty acid synthesis (Bauman et al., 1970). A third source of NADPH for fatty acid synthesis in non-
ruminants is through the conversion of malate to pyruvate in the cytosol, a reaction catalyzed by NADP-malate dehydrogenase (EC 1.1.1.40).

The inability of ruminant adipose tissue to utilize glucose for fatty acid synthesis has been attributed to low activities of two cytosolic enzymes essential for lipogenesis: ATP-citrate lyase and NADP-malate dehydrogenase (Hanson and Ballard, 1968). The net result of low activities of these enzymes is two-fold: 1) mitochondrial acetyl CoA arising from glycolysis cannot be utilized in the cytosol for fatty acid synthesis, and 2) reducing equivalents that would be generated by the action of NADP-malate dehydrogenase are not produced. In ruminants, the isocitrate pathway provides at least one-fourth of the NADPH necessary for fatty acid synthesis with the majority generated from the pentose phosphate pathway (Baldwin et al., 1973; Yang and Baldwin, 1973).

Fatty acid esterification to form triacylglycerols in ruminants proceeds by way of the α-glycerol phosphate pathway (Benson and Emery, 1971). Because of negligible activity of glycerol kinase (EC 2.7.1.30) in adipose tissue, free glycerol is not a quantitatively significant source of α-glycerol phosphate but, instead, glucose is the major source of glycerol moieties of triacylglycerol molecules (Hood et al., 1972; Martin and Wilson, 1974).

As has been discussed above, the generally accepted view is that acetate and not glucose is the major substrate for fatty acid synthesis in ruminant adipose tissue and that glucose is not utilized to a significant extent because of low activity of ATP-citrate lyase. This conception has been challenged in recent years. Whitehurst et al. (1978) have obtained substantial experimental evidence to indicate that lactate is a precursor
for fatty acid synthesis and for glycerogenesis in bovine adipose tissue. Prior (1978) showed that lactate is used as a substrate for lipogenesis in vivo in sheep. Furthermore, Whitehurst et al. (1981) found activities of ATP-citrate lyase in bovine adipose tissue to be more than adequate to support fatty acid synthesis from lactate. Prior and Jacobson (1979a) have shown also that adipose tissue contains a significant concentration of specific isoenzymes of lactate dehydrogenase (EC 1.1.1.27) such that lactate would be provided for lipogenesis as a product of anaerobic glycolysis in skeletal muscle, erythrocytes, and lungs. In addition to lactate as an alternative substrate for lipogenesis in bovine adipose tissue, Prior and Jacobson (1979a,b) found that pyruvate was utilized for fatty acid synthesis at greater rates than was acetate. The previously accepted idea that low ATP-citrate lyase activity in bovine adipose tissue limits lipogenesis from glucose deserves careful reexamination. The inability of ruminant adipocytes to synthesize fatty acids from glucose may be more accurately explained on the basis of inhibition of a step in glycolysis, resulting in impairment of conversion of glucose to pyruvate (Whitehurst et al., 1981).

During growth, protein deposition plateaus and is exceeded by fat accretion (Bergen, 1974). Increases in body weight with increasing age, therefore, are primarily the result of fat accretion. While overall fat deposition increases with age, rates of fatty acid synthesis, when expressed on a tissue weight basis or in terms of milligrams of soluble protein, decrease in all adipose tissue sites of steers (Pothoven and Beitz, 1973). The apparent dichotomy in the above two statements is explained by the fact that triacylglycerol deposition in adipose tissue is
a result of increased filling of adipocytes with lipid, i.e., hypertrophy, while cytosolic protein content, including lipogenic enzyme concentration, remains constant (Allee et al., 1971). The net result is that, although an animal may continue to deposit fat and thereby increase adipocyte hypertrophy, lipogenic capacity per unit of cytosolic protein actually decreases with increasing age of cattle.

Experiments designed to study rates of fatty acid synthesis in animal tissues classically are in vitro experiments in which radioactively labelled substrates are incubated with liver tissue slices or sections of adipose tissue or mammary gland (Pothoven and Beitz, 1973). Incubated tissues are extracted for total lipids and separated into fatty acid and glycerol fractions and, radioactivity in each fraction is quantified by liquid scintillation spectrophotometry. The incubation system is conducive also to determination of rates of oxidation of substrate to carbon dioxide (CO₂; Buhler, 1962). Radioactive CO₂ is volatilized from media in incubation flasks and is collected on filter paper saturated with sodium hydroxide. The filter paper is dried and radioactive carbon incorporated into CO₂ is quantified, as with fatty acid and glycerol fractions, by liquid scintillation spectrophotometry. Incorporation rates by adipocytes of substrate into long-chain fatty acids or CO₂, therefore, are indices of fatty acid synthetic rates and capacity to generate ATP by way of the TCA cycle. Details of tissue incubation procedures can be found in the Materials and Methods sections of this dissertation.

Growth of Skeletal Muscle

A second component of growth, in addition to fat accretion, in food-producing animals is growth of skeletal muscle, and energy-efficient
deposition of skeletal muscle is a fundamental goal in meat animal pro-
duction. Skeletal muscle growth is the result of two processes, protein
accretion, which can be thought of as the net result of protein synthesis
and protein degradation, and muscle cell proliferation (Allen et al., 1979).
Protein accretion is influenced by interactions between nutritional and
endocrine status of an animal, the explanations of which are complex and
not completely resolved and, therefore, will not be dealt with here.
Proliferation of myogenic cells (precursors of muscle fibers) can be
divided into a prenatal phase, when muscle fibers (cells) are undergoing
hyperplasia, and a postnatal period of muscle fiber hypertrophy
(Swatland, 1976). No substantial increase in number of muscle fibers
occurs after birth but, on the basis of deoxyribonucleic acid (DNA)
accumulation in muscle, the postnatal period is an important time of
addition of nuclear material for several species (Winick and Noble, 1966;
Harbison et al., 1976; Johns and Bergen, 1976). The source of DNA for
accumulation in skeletal muscle during the postnatal period was not
evident because muscle fiber nuclei are incapable of DNA synthesis or
mitosis, and muscle fiber number remains constant (Allen et al., 1979).
Mauro (1961) detected in muscle what we know now as satellite cells. Sub-
sequently, satellite cells were shown to divide and fuse with existing
Furthermore, satellite cell populations in an animal decrease with in-
creasing age (Allbrook et al., 1971; Snow, 1977; Young et al., 1979). But,
because repair of muscle after injury occurs, even in older animals, a
proportion of satellite cells evidently remains functional into old age
(Schamlbruch and Hellhammer, 1976; Snow, 1977).
The capacity of myogenic or satellite cells to proliferate in muscle carries significant implications for postnatal stimulation of muscle growth (Allen et al., 1979). In the meat animal industry, where the proportion of muscle to adipose tissue an animal gains is of unquestionable economic importance, the potential for improvements in efficiency of production by increasing lean tissue growth may reside in manipulation of myogenic or satellite cell proliferation (Allen, 1976; Allen et al., 1979). Several agents are known to affect myogenic cell proliferation in vitro. Agents with a stimulatory effect include testosterone (Powers and Florini, 1975), growth hormone and insulin in greater than physiological concentrations (Gospodarowicz et al., 1975, 1976; Florini et al., 1977), and several growth factors (Dulak and Temin, 1973a,b; Gospodarowicz et al., 1975, 1976). Agents that are not mitogenic (stimulatory to proliferation) with respect to myogenic cells include 17-β-estradiol (Powers and Florini, 1975) and physiological concentrations of either growth hormone or insulin (Gospodarowicz et al., 1975, 1976; Florini et al., 1977). Because the results regarding stimulation of myogenic cell proliferation by exogenous agents have been obtained largely from cell culture experiments, the physiological significance of results remains unclear.

Experiments have been conducted recently to determine the effects of prenatal decapitation, which effectively removes neuroendocrine influences on subsequent fetal development in the pig, including development and growth of skeletal muscle (Campion et al., 1981). Indices of muscle growth and development in studies by Campion et al. (1981) included determinations of muscle weight and length, minimum fiber diameter, concentrations of DNA, ribonucleic acid (RNA), and protein, and numbers of
muscle cell or satellite cell nuclei. With respect to organ development, decapitation of the fetal pig at 45 days of gestation results in significantly decreased weights of heart, liver, thyroid gland, and kidney as well as in testicular and adrenal gland growth retardation (Kraeling et al., 1978; Colenbrander et al., 1979), indicating that decapitation produces measurable effects on endocrine gland development. No differences in somatic growth or skeletal muscle development were evident, however, between decapitated and sham-operated fetal pigs, suggesting that an intact neurohypophyseal axis is not crucial for development of skeletal muscle in fetal pigs beyond 45 days in utero (Campion et al., 1981). Campion et al. (1981) cautiously indicate that their findings do not prove unequivocally the absence of hormonal control of somatic growth in the fetus. Rather, the mechanism whereby somatic growth is regulated differs between the pre- and postnatal animal.

Skeletal muscle is versatile with respect to substrate utilization because glucose, free fatty acids, and ketone bodies all serve as fuel for oxidation and subsequent production of ATP. All vertebrate muscles are capable of aerobic and anaerobic metabolism depending on nutritional and endocrine status of the animal. Under aerobic conditions, free fatty acids are the primary substrates for oxidation in skeletal muscle, while glucose is preferred during hypoglycemia or under anaerobic conditions. Energy is stored in muscle primarily as glycogen with some triacylglycerol present. Glucose, therefore, contributes to glycogen synthesis and also is a source of glycerol for triacylglycerol synthesis. Fatty acids derived from plasma are incorporated into triacylglycerol or oxidized by skeletal muscle (Lehninger, 1975; Atkins, 1981).
Experiments conducted recently have shown that skeletal muscle tissue sections could be incubated with radioactive substrates and incorporation rates of substrate into product could be determined using procedures similar to those described above for adipose tissue incubation experiments (Harmon et al., 1982; Trankina and Beitz, 1982).

**Summary**

The objectives of this review have been 1) to survey the variety of agents that have been used successfully as feed additives to improve growth performance of livestock and to describe the biochemical and (or) physiological mechanisms whereby these agents increase growth rates and improve feed efficiency and 2) to provide a brief overview of the components of somatic growth-accretion of fat and deposition of skeletal muscle. With a knowledge of how proven growth-promoting agents function, and in light of the economic significance of increasing the proportion of lean to trimmable fat in food-producing animals, we are equipped to search for other compounds that have the potential to affect the meat animal industry with the impact of diethylstilbestrol.
PART I. EFFECTS OF RONNEL ON METABOLIC ACTIVITY OF SUBCUTANEOUS ADIPOSE TISSUE AND SKELETAL MUSCLE FROM 6-, 12-, AND 18-MONTH-OLD STEERS
INTRODUCTION

A fundamental goal in livestock production is to introduce management practices that result in improved growth performance of food-producing animals. Supplementation of animal feeds with additives that promote growth and improve efficiency of feed energy conversion to body weight gain has played a major role in improving meat animal production as well as in decreasing production costs. A variety of agents has been shown to promote growth and to improve feed efficiency when administered to cattle. Perhaps the most well-known agent is the anabolic, synthetic estrogen, diethylstilbestrol (Burroughs et al., 1955), which promotes lean tissue gain in cattle by increasing nitrogen retention (Clegg and Carroll, 1956; Oltjen and Lehmann, 1968; Byers, 1979). In the last 10 years, organophosphate pesticides have received attention because of their ability to promote growth when fed to cattle (Johnson et al., 1971; Johnson et al., 1974). Ronnel, [0,0-dimethyl 0-(2,4,5-trichlorophenyl) phosphorothioate], is an anthelmintic and insecticidal organophosphate that improves growth performance of cattle (Rumsey et al., 1975; Thomas and Ware, 1978; Rumsey, 1979; Rumsey et al., 1981a). While little information is available describing exactly how ronnel produces growth-promoting effects, substantial evidence exists to suggest that ronnel acts by way of a postruminal mechanism (Plapp and Casida, 1958; Rumsey, 1979; Trankina et al., 1981, 1982). Furthermore, positive effects on growth are independent of ronnel's benefits as a pesticide (Rumsey, 1979). A plausible explanation is that ronnel's effects on growth and feed efficiency may be related to alterations in proportions of deposited fat
and muscle protein. No studies to determine how ronnel might affect metabolism in adipose tissue and skeletal muscle of growing steers have been carried out. The purpose of the present study, therefore, was to test the hypothesis that ronnel alters substrate utilization and metabolic activity in subcutaneous adipose tissue and skeletal muscle from steers at 6, 12, and 18 months of age.
MATERIALS AND METHODS

Experimental Animals

Six-, 12-, and 18-month-old steers originated from a herd designed to produce three body frame sizes: large, medium, and small. Steers used in the study were large-frame, Angus x Simmental. Steers were housed outdoors at the McNay Research Center in Chariton, IA and were fed a high-energy diet ad libitum. The diet consisted of 68.7% shelled corn, 25% corn cobs, 5% molasses, and 1.3% minerals. Five steers at each age were transported from the McNay Research Center to Ames three weeks before slaughter and housed at the Iowa State Beef Nutrition Research Farm. At the time of slaughter, at the Iowa State University Meats Laboratory, steers had average body weights of 185.9, 426.5, and 595.6 kg at 6, 12, and 18 months of age, respectively.

Tissue Sampling

Subcutaneous adipose tissue (backfat) and skeletal muscle (semitendinosus) were obtained within 30 minutes after stunning and exsanguination. Subcutaneous adipose tissue was maintained in 0.15 M NaCl at 37° C, and skeletal muscle was kept on ice before sectioning and subsequent incubation.

Incubation Procedures

A 100 to 200 mg (wet weight) thin section of adipose tissue or a thin, longitudinal slice of skeletal muscle was incubated in each flask under an atmosphere of 95% O₂ and 5% CO₂ with constant shaking for 2 hours at 37° C. Each flask for adipose tissue incubations contained 3 ml of Krebs-Ringer bicarbonate buffer (Ca²⁺-free, pH 7.4; Laser, 1961), 75 μmoles of sodium
acetate, and 15 μmoles of glucose to provide a source of glyceride-glycerol and as a substrate for generation of reducing equivalents (NADPH) by way of the pentose phosphate pathway (Bauman et al., 1970; Yang and Baldwin, 1973) plus 1 μCi of sodium-[1-14C]-acetate (New England Nuclear, Boston, MA). Each flask for skeletal muscle incubations contained 3 ml of Krebs-Ringer bicarbonate buffer (Ca2+-free, pH 7.4; Laser, 1961) and 15 μmoles of glucose plus 1 μCi of D-[U-14C]-glucose (New England Nuclear, Boston, MA). Bovine insulin (0.3 IU; Sigma, St. Louis, MO) was added to all flasks. Ronnel was dissolved in minimal quantities of 100% ethanol such that a 1 μl aliquot in each flask would deliver 2.5, 5, or 10 mg of ronnel per kg of adipose tissue or skeletal muscle. Flasks containing 3 ml of Krebs-Ringer bicarbonate buffer, 1 μl of 100% ethanol, and no ronnel served as controls. Triplicate incubations were performed for each tissue sample and ronnel dose combination. A set of zero-time incubations was included for each of the two substrates. To collect 14CO2, 0.1 ml of 30% NaOH was injected onto filter paper situated inside a hanging well in each flask. Incubations were terminated by addition of 0.5 ml of 1.5 N H2SO4.

Analytical Procedures

Oxidation of acetate or glucose to CO2 was quantified using the method of Buhler (1962). After incubation, 13.2 ml of chloroform: methanol (1:2, v/v) and 2.3 ml of chloroform:methanol:water (1:2:0.8, v/v/v) were added to each flask (Bligh and Dyer, 1959). Flask contents were transferred to screw-cap extraction tubes, and tissue sections and media were homogenized with a Polytron homogenizer (Brinkman Instruments,
(Westbury, NY). The shaft of the homogenizer was rinsed with 5 ml of chloroform, and this rinse was collected in extraction tubes. Tissue samples were washed and lipids were extracted using a modification of the Folch wash procedure (Folch et al., 1957). Each sample was washed with one 5-ml and three 10-ml aliquots of deionized H_2O resulting in formation of an upper, aqueous layer and a lower, chloroform layer. Samples were evaporated to dryness, and 10 ml of chloroform were added to each tube. Total lipids were transesterified with 1.25 M sodium methoxide and subsequently neutralized with 10% methanolic HCl to split triacylglycerols into fatty acid methyl esters and free glycerol. Ten ml of deionized H_2O were added to each tube to separate the upper, aqueous, glycerol-containing layer from the lower, chloroform, fatty acid-containing layer. Fatty acid and glycerol fractions were evaporated to dryness and, after addition of Beckman Ready-Solv™ scintillation cocktail, were assayed for radioactivity by liquid scintillation counting (Model LS-8000 liquid scintillation spectrophotometer, Beckman Instruments, Irvine, CA). Quenching was corrected by the external standard method.

Statistical Analysis

Data were analyzed by analysis of variance (Barr et al., 1979) and Duncan's new multiple range test (Steel and Torrie, 1960).
RESULTS

In vitro rates of substrate conversion to product were used to assess the effects of ronnel on metabolic activity in subcutaneous adipose tissue and in skeletal muscle. The data are organized as histograms with the accompanying format of substrate conversion to product versus ronnel dose. Conversion rates are expressed on a tissue-weight basis. Rates represented by bars with different letters are significantly different at 5% probability. Tissue site and age of steers are noted on each graph.

Metabolic Activity in Subcutaneous Adipose Tissue

With respect to oxidation of acetate to CO$_2$ in 6-month-old steers, shown in figure 1, ronnel did not produce statistically significant effects (P>.05). A nearly significant (P<.06) linear decrease in acetate oxidation to CO$_2$ with increasing ronnel dose, however, was observed. In experiments with adipose tissue from 12- and 18-month-old steers, only the 2.5 and 5 mg/kg doses of ronnel were tested. Effects of ronnel dose on oxidation of acetate to CO$_2$ in adipose tissue from 12-month-old steers (figure 2) were similar to those for 6-month-old steers, but oxidation rates were 30 to 50% greater in 12-month-old steers than in 6-month-old steers. Figure 3 shows rates of oxidation of acetate to CO$_2$ in adipose tissue from 18-month-old steers. No statistically significant (P>.05) effects of ronnel on oxidation rates were observed. Rates of oxidation of acetate to CO$_2$ in adipose tissue from 18-month-old steers quantitatively were similar to rates in adipose tissue from 6-month-old steers with rates in both ages of steers ranging from 400 to 600 nmoles of CO$_2$ produced per 2 hours per gram of adipose tissue.
Fatty acid synthesis rates were determined to provide a second index of metabolic activity in adipose tissue as a function of ronnel dose. Figures 4 and 5 show that, in adipose tissue from 6- and 12-month-old steers, increasing doses of ronnel added to incubation media produced significant (P<.05) decreases in rates of fatty acid synthesis from acetate. Furthermore, in adipose tissue from 12-month-old steers, a highly significant (P<.001) linear decrease in rates of fatty acid synthesis from acetate occurred with increasing dose of ronnel. In adipose tissue from 12-month-old steers, rates of fatty acid synthesis from acetate were 50 to 60% lower than rates in adipose tissue from 6-month-old steers. No statistically significant (P>.05) effects of ronnel on rates of fatty acid synthesis were observed in adipose tissue from 18-month-old steers (figure 6); rates, however, were 50 to 75% lower in adipose tissue from 18-month-old steers versus rates in adipose tissue from 6-month-old steers and 55 to 65% lower in adipose tissue from 18-month-old versus 12-month-old steers. Rates of fatty acid synthesis in adipose tissue, therefore, decreased with increasing age of steers.

Radioactivity present in glycerol (figures 7, 8, and 9) is not the result of de novo glycerogenesis from acetate but, instead, represents recycling of radioactive carbon (14C), derived from acetate, through tricarboxylic acid (TCA) cycle intermediates with eventual incorporation of 14C into glycerol. In adipose tissue from the three ages of steers, recycling of acetate carbon into glycerol decreased with increasing dose of ronnel. Rates of incorporation of radioactive carbon from acetate into glycerol decreased with increasing age of steers.
Metabolic Activity in Skeletal Muscle

Effects of ronnel in another metabolically active tissue, skeletal muscle, were examined. Because glucose is an important substrate for energy utilization in skeletal muscle, glucose was used as the substrate for oxidation measurements. Figures 10, 11, and 12 are composite representations of metabolic activity in skeletal muscle, and each graph shows rates of oxidation of glucose to CO₂, rates of fatty acid synthesis, and rates of glycerol production from glucose.

Addition of ronnel to incubation media produced a significant (P<.04) linear decrease in oxidation of glucose to CO₂ in skeletal muscle from 6-month-old steers (figure 10). Oxidation of glucose to CO₂ tends to increase with increasing dose of ronnel in skeletal muscle from 12-month-old steers (figure 11). No statistically significant (P>.05) effect of ronnel occurred in skeletal muscle from 18-month-old steers (figure 12). Quantitatively, rates of oxidation of glucose to CO₂ were greatest in skeletal muscle from 6-month-old steers and lowest in skeletal muscle from 12-month-old steers.

Rates of fatty acid synthesis from glucose in skeletal muscle from 6-, 12-, and 18-month-old steers were low relative to rates of fatty acid synthesis from acetate in adipose tissue. Furthermore, rates of fatty acid synthesis in skeletal muscle decreased with increasing age of steers; rates were negligible in skeletal muscle from 18-month-old steers. Addition of ronnel to incubation media decreased fatty acid synthesis rates significantly (P<.05) in skeletal muscle from 12-month-old steers; ronnel had no significant (P>.05) effect on rates of fatty acid synthesis in skeletal muscle from 6- or 18-month-old steers. At both the 6- and 12-
month ages, 2.5 and 5 mg/kg doses of ronnel depressed de novo synthesis of fatty acids in skeletal muscle, although statistically significant (P<.05) decreases occurred only in muscle from 12-month-old steers.

Glycerol production from glucose as influenced by ronnel addition to incubation media also was observed. As might be expected, rates of glycerol production in skeletal muscle certainly were measureable when glucose was the substrate (figures 10, 11, and 12). In skeletal muscle from 6-month-old steers, an increase in glycerol production occurred with increasing dose of ronnel. When ronnel dose was 10 mg/kg, glycerol production was significantly greater (P<.05) than when ronnel dose was 0 or 2.5 mg/kg (figure 10). In skeletal muscle from 12-month-old steers, rates of glycerol production from glucose were not affected by addition of ronnel to incubation media. Glycerol production rates in skeletal muscle from 18-month-old steers increased when ronnel was added to incubation media, but the increase was statistically significant (P<.05) only for the 2.5 mg/kg dose. Rates of glycerol production were 4- to 8-fold greater in skeletal muscle from 6-month-old steers than in skeletal muscle from either 12- or 18-month-old steers.
Figure 1. Effect of ronnel on oxidation of acetate to CO$_2$ in subcutaneous adipose tissue from 6-month-old steers
ADIPOSE TISSUE FROM 6-MONTH-OLD STEERS

ACETATE → CO₂ (nmoles/2 hr/g)

RONNEL DOSE (mg/kg tissue)
Figure 2. Effect of ronnel on oxidation of acetate to $\text{CO}_2$ in subcutaneous adipose tissue from 12-month-old steers
ADIPOSE TISSUE FROM 12-MONTH-OLD STEERS

ACETATE → CO₂ (nmoles/2hr×g)

RONNEL DOSE (mg/kg tissue)

0

2.5

5

B

AB
Figure 3. Effect of ronnel on oxidation of acetate to CO₂ in subcutaneous adipose tissue from 18-month-old steers.
ADIPOSE TISSUE FROM 18-MONTH-OLD STEERS

ACETATE → CO₂ (nmole/2hr×g)

RONNEL DOSE (mg/kg tissue)
Figure 4. Effect of ronnel on fatty acid synthesis from acetate in subcutaneous adipose tissue from 6-month-old steers.
ADIPOSE TISSUE FROM 6-MONTH-OLD STEERS

ACETATE→FATTY ACIDS (nmoles/2hr×g)

RONNEL DOSE (mg/kg tissue)

0 2.5 5 10
Figure 5. Effect of ronnel on fatty acid synthesis from acetate in subcutaneous adipose tissue from 12-month-old steers
ADIPOSE TISSUE FROM 12-MONTH-OLD STEERS

ACETATE → FATTY ACIDS (nmol/2hr×g)

RONNEL DOSE (mg/kg tissue)

0

2.5

5

A

B

B
Figure 6. Effect of ronnel on fatty acid synthesis from acetate in subcutaneous adipose tissue from 18-month-old steers
ADIPOSE TISSUE FROM 18-MONTH-OLD STEERS

ACETATE ➔ FATTY ACIDS (nmol/2hr×g)

RONNEL DOSE (mg/kg tissue)

0  2.5  5
Figure 7. Effect of ronnel on recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 6-month-old steers
ADIPOSE TISSUE FROM 6-MONTH-OLD STEERS

ACETATE → GLYCEROL (nmol/2 hr/g tissue)

RONNEL DOSE (mg/kg tissue)

0  2.5  5  10
Figure 8. Effect of ronnel on recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 12-month-old steers
ADIPOSE TISSUE FROM 12-MONTH-OLD STEERS

ACETATE → GLYCEROL (nmol/2 hr/g)

RONNEL DOSE (mg/kg tissue)

0 2.5 5
Figure 9. Effect of ronnel on recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 18-month-old steers
ADIPOSE TISSUE FROM 18-MONTH-OLD STEERS

ACETATE → GLYCEROL (nmoles/2 hr × g)

RONNEL DOSE (mg/kg tissue)
Figure 10. Effect of ronnel on conversion of glucose to product in skeletal muscle from 6-month-old steers
Figure 11. Effect of ronnel on conversion of glucose to product in skeletal muscle from 12-month-old steers
CARBON DIOXIDE
SKELETAL MUSCLE FROM 12-MONTH-OLD STEERS

GLUCOSE→PRODUCT (nmoles/2hr×g)

CARBON DIOXIDE
FATTY ACIDS
GLYCEROL

RONNEL DOSE (mg/kg tissue)

0 2.5 5

0 2.5 5

0 2.5 5
Figure 12. Effect of ronnel on conversion of glucose to product in skeletal muscle from 18-month-old steers
GLUCOSE PRODUCT (nmol/2 hr x g)

CARBON DIOXIDE SKELETAL MUSCLE FROM 18-MONTH-OLD STEERS

FATTY ACIDS

GLYCEROL

RONNEL DOSE (mg/kg tissue)
DISCUSSION

The mechanisms of action of several known growth promotants have been studied extensively (Trenkle, 1969; Chalupa, 1977). Relatively little information is available, however, to describe how organophosphate compounds promote growth and improve feed efficiency in cattle. Data from preliminary experiments indicate that ronnel might alter substrate utilization and rates of fatty acid synthesis in adipose tissue and skeletal muscle from rats and a steer (Trankina and Beitz, Department of Animal Science, Iowa State University, Ames, Iowa). The objective of the present experiment, therefore, was to determine the effects of an organophosphate, ronnel, on metabolic activity in subcutaneous adipose tissue and skeletal muscle from large-frame steers at 6, 12, and 18 months of age. Rates of oxidation of acetate to CO₂ and rates of fatty acid synthesis were used as the primary indices of metabolic activity.

In adipose tissue from 6-month-old steers, a nearly significant (P<.06) linear decrease in rates of oxidation of acetate to CO₂ occurred with increasing dose of ronnel (figure 1). The 2.5 mg/kg dose of ronnel tended to produce greater rates of oxidation of acetate to CO₂ (P>.05). The 5 and 10 mg/kg doses of ronnel produced rates of oxidation of acetate to CO₂ that were less than control rates, although not statistically significantly so (P>.05). The pattern of oxidation rates of acetate to CO₂ in adipose tissue from 12-month-old steers as influenced by ronnel (figure 2) is similar to that which occurred in adipose tissue from 6-month-old steers. The addition of 2.5 mg of ronnel per kg of adipose tissue to incubation media produced greater (P<.05) rates of acetate oxidation to
CO_2. The 5 mg/kg dose produced oxidation rates that were similar to control rates. No statistically significant (P>.05) effects of ronnel on rates of acetate oxidation to CO_2 were observed in adipose tissue from 18-month-old steers. Because of high standard errors of the means, these tendencies were not statistically significant at 5% probability (figure 3). Quantitatively, rates of oxidation of acetate to CO_2 per unit weight of tissue were 30 to 50% greater in adipose tissue as steers grew from 6 to 12 months of age. Rates in adipose tissue from 18-month-old steers were quantitatively similar to rates in adipose tissue from 6-month-old steers.

The principal result of oxidation of substrates, by way of the TCA cycle, is the production of adenosine triphosphate (ATP) by oxidative phosphorylation. Production of CO_2, therefore, is a measure of the capacity to generate ATP. The need for ATP is continuous throughout an animal's life; with increasing age, however, necessity of ATP for body functions associated with growth and muscular activity decreases. Younger, more active animals, therefore, have greater capacities for ATP production and characteristically exhibit greater metabolic rates than do older, more sedentary animals (Guyton, 1976; Atkins, 1981). In the present investigation, metabolic activity of subcutaneous adipose tissue as reflected by rates of oxidation of acetate to CO_2 tend to be more suppressed by ronnel at 6 than at 12 or 18 months of age. The effects of ronnel may be more conspicuous in adipose tissue from younger, more metabolically active steers and may become relatively more subtle and less easily detected with advancing age. That metabolic activity declines with advancing age is supported by the fact that overall rates of oxidation of acetate to CO_2 increased as steers grew
from 6 to 12 months of age but, then, returned to rates that occurred in adipose tissue from 6-month-old steers.

Because adipose tissue is the primary site of lipogenesis and acetate is the principal substrate for lipogenesis in ruminants (Hanson and Ballard, 1967, 1968; Hood et al., 1972), the capacity of adipose tissue to synthesize fatty acids from acetate represents a second important index of metabolic activity. Additionally, the significance of subcutaneous adipose tissue as a site of fatty acid synthesis and subsequent lipid accretion is well-established (Pothoven and Beitz, 1973; Hood and Allen, 1975; Whitehurst et al., 1981). In adipose tissue from 6- and 12-month-old steers, significant (P<.05) decreases in rates of fatty acid synthesis occurred as ronnel dose increased (figures 4 and 5). Furthermore, the decrease in rates of fatty acid synthesis as influenced by addition of ronnel to incubation media was linear (P<.001) in adipose tissue from 12-month-old steers. No statistically significant (P>.05) effects of ronnel on rates of fatty acid synthesis were observed in adipose tissue from 18-month-old steers (figure 6). Ronnel, therefore, depressed metabolic activity in adipose tissue from 6- and 12-month-old steers but not in adipose tissue from 18-month-old steers. Once again, the lack of response in older animals to potential metabolic actions of ronnel may be the result of a general decline in metabolic responsiveness that occurs with age. Ronnel had the greatest effect on fatty acid synthesis in adipose tissue from 12-month-old steers, i.e., the greatest difference between rates of fatty acid synthesis with and without ronnel added to incubation media occurred in adipose tissue from 12-month-old steers. Quantitatively, rates of fatty acid synthesis were greatest in adipose tissue from 6-month-old
steers and least in adipose tissue from 18-month-old steers. In the present study, therefore, rates of fatty acid synthesis expressed on a tissue-weight basis decreased with increasing age of steers. With respect to rates of fatty acid synthesis expressed on the basis of adipose tissue weight, the results of the present investigation are in agreement with the findings of others. Fatty acid synthesis rates, expressed on a tissue-weight basis, decreased with advancing age in all adipose tissue depots examined (subcutaneous, perirenal, omental, and intermuscular) in Holstein steers (Pothoven and Beitz, 1973). Decreases in fatty acid synthesis rates occurring with increasing age also have been observed in adipose tissue from lambs (Ingle et al., 1972) and from rats (Benjamin et al., 1961). Based on overall rates of fatty acid synthesis in the present investigation, adipose tissue from younger steers was more metabolically active than adipose tissue from older steers, thus lending support to the contention that ronnel's effects on metabolic activity in adipose tissue from younger steers may be more easily detected than effects in older steers.

Radioactivity present in glycerol in adipose tissue (figures 7, 8, and 9) is not the result of net production of glycerol from acetate, because acetate cannot be glycerogenic. The observed rates of glycerol production are, instead, presumed to represent recycling of $^{14}$C, derived from acetate, through TCA cycle intermediates. Because succinate, a TCA cycle intermediate, is a symmetrical molecule, $^{14}$C passing through the TCA cycle will be equally distributed in the two carboxyl groups of succinate. Subsequent metabolism of succinate to fumarate and fumarate to malate occurs. Malate can leave the mitochondria and is converted to oxaloacetate (OAA)
in the cytosol. Radioactive carbon from OAA is incorporated into a series of intermediate compounds with eventual incorporation of $^{14}C$ into glycerol. In adipose tissue from steers at all three ages, recycling of acetate carbon into glycerol decreased ($P<.05$) with increasing dose of ronnel. If recycling of $^{14}C$ into glycerol is considered a metabolic process, then the diminished capacity for adipose tissue to incorporate acetate carbon into glycerol by way of the TCA cycle when ronnel is added to incubation media provides additional support for the concept that ronnel depresses metabolic activity of adipose tissue. For all treatments, rates of incorporation of $^{14}C$ from acetate into glycerol decreased with increasing age of steers. This decrease may be the result of a general decrease in metabolic activity with age. Another possible explanation for the observed decrease in glycerol radioactivity that occurred with increasing age might be a result of the techniques used to separate fatty acid and glycerol fractions as these techniques were improved at the time of analysis of tissue from 18-month-old steers. Furthermore, rates of recycling of acetate carbon into glycerol in adipose tissue from 6- and 12-month-old steers in the present study are greater than those reported elsewhere, while rates in adipose tissue from 18-month-old steers are in agreement with reported values (Whitehurst et al., 1978).

Effects of ronnel were examined in another metabolically active tissue, skeletal muscle. Rates of oxidation of glucose to CO$_2$, fatty acid synthesis rates, and glycerol production were determined (figures 10, 11, and 12). In skeletal muscle, glucose is an important substrate for energy utilization and is a precursor for glyceride-glycerol production necessary for synthesis of triacylglycerols, which are storage forms of energy in
muscle (Lehninger, 1975; Atkins, 1981). Addition of ronnel to incubation media was associated with a significant (P<.04) decrease in oxidation of glucose to CO₂ in skeletal muscle from 6-month-old steers. Oxidation of glucose to CO₂ tended to increase with increasing dose of ronnel in skeletal muscle from 12-month-old steers (P>.05) and tended to decrease with increasing dose of ronnel in skeletal muscle from 18-month-old steers (P>.05). On the basis of glucose oxidation to CO₂, ronnel decreased metabolic activity in skeletal muscle from 6-month-old steers but increased metabolic activity as steers grew to 12 months of age. By 18 months of age, ronnel had little effect on skeletal muscle, although a tendency toward decreased metabolic activity was observed.

Rates of fatty acid synthesis from glucose in skeletal muscle from all three ages of steers were low relative to rates of fatty acid synthesis from acetate in adipose tissue. Although skeletal muscle has the capacity to store energy as triacylglycerol molecules, the fatty acids required for triacylglycerol synthesis are derived primarily from plasma as fatty acids or from triacylglycerol in plasma. Rates of fatty acid synthesis comparable to rates in adipose tissue, therefore, would not be expected to occur in skeletal muscle, but rates were certainly measureable in the present study. Rates of fatty acid synthesis in skeletal muscle decreased with increasing age of steers to negligible rates in skeletal muscle from 18-month-old steers. The significance of the decrease in fatty acid synthesis with age may be another manifestation of decreasing metabolic activity accompanying longevity. Addition of ronnel to incubation media decreased fatty acid synthesis rates significantly (P<.05) in skeletal muscle from 12-month-old steers but had no significant (P>.05) effect on
rates of fatty acid synthesis in skeletal muscle from 6- or 18-month-old steers.

Glycerol production from glucose in skeletal muscle as influenced by ronnel also was examined. As in adipose tissue, where $^{14}$C derived from acetate was recycled into glycerol, incorporation of radioactive glucose carbon into glycerol in skeletal muscle may be partly a result of recycling of $^{14}$C into TCA cycle intermediates with eventual incorporation into glycerol. The significance of recycling of glucose carbon into glycerol, however, is probably minimal and, rather, the majority of radioactive glycerol arises from net synthesis from $^{14}$C-glucose. In addition to serving as a substrate for oxidation and as a precursor for glyceride-glycerol synthesis, glucose is required in skeletal muscle as a source of glycerol for cell membrane phospholipid synthesis. In skeletal muscle from 6-month-old steers, an increase in glycerol production occurred with increasing dose of ronnel (figure 10; P<.05) when ronnel dose was 10 mg/kg of tissue. Rates of glycerol production from glucose in skeletal muscle from 12-month-old steers were not affected by addition of ronnel to incubation media (figure 11). In skeletal muscle from 18-month-old steers, glycerol production rates were greatest (P<.05) when ronnel dose was 2.5 mg/kg of tissue. In general, addition of ronnel was associated with greater rates of glycerol synthesis in skeletal muscle from 18-month-old steers than when no ronnel was present in incubation media. The potential for increased glycerol synthesis may indicate increased capacity of growing skeletal muscle to synthesize cell membranes. Rates of glycerol production were 4- to 8-fold greater in skeletal muscle from 6-month-old steers than in skeletal muscle from either 12- or 18-month-old steers, perhaps reflecting
a decreased capacity for muscle cell membrane synthesis and, in turn, a
decrease in muscle growth that occurs during the postnatal period
(Swatland, 1976).

Ronnel clearly promotes growth in cattle, but the exact way in which
it does so remains relatively unexplored. Results of the present in-
vestigation show that ronnel depresses metabolic activity in adipose tissue
from 6- and 12-month-old growing steers without a concomitant decrease in
metabolic activity of skeletal muscle and that metabolic activity of
adipose tissue and skeletal muscle in older (18-month-old) steers is less
affected by ronnel. The latter result may be a reflection of the general
decrease in metabolic activity accompanying longevity, or perhaps tissues
from older animals become refractory toward potential manipulators of
metabolism.

In light of recent economic considerations in the meat animal industry
regarding appropriate amounts of carcass fat that an animal is to gain be-
fore slaughter, a desirable approach to improvements in animal production
would be to search for agents that promote growth by selectively distribu-
ting nutrients between adipose tissue and skeletal muscle, thereby in-
creasing the proportion of lean to adipose tissue. Data from the present
investigation suggest that ronnel does indeed alter the partitioning of
acetate and glucose from major metabolic processes of adipose tissue to
skeletal muscle. Furthermore, because no previous studies are available
relating effects of growth-promoting compounds to metabolic activity of
adipose tissue and skeletal muscle, results of the present experiments
represent an introductory contribution to the study of effects of growth
promotants, at the level of body tissues. Because the actions of non-
organophosphate growth promotants, i.e., the anabolic agents, are closely related to effects on hormonal status, additional experiments designed to test for possible interactions of growth-promoting organophosphates with hormones known to influence growth should be undertaken.
PART II. EFFECTS OF DURSEAN® ON METABOLIC ACTIVITY OF SUBCUTANEOUS ADIPOSE TISSUE AND SKELETAL MUSCLE FROM 12- AND 18-MONTH-OLD STEERS
INTRODUCTION

Improvements in growth rate and feed efficiency of animals used for human consumption are of unquestionable economic importance. Several agents, when administered to cattle, produce improvements in growth performance; perhaps the best-known of these agents is the anabolic synthetic estrogen, diethylstilbestrol (Burroughs et al., 1955), which has been shown to stimulate nitrogen retention and lean body tissue gain (Clegg and Carroll, 1956; Oltjen and Lehmann, 1968; Byers, 1979). The search for growth-promoting agents is continual and, recently, organophosphate pesticides have been examined as potential growth-promotants. Ronnel [O,O-dimethyl O-(2,4,5-trichlorophenyl) phosphorothioate] is one such organophosphate that promotes growth and improves feed efficiency in cattle (Rumsey, 1979; Rumsey et al., 1981a). Little information describing ronnel's mechanism of action is available. A recent report, however, has suggested that ronnel may alter the partitioning of nutrients between adipose tissue and skeletal muscle of cattle and, thereby, increase the proportion of lean to adipose tissue (Trankina and Beitz, 1982). Dursban® [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] commonly known as chlorpyrifos, is an organophosphate pesticide with a chemical structure very similar to the structure of ronnel. Unpublished data from Dow Chemical, U.S.A., the manufacturer of Dursban®, indicate that, like ronnel, Dursban® feeding to livestock results in improved growth performance. Also, like ronnel, the mechanism of action whereby Dursban® influences growth is unknown. Because ronnel and Dursban® are chemically similar, they may affect metabolism by way of a common mechanism of action.
The objective of the present investigation, therefore, was to examine substrate utilization and metabolic activity as influenced by Dursban® in subcutaneous adipose tissue and skeletal muscle from steers at 12 and 18 months of age.
MATERIALS AND METHODS

Experimental Animals

Five 12-month-old and five 18-month-old large-frame, Angus x Simmental steers originating from a herd designed to produce large, medium, and small body frame sizes were used in the study. Steers were housed at the McNay Research Center in Chariton, IA, where they were fed a high-energy diet consisting of 68.7% shelled corn, 25% corn cobs, 5% molasses, and 1.3% minerals. Three weeks before slaughter, steers were transported from the McNay Research Center to Ames and were housed at the Iowa State Beef Nutrition Research Farm. Twelve- and 18-month-old steers had average body weights of 426.5 and 595.6 kg, respectively, at the time of slaughter at the Iowa State University Meats Laboratory.

Tissue Sampling

Tissues were obtained within 30 minutes after stunning and exsanguination. Subcutaneous adipose tissue (backfat) was maintained in 0.15 M NaCl at 37° C, and skeletal muscle was kept on ice before sectioning and subsequent incubation.

Incubation Procedures

A 100 to 200 mg (wet weight) thin section of adipose tissue or a thin, longitudinal slice of skeletal muscle was incubated in each flask under an atmosphere of 95% O₂ and 5% CO₂ with constant shaking for 2 hours at 37° C. Each flask for adipose tissue incubations contained 3 ml of Krebs-Ringer bicarbonate buffer (Ca²⁺-free, pH 7.4; Laser, 1961), 75 μmoles of sodium acetate, and 15 μmoles of glucose to provide a source of glyceride-
glycerol and as a substrate for generation of reducing equivalents (NADPH) by way of the pentose phosphate pathway (Bauman et al., 1970; Yang and Baldwin, 1973) plus 1 μCi of sodium-[1-14C]-acetate (New England Nuclear, Boston, MA). Each flask for skeletal muscle incubations contained 3 ml of Krebs-Ringer bicarbonate buffer (Ca2+-free, pH 7.4; Laser, 1961) and 15 μmoles of glucose plus 1 μCi of D-[U-14C]-glucose (New England Nuclear, Boston, MA). Bovine insulin (0.3 IU; Sigma, St. Louis, MO) was added to all flasks. Dursban® was dissolved in minimal quantities of 100% ethanol such that a 1 μl aliquot in each flask would deliver 0.5, 1, or 2 mg of Dursban® per kg of adipose tissue or skeletal muscle. Control flasks contained 3 ml of Krebs-Ringer bicarbonate buffer, 1 μl of 100% ethanol, and no Dursban®. Each tissue sample and Dursban® dose combination was incubated in triplicate, and a set of zero-time incubations was included for each of the two substrates. Incubations were terminated by addition of 0.5 ml of 1.5 N H2SO4, and 14CO2 was collected after injecting 0.1 ml of 30% NaOH onto filter paper situated inside a hanging well in each flask.

Analytical Procedures

Oxidation of acetate or glucose to CO2 was determined using the method of Buhler (1962). After incubation, 13.2 ml of chloroform:methanol (1:2, v/v) and 2.3 ml of chloroform:methanol:water (1:2:0.8, v/v/v) were added to each flask (Bligh and Dyer, 1959). Flask contents were transferred to screw-cap extraction tubes, and tissue sections and media were homogenized with a Polytron homogenizer (Brinkman Instruments, Westbury, NY). The shaft of the homogenizer was rinsed with 5 ml of chloroform, and this rinse was collected in extraction tubes. Tissue samples were
washed, and lipids were extracted using a modification of the Folch wash procedure (Folch et al., 1957). Each sample was washed with one 5-ml and three 10-ml aliquots of deionized H₂O, resulting in formation of an upper, aqueous layer and a lower, chloroform layer. Samples were evaporated to dryness, and 10 ml of chloroform were added to each tube. Total lipids were transesterified with 1.25 M sodium methoxide and subsequently neutralized with 10% methanolic HCl to split triacylglycerols into fatty acid methyl esters and free glycerol. Ten ml of deionized H₂O were added to each tube to separate the upper, aqueous, glycerol-containing layer from the lower, chloroform, fatty acid-containing layer. Fatty acid and glycerol fractions were evaporated to dryness and, after addition of Beckman Ready-Solv™ scintillation cocktail, were assayed for radioactivity by liquid scintillation counting (Model LS-8000 liquid scintillation spectrophotometer, Beckman Instruments, Irvine, CA). Quenching was corrected by the external standard method.

Statistical Analysis

Data were analyzed by analysis of variance (Barr et al., 1979) and Duncan's new multiple range test (Steel and Torrie, 1960).
RESULTS

To assess the effects of ronnel on metabolic activity in subcutaneous adipose tissue and skeletal muscle, in vitro rates of substrate conversion to product were determined. Data are organized as histograms in the format of substrate conversion to product versus ronnel dose, and conversion rates are expressed on a tissue-weight basis. Representation of statistical significance at 5% probability is by bars with different letters. Tissue site and age of steers are noted on each graph.

Metabolic Activity in Subcutaneous Adipose Tissue

Dursban® had no statistically significant (P > .05) effects on oxidation of acetate to CO₂ in adipose tissue from either 12- or 18-month-old steers (figures 13 and 14); opposite trends in oxidation rates, however, occurred at each age. A tendency (P > .05) toward increased rates of oxidation of acetate to CO₂ with increasing Dursban® dose occurred in adipose tissue from 12-month-old steers, and a tendency (P > .05) toward decreased rates of oxidation of acetate to CO₂ with increasing Dursban® dose was observed in adipose tissue from 18-month-old steers. Rates of oxidation of acetate to CO₂ decreased from 12 to 18 months of age.

In addition to rates of oxidation of acetate to CO₂ as an index of metabolic activity, fatty acid synthesis rates, as a function of Dursban® dose, were determined in adipose tissue. In 12-month-old steers, Dursban®, at doses of 0.5 and 1 mg/kg of tissue, produced dramatic and statistically significant (P < .05) decreases in fatty acid synthesis rates when compared with control rates (figure 15). When Dursban® dose was 2 mg/kg of tissue, however, rates of fatty acid synthesis were similar to control rates. In contrast, rates of fatty acid synthesis in adipose tissue from 18-month-old...
steers were similar at Dursban® doses of 0, 0.5, 1, and 2 mg/kg of tissue (figure 16). Dursban® dose of 2 mg/kg of tissue resulted in greatest rates of fatty acid synthesis in adipose tissue from 18-month-old steers. Rates of fatty acid synthesis, on the basis of tissue weight, were greater in adipose tissue from 12- than from 18-month-old steers.

Radioactivity present in glycerol fractions also was determined (figures 17 and 18). An important point to note is that radioactive glycerol does not arise from de novo glycerogenesis from radioactive acetate but, rather, represents recycling of acetate carbon, by way of intermediates of the TCA cycle, into glycerol. Incorporation of $^{14}C$ derived from acetate into glycerol increased linearly (P<.0001) with increasing dose of Dursban® in adipose tissue from 12-month-old steers. This increase in incorporation was statistically significant (P<.05) when Dursban® dose was 2 mg/kg of tissue (figure 17). The opposite pattern occurred in adipose tissue from 18-month-old steers, i.e., incorporation of $^{14}C$ from acetate into glycerol decreased linearly (P<.01) with increasing dose of Dursban®. Rates of incorporation of $^{14}C$ from acetate into glycerol decreased from 12 to 18 months of age in steer adipose tissue.

Metabolic Activity in Skeletal Muscle

Figures 19 and 20 are composite representations of metabolic activity in skeletal muscle as influenced by Dursban®. Glucose was chosen as the substrate for determinations of metabolic activity, because skeletal muscle utilizes glucose for a number of metabolic functions. Each graph shows rates of oxidation of glucose to CO$_2$, rates of fatty acid synthesis, and rates of glycerol production from glucose.

With respect to rates of oxidation of glucose to CO$_2$, a tendency
(P>.05) for increased oxidation rates with increasing dose of Dursban® occurred in skeletal muscle from 12-month-old steers (figure 19). In skeletal muscle from 18-month-old steers (figure 20), 0.5 and 1 mg/kg doses of Dursban® produced rates of oxidation of glucose to CO₂ that were less than control rates, although not statistically so (P>.05). The 2 mg/kg dose of Dursban® was associated with rates of oxidation of glucose to CO₂ that were similar to control rates and to rates obtained with the 1 mg/kg dose but significantly (P<.05) greater than rates produced with the 0.5 mg/kg dose. Quantitatively, rates of oxidation of glucose to CO₂ were greater in skeletal muscle from 18-month-old than from 12-month-old steers.

Rates of fatty acid synthesis from glucose in skeletal muscle from 12- and 18-month-old steers were much lower than rates in adipose tissue from both ages of steers. A decrease in fatty acid synthesis rates in skeletal muscle occurred from 12 to 18 months of age; rates in skeletal muscle from 18-month-old steers were negligible. Addition of Dursban® to incubation media tended to decrease rates of fatty acid synthesis from glucose in skeletal muscle from 12-month-old steers (P>.05) but had no effect in skeletal muscle from 18-month-old steers.

Rates of glycerol production from glucose were low in skeletal muscle from 12- and 18-month-old steers. Furthermore, rates of glycerol production were quantitatively similar in skeletal muscle from both ages of steers. No effects of Dursban® on glycerol production rates were observed in skeletal muscle from either 12- or 18-month-old steers.
Figure 13. Effect of Dursban $^\text{\textregistered}$ on oxidation of acetate to CO$_2$ in subcutaneous adipose tissue from 12-month-old steers
ADIPOSE TISSUE FROM 12-MONTH-OLD STEERS

ACETATE → CO₂ (mmoles/2 hr x g)

DURSBAN DOSE (mg/kg tissue)

0 0.5 1 2
Figure 14. Effect of Dursban® on oxidation of acetate to CO₂ in subcutaneous adipose tissue from 18-month-old steers
ADIPOSE TISSUE FROM 18-MONTH-OLD STEERS

ACETATE-\textsubscript{CO}_2 (nmoles/2 hr x g)

DURSBAN DOSE (mg/kg tissue)

0 0.5 1 2
Figure 15. Effect of Dursban® on fatty acid synthesis from acetate in subcutaneous adipose tissue from 12-month-old steers
ADIPOSE TISSUE FROM 12-MONTH-OLD STEERS

ACETATE → FATTY ACIDS (nmol/2 hr/g)

DURSBAN DOSE (mg/kg tissue)

- 0
- 0.5
- 1
- 2

A
B
A
Figure 16. Effect of Dursban® on fatty acid synthesis from acetate in subcutaneous adipose tissue from 18-month-old steers
ADIPOSE TISSUE FROM 18-MONTH-OLD STEERS

ACETATE → FATTY ACIDS (nmoles/2hr×g)

DURSBAN DOSE (mg/kg tissue)

0 0.5 1 2
Figure 17. Effect of Dursban® on recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 12-month-old steers.
ADIPOSE TISSUE FROM 12-MONTH-OLD STEERS

ACETATE ➔ GLYCEROL (nmole2/2hr×g)

DURSBAN DOSE (mg/kg tissue)

0 0.5 1 2

A A A B
Figure 18. Effect of Dursban® on recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 18-month-old steers
ADIPOSE TISSUE FROM 18-MONTH-OLD STEERS

ACETATE—GLYCEROL (nmol/2 hr x g)

DURSBAN DOSE (mg/kg tissue)

0 0.5 1 2

A

B

B

B
Figure 19. Effect of Dursban® on conversion of glucose to product in skeletal muscle from 12-month-old steers
CARBON DIOXIDE

FATTY ACIDS

GLYCEROL

GLUCOSE → PRODUCT (nmol/2hr×g)

DURSBAN DOSE (mg/kg tissue)

SKELETAL MUSCLE FROM 12-MONTH-OLD STEERS
Figure 20. Effect of Dursban® on conversion of glucose to product in skeletal muscle from 18-month-old steers
CARBON DIOXIDE

SKELETAL MUSCLE FROM 18-MONTH-OLD STEERS

GLUCOSE PRODUCT (nmols/2 hr × g)

DURBAN DOSE (mg/kg tissue)

FATTY ACIDS

GLYCEROL

AB

A
DISCUSSION

Several of the well-known growth-promoting agents and their modes of action have been studied extensively (Burgess and Lamming, 1960; Trenkle, 1969; Fowler et al., 1970; Sharp and Dyer, 1971; Chalupa, 1977). The more recently discovered growth-promoting effects of organophosphate compounds, such as roxcel and Dursban®, however, remain relatively unexplored with respect to mechanisms of action. Results from recent experiments suggest that roxcel affects metabolic activity in adipose tissue and skeletal muscle from growing steers (Trankina and Beitz, 1982). The possibility that Dursban®, an organophosphate structurally similar to roxcel, also could alter substrate utilization in two metabolically active tissues, adipose tissue and skeletal muscle, was examined in the present study.

An increase in rates of oxidation of acetate to CO₂ with increasing Dursban© dose occurred in adipose tissue from 12-month-old steers, and a decrease in rates of oxidation with increasing Dursban© dose occurred in adipose tissue from 18-month-old steers (figures 13 and 14). The reason for the dichotomous results in 12- and 18-month-old steers is not evident. An increase in oxidation rates in adipose tissue from 12-month-old steers also occurred in a previous study when roxcel was added to incubation media (Trankina and Beitz, 1982), indicating that both organophosphates produce similar effects on rates of oxidation of acetate to CO₂ in adipose tissue from 12-month-old steers. Effects of roxcel and Dursban© in adipose tissue from 18-month-old steers were dissimilar, because roxcel addition to incubation media had no effect on rates of oxidation of acetate to CO₂. Quantitatively, rates of oxidation of acetate to CO₂ in adipose tissue from 12-month-old steers were greater than rates in adipose tissue from 18-month-
old steers when expressed on the basis of tissue weight in the present investigation. The same pattern of decrease in oxidation rates with increasing age of steers occurred in the ronnel study. A plausible explanation for the decreased oxidation rates is a general decline in metabolic activity that occurs with advancing age. Rates of oxidation of acetate to CO$_2$ in adipose tissue from 12- and 18-month-old steers in the present study were quantitatively similar to oxidation rates in adipose tissue in the previous study with ronnel.

The capacity of adipose tissue to synthesize fatty acids from acetate is a second important index of metabolic activity in this tissue. Effects of Dursban® on fatty acid synthetic capacity, therefore, were tested. In adipose tissue from 12-month-old steers, significant decreases in fatty acid synthesis occurred when Dursban® dose was either 0.5 or 1 mg/kg of tissue (figure 15). Dursban® dose of 2 mg/kg of tissue was associated with fatty acid synthesis rates that closely paralleled control values. A similar decrease in rates of fatty acid synthesis in adipose tissue from 12-month-old steers occurred with increasing dose of ronnel. The significance of the return of fatty acid synthesis rates to control values when Dursban® dose was 2 mg/kg of tissue is not clear, but a possible explanation is that 2 mg of Dursban® per kg of adipose tissue or some dose between 1 and 2 mg/kg of tissue may be a threshold at which Dursban® no longer depresses fatty acid synthesis. No statistically significant (P>.05) effects of Dursban® on rates of fatty acid synthesis in adipose tissue from 18-month-old steers were observed, although rates were greatest when Dursban® dose was 2 mg/kg of tissue (figure 16). Data from the present experiments testing Dursban® are in good agreement with results of previous
experiments with ronnel where ronnel was shown to have no statistically significant (P>.05) effects on rates of fatty acid synthesis in adipose tissue from 18-month-old steers, although rates were greatest with the greatest dose of ronnel. Dursban®, like ronnel, depressed metabolic activity when fatty acid synthesis rates were used as criteria for metabolic activity in adipose tissue from 12-month-old steers but not in adipose tissue from 18-month-old steers. The apparent refractoriness of adipose tissue from older animals may be a manifestation of general decline in metabolic responsiveness that occurs with age (Guyton, 1976; Atkins, 1981).

With respect to rates of fatty acid synthesis expressed on a tissue-weight basis, results of the present investigation with Dursban® are in agreement with other reports (Ingle et al., 1973; Pothoven and Beitz, 1973) as well as with an earlier report involving ronnel (Trankina and Beitz, 1982); i.e., rates of fatty acid synthesis decrease with advancing age. Adipose tissue from younger steers was more metabolically active than was adipose tissue from older steers, thus contributing to the contention that metabolic activity declines during the aging process.

Radioactivity present in glycerol in adipose tissue also was quantified. Observed rates of glycerol production represent recycling of $^{14}$C from acetate through the TCA cycle with eventual incorporation of $^{14}$C into glycerol; net synthesis of glycerol from acetate does not occur. A linear (P<.0001) increase in recycling of acetate carbon into glycerol occurred in adipose tissue from 12-month-old steers, whereas a linear (P<.01) decrease in recycling of acetate carbon into glycerol occurred in adipose tissue from 18-month-old steers. In the previous experiment, where effects of ronnel on recycling of acetate carbon into glycerol were determined, increasing
dose of ronnel produced decreased (P<.05) rates of incorporation of $^{14}$C into glycerol in adipose tissue from both 12- and 18-month-old steers. If recycling of $^{14}$C into glycerol is considered contributory to adipose tissue metabolism, the depression of the capacity of adipose tissue to incorporate acetate carbon into glycerol provides additional support for the concept of decreased metabolic activity of adipose tissue; in the present study, decreased metabolic activity, as a result of Dursban® addition to incubation media, was observed in adipose tissue from 18- but not from 12-month-old steers. Rates of incorporation of $^{14}$C from acetate into glycerol decreased with increasing age of steers, a result that also occurred in experiments with ronnel. In the earlier study with ronnel, and in the present investigation with Dursban®, the decrease in recycling of acetate carbon into glycerol that occurred with age may be the result of a general decrease in metabolic activity of tissue with age or may have occurred as a result of improvements that were made at the time of analysis of tissues from 18-month-old steers in techniques for separating fatty acid and glycerol fractions. The latter explanation is supported by the fact that rates of recycling of acetate carbon into glycerol in adipose tissue from 12-month-old steers were substantially greater than those reported elsewhere, whereas rates in adipose tissue from 18-month-old steers are in agreement with reported values (Whitehurst et al., 1978).

The effects of Dursban® in skeletal muscle also were examined (figures 19 and 20). Rates of oxidation of glucose to CO$_2$, fatty acid synthesis rates, and glycerol production were used as indices of metabolic activity. In skeletal muscle, glucose can be oxidized to produce ATP and serves as a precursor for glyceride-glycerol synthesis necessary for triacylglycerol
formation. Additionally, glucose is a source of glycerol for cell membrane phospholipid biosynthesis (Lehninger, 1975). In skeletal muscle from 12-month-old steers, a tendency (P>.05) for increased rates of oxidation of glucose to CO₂ occurred with increasing dose of Dursban®. Skeletal muscle from 18-month-old steers exhibited rates of oxidation of glucose to CO₂ at Dursban® doses of 0.5 and 1 mg/kg of tissue that were less than control values (P<.05). The 2 mg/kg dose of Dursban® produced rates of oxidation of glucose to CO₂ that were similar to control values. The patterns of oxidation rates in skeletal muscle from 12- and 18-month-old steers, therefore, were similar under the influence of either ronnel or Dursban®. On the basis of glucose oxidation to CO₂, Dursban®, like ronnel, tended to increase metabolic activity of skeletal muscle from 18-month-old steers. Rates of CO₂ production from glucose were quantitatively similar in studies involving both ronnel and Dursban® for 12- and 18-month-old steers.

Rates of fatty acid synthesis from glucose in skeletal muscle from 12- and 18-month-old steers were low relative to rates of fatty acid synthesis from acetate in adipose tissue. Because the majority of fatty acids required for triacylglycerol synthesis in skeletal muscle are derived from blood plasma, low rates of fatty acid synthesis from glucose in skeletal muscle relative to rates in adipose tissue were expected. Rates of fatty acid synthesis in skeletal muscle from steers decreased from 12 to 18 months of age, perhaps reflecting a general decline in metabolic activity. Dursban® addition to incubation media tended (P>.05) to decrease fatty acid synthesis rates in skeletal muscle from 12-month-old steers but had no effect on skeletal muscle from 18-month-old steers. The same pattern occurred in an earlier study where effects of ronnel on fatty acid synthesis
rates in skeletal muscle were tested. The depression in fatty acid
synthesis rates in skeletal muscle of younger steers, as influenced by
ronnel or Dursban®, may indicate direction of glucose away from fatty acid
synthesis and toward oxidation with subsequent ATP production; rates of
CO₂ production were 4- to 16-fold greater than rates of fatty acid
synthesis in skeletal muscle in studies involving both ronnel and Dursban®.
Rates of fatty acid synthesis in each study were quantitatively similar
for both the 12- and 18-month-old steers.

Incorporation of radioactive carbon from glucose into glycerol in
skeletal muscle was quantified. A minimal quantity of radioactive glycerol
arises from recycling of ¹⁴C from glucose through TCA cycle intermediates
with eventual incorporation into glycerol; the majority, however, involves
new synthesis from ¹⁴C-glucose. Glycerol in muscle is necessary for tri-
acylglycerol synthesis and contributes to cell membrane phospholipid bio-
synthesis. Rates of glycerol production were low but quantitatively similar
in skeletal muscle from 12- and 18-month-old steers. No effect of Dursban®
was evident in skeletal muscle from either age of steers. The latter
observation agrees with findings in skeletal muscle from 12-month-old
steers in the study with ronnel. Rates of glycerol production as influenced
by either ronnel or Dursban® were quantitatively similar.

Dursban® tends to decrease metabolic activity of adipose tissue and to
increase metabolic activity of skeletal muscle with many of the same
specific manifestations produced in tissues when ronnel is added to incu-
bation media. Because of similarities in results from experiments with
both ronnel and Dursban®, the suggestion that organophosphate, growth-
promoting pesticides act by way of alterations in partitioning of acetate and
glucose between metabolic processes in adipose tissue and skeletal muscle is further supported. Additionally, a decline in metabolic activity of adipose tissue and skeletal muscle occurring with advancing age was observed in experiments testing the effects of both ronnel and Dursban®.
PART III. EFFECTS OF THYROID HORMONE, GROWTH HORMONE, AND ORGANOPHOSPHATES ON METABOLIC ACTIVITY OF SUBCUTANEOUS ADIPOSE TISSUE FROM STEERS
INTRODUCTION

One level at which improvements can be made in livestock production is through increased understanding of the significance of nutrient utilization and partitioning of energy substrates in growth of adipose tissue and skeletal muscle. Such processes are governed by endocrine regulation of metabolic pathways within cells (Kahn, 1976; Goldfine, 1978; Catt et al., 1979). The capacity to effect changes in concentrations of circulating hormones and to thereby alter precursor supply to metabolic processes within cells is one way in which growth-promotants may affect nutrient partitioning and result in deposition of more protein and less trimmable fat in carcasses of food-producing animals. Growth hormone (GH) and thyroxine (T₄) are involved intimately in the growth processes of mammals (Guyton, 1976). Furthermore, one manifestation of administration of estrogens, known to be anabolic in ruminants, is increased secretion of growth hormone; estrogen administration is associated with increased protein content and decreased lipid content of carcasses (Trenkle, 1970; Borger et al., 1973). Feeding of ronnel, an organophosphate pesticide that improves growth performance of cattle (Rumsey et al., 1975; Thomas and Ware, 1978; Rumsey, 1979; Rumsey et al., 1981a), results in significant increases in plasma concentrations of T₄ in steers (Rumsey et al., 1981a). Because of the established effects of T₄ and GH at the level of body tissues and because one way in which anabolic agents promote growth is by way of altered hormone status, the present study was designed to investigate possible interactions of the growth-promoting organophosphate pesticides,
ronnel and Dursban®, with T₄ or GH on substrate utilization and fatty acid synthesis in adipose tissue from steers.
MATERIALS AND METHODS

Tissue Sampling

Subcutaneous adipose tissue (backfat) was obtained from eight steers with average body weight of 515 kg and that had been fed an 80% concentrate and corn silage diet. Tissue samples were obtained from the Iowa State University Meats Laboratory within 30 minutes after stunning and exsanguination and were maintained at 37° C in 0.15 M NaCl.

Incubation Procedures

A 100 to 200 mg (wet weight) thin section of adipose tissue was incubated in each flask under an atmosphere of 95% O₂ and 5% CO₂ with constant shaking for 2 hours in a 37° C water bath. Each flask contained 3 ml of Krebs-Ringer bicarbonate buffer (Ca²⁺-free, pH 7.4; Laser, 1961), 75 µmoles of sodium acetate, 15 µmoles of glucose to provide a substrate for generation of reducing equivalents (NADPH) by way of the pentose phosphate pathway and as a precursor for glyceride-glycerol synthesis in adipose tissue (Bauman et al., 1970; Yang and Baldwin, 1973), 0.3 IU of bovine insulin (Sigma, St. Louis, MO), 1 µCi of sodium-[1-¹⁴C]-acetate (New England Nuclear, Boston, MA), and either 21 mg of L-sodium thyroxine (Nutritional Biochemicals, Cleveland, OH) or 1.8 mg of bovine growth hormone (prepared by A. Trenkle). Each organophosphate was dissolved in a minimal quantity of 100% ethanol such that a 1 µl aliquot in each flask would deliver either 5 mg of ronnel or 2 mg of Dursban® per kg of adipose tissue. Flasks containing 3 ml of Krebs-Ringer bicarbonate buffer, 1 µl of 100% ethanol, and no hormones or organophosphates served as controls. A set of flasks containing hormones but no organophosphates also was prepared.
and incubated. Triplicate incubations were performed for each tissue sample, hormone, and organophosphate combination. A set of zero-time incubations was included for T₄ and for GH. Incubations were terminated by addition of 0.5 ml of 1.5 N H₂SO₄ to incubation media; ¹⁴CO₂ was collected after injecting 0.1 ml of 30% NaOH onto filter paper situated inside a hanging well in each flask.

Analytical Procedures

Oxidation of acetate to CO₂ was quantified using the method of Buhler (1962). After incubation, 13.2 ml of chloroform:methanol (1:2 v/v) and 2.3 ml of chloroform:methanol:water (1:2:0.8, v/v/v) were added to each flask (Bligh and Dyer, 1959). Flask contents were transferred to screw-cap extraction tubes, and tissue sections and media were homogenized with a Polytron homogenizer (Brinkman Instruments, Westbury, NY). The shaft of the homogenizer was rinsed with 5 ml of chloroform, and this rinse was collected in extraction tubes. Tissue samples were washed, and lipids were extracted using a modification of the Folch wash procedure (Folch et al., 1957). Each sample was washed with one 5-ml and three 10-ml aliquots of deionized H₂O, resulting in formation of an upper, aqueous layer and a lower, chloroform layer. Samples were evaporated to dryness, and 10 ml of chloroform were added to each tube. Total lipids were transesterified with 1.25 M sodium methoxide and subsequently neutralized with 10% methanolic HCl to split triacylglycerols into fatty acid methyl esters and free glycerol. Ten ml of deionized H₂O were added to each tube to separate the upper, aqueous, glycerol-containing layer from the lower, chloroform, fatty acid-containing layer. Fatty acid-containing fractions were evaporated
to dryness and, after addition of Beckman Ready-Solv™ scintillation cocktail, were assayed for radioactivity by liquid scintillation counting (Model LS-8000 liquid scintillation spectrophotometer, Beckman Instruments, Irvine, CA). Quenching was corrected by the external standard method.

Statistical Analysis

Data were analyzed by analysis of variance (Barr et al., 1979) and Duncan's new multiple range test (Steel and Torrie, 1960).
RESULTS

Rates of oxidation of acetate to CO₂ and rates of fatty acid synthesis from acetate in subcutaneous adipose tissue were used to determine effects of hormones and organophosphate-hormone combinations on metabolic processes in adipose tissue and are presented in table 1. Results are expressed on the basis of tissue weight.

Effects of T₄ and T₄ Plus Organophosphates

Rates of oxidation of acetate to CO₂ in adipose tissue were significantly (P<.05) greater than control rates when T₄ was added to incubation media. Thyroxine plus ronnel produced rates of oxidation of acetate to CO₂ that were significantly (P<.05) greater than control rates and significantly (P<.05) less than rates produced when only T₄ was added to incubation media. Rates of oxidation of acetate to CO₂ that occurred when T₄ plus Dursban® were added to incubation media were significantly (P<.05) greater than control rates. Addition of T₄ or T₄ plus either ronnel or Dursban®, therefore, produced rates of oxidation of acetate to CO₂ that were significantly (P<.05) greater than when neither T₄ nor organophosphates were added to incubation media. Furthermore, rates of oxidation, when T₄ was combined with either ronnel or Dursban®, were less than oxidation rates produced when only T₄ was added to incubation media.

Rates of fatty acid synthesis from acetate in adipose tissue also were determined. Addition of T₄ to incubation media produced rates of fatty acid synthesis that were significantly (P<.05) less than control rates. Thyroxine plus ronnel had no significant (P>.05) stimulatory or depressive effect on fatty acid synthesis from acetate relative to controls. Rates of
fatty acid synthesis were significantly (P<.05) greater when T₄ plus ronnel were added to incubation flasks when compared with addition of T₄ alone. No significant (P>.05) effects compared with control or other treatment groups on rates of fatty acid synthesis from acetate in adipose tissue occurred when T₄ was combined with Dursban®. Thyroxine addition, therefore, had a depressive effect on rates of fatty acid synthesis in steer adipose tissue. Combining T₄ with organophosphates was associated with rates of fatty acid synthesis that were slightly less than control rates; because of large standard errors of the means, however, the depressed rates of fatty acid synthesis were not statistically significant (P>.05).

Effects of GH and GH Plus Organophosphates

Whereas addition to incubation media of T₄ or combinations of T₄ with organophosphates resulted in significant (P<.05) effects on rates of oxidation of acetate to CO₂ and on rates of fatty acid synthesis from acetate in steer adipose tissue, addition of GH or GH plus organophosphates did not produce statistically significant effects when rates were compared with control rates. Rates of oxidation of acetate to CO₂ tended (P>.05) to be less than control rates when GH was added or when GH plus ronnel were added to incubation media. Rates of oxidation when GH plus Dursban® were added to incubation media tended (P>.05) to be greater than control rates or rates for GH or GH plus ronnel.

With respect to rates of fatty acid synthesis, no significant (P>.05) effects of GH or GH plus ronnel or Dursban® were evident. A tendency was present (P>.05) for lower rates of fatty acid synthesis with GH plus Dursban® versus control rates.
Effects of T4 and T4 Plus Organophosphates Versus GH and GH Plus Organophosphates

Rates of oxidation of acetate to CO2 in steer adipose tissue were greater (P<.05) when T4 was added to incubation media than when GH was added. Oxidation rates also were greater (P<.05) when T4 was added than when GH plus either organophosphate was added to incubation media. Additionally, T4 plus either organophosphate produced rates of oxidation of acetate to CO2 that were greater (P<.05) than rates produced when GH or GH plus either organophosphate was present in incubation media. In general, then, T4 or T4 plus organophosphates produced rates of oxidation of acetate to CO2 that were greater (P<.05) than rates produced when GH or GH plus organophosphates were present in incubation media.

Rates of fatty acid synthesis from acetate in steer adipose tissue were greater (P<.05) when GH was present in incubation media than when T4 was present. Addition of GH plus ronnel but not GH plus Dursban® to incubation media resulted in rates of fatty acid synthesis that were greater (P<.05) than rates occurring when T4 was added. Similar rates of fatty acid synthesis from acetate occurred when T4 plus organophosphates or GH plus organophosphates were added to incubation media.
Table 1. Interactions between thyroxine (T₄), growth hormone (GH), and organophosphates (ronnel and Dursban®) on oxidation of acetate to CO₂ and rates of fatty acid synthesis in subcutaneous adipose tissue from steers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CO₂</th>
<th>Fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmoles/2hr x g</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>625.4 ± 148.8 a, b</td>
<td>913.1 ± 137.4 a</td>
</tr>
<tr>
<td>T₄</td>
<td>1870.1 ± 189.7 b</td>
<td>456.2 ± 120.5 b</td>
</tr>
<tr>
<td>T₄ + ronnel</td>
<td>1239.0 ± 154.9 c</td>
<td>841.7 ± 116.2 a</td>
</tr>
<tr>
<td>T₄ + Dursban®</td>
<td>1551.4 ± 169.7 b, c</td>
<td>698.0 ± 102.4 a, b</td>
</tr>
<tr>
<td>GH</td>
<td>561.7 ± 154.9 a</td>
<td>874.5 ± 125.5 a</td>
</tr>
<tr>
<td>GH + ronnel</td>
<td>558.4 ± 148.8 a</td>
<td>906.8 ± 131.0 a</td>
</tr>
<tr>
<td>GH + Dursban®</td>
<td>704.3 ± 123.1 a</td>
<td>665.7 ± 125.5 a, b</td>
</tr>
</tbody>
</table>

¹Values are means of triplicate incubations (n = 8 steers).

²Means in the same column with different superscripts are significantly different (P<.05).
DISCUSSION

The physiological mechanisms governing growth-promoting actions of organophosphate compounds are unknown or poorly defined. The prediction that growth-promoting activity may be linked closely to alterations in the status of hormones involved in animal growth is logical and deserves consideration. Results discussed in previous sections of this dissertation suggest that two growth-promoting, organophosphate pesticides, ronnel and Dursban®, alter metabolic activity in adipose tissue and skeletal muscle from growing steers. Adipose tissue classically has been regarded primarily as a site of lipid storage and neither as a site of significant metabolic activity nor as a target for hormonal effects (Goodman and Schwartz, 1974). That adipose tissue is one of the most metabolically dynamic body tissues and is a site of significant hormone action now have been acknowledged. The present study was undertaken to investigate possible relationships between ronnel and Dursban® and two hormones intimately involved in animal growth, T₄ and GH, on metabolic events in adipose tissue from steers. Rates of substrate oxidation to CO₂ and fatty acid synthesis rates were chosen as criteria with which metabolic activity was assessed.

Significant (P<.05) increases in rates of oxidation of acetate to CO₂, when compared with control rates, occurred when T₄ was added to incubation media. In general, thyroid hormones increase metabolic rate in almost all body tissues; rapid increases in size and number of mitochondria result in enhanced capacity for oxidative phosphorylation with subsequent increased production of ATP (Hoch, 1974; Guyton, 1976). Increased rates of oxidation of acetate to CO₂ when T₄ was present in incubation media, therefore, are in agreement with general increases in metabolic rate.
induced by $T_4$. Addition of $T_4$ plus either ronnel or Dursban$^\circledR$ to incubation media resulted in rates of oxidation of acetate to CO$_2$ that were significantly (P<.05) greater than control rates. Furthermore, combination of $T_4$ with either ronnel or Dursban$^\circledR$ produced rates of oxidation of acetate to CO$_2$ that were less than rates observed with $T_4$ alone (P<.05 for $T_4$ plus ronnel versus $T_4$ alone), suggesting that organophosphates, when present in incubation media along with $T_4$, depressed the $T_4$-induced increased oxidation rates in adipose tissue. Combining this contention with results from previously described experiments testing effects of organophosphates on metabolic activity of adipose tissue and skeletal muscle, further evidence is provided to suggest that organophosphates act to decrease metabolic activity, even in the present of $T_4$, a known activator of metabolic processes.

With respect to rates of fatty acid synthesis in adipose tissue in the present investigation, a significant (P<.05) decrease in fatty acid synthesis rates versus control rates occurred when $T_4$ was added to incubation media. Thyroxine is a potent stimulator of lipolysis in adipocytes (Goodman and Bray, 1966), and thereby increases lipid mobilization. A hyperthyroid state results in increased energy requirements and favors lipolysis to mobilize free fatty acids for oxidation in body tissues (Goodman and Bray, 1966; Guyton, 1976). Decreased direction of substrate utilization toward lipogenesis, therefore, would be expected. Combining $T_4$ with either ronnel or Dursban produced rates of fatty acid synthesis from acetate that were comparable to control rates but greater than rates produced when only $T_4$ was added to incubation media (P<.05 for $T_4$ plus ronnel versus $T_4$ alone). A logical conclusion that might be drawn is that
organophosphates somehow negate or inhibit the depression of fatty acid synthesis rates induced by T₄. This conclusion, however, does not support previous results suggesting that ronnel and Dursban® depress metabolic activity, i.e., fatty acid synthesis rates, in adipose tissue from steers. The reason why addition of T₄ or organophosphates, separately, to incubation media depressed fatty acid synthesis rates relative to control rates, but combination of T₄ with organophosphates resulted in rates that were similar to control rates, remains enigmatic.

Possible relationships between improved growth performance and altered thyroid function in steers have been examined by others. Kahl et al. (1978) found significantly (P<.01) greater plasma concentrations of T₄ in steers implanted with the anabolic agent, Synovex-S, versus nonimplanted steers. Rumsey et al. (1981a) also have suggested that the growth-promoting effect of ronnel in steers may be related to alterations in thyroid function. Plasma concentrations of T₄ were significantly (P<.01) greater in ronnel-fed steers than in control steers (Rumsey et al., 1981a). Studies by Kahl et al. (1978) and Rumsey et al. (1981a) have considered changes in plasma T₄ concentrations. The present study has dealt with specific metabolic consequences of T₄ or T₄ plus organophosphates at the tissue level. Addition of T₄ to incubation media resulted in greater rates of oxidation of acetate to CO₂ in adipose tissue than when T₄ was not present. This result is consistent with the general increase in metabolic rates of most body tissues under the influence of T₄. Organophosphates lessened the T₄-induced increase in oxidative capacity of adipose tissue. Although effects of T₄ and T₄ plus organophosphates on metabolic activity in skeletal muscle were not tested in the present investigation, the depression in metabolic
activity of adipose tissue associated with addition of organophosphates to incubation media observed in this and two previous studies (see Parts I and II of this dissertation) may be tissue-specific. To further explain, trends toward decreased metabolic activity in adipose tissue and increased metabolic activity in skeletal muscle associated with addition of organophosphates to incubation media and evidence that rommel feeding to steers produces increased plasma T₄ concentrations may indicate that organophosphates act by increasing plasma T₄ concentrations while simultaneously influencing the responsiveness of specific tissues to T₄ stimulation. In this way, organophosphates may affect nutrient partitioning between adipose tissue and skeletal muscle, ultimately resulting in deposition of more protein and less trimmable fat in animal carcasses, a fundamental goal in producing more efficient animal growth.

Reported effects of GH on substrate oxidation to CO₂ are equivocal. Studies by Goldman and Bressler (1967) indicated that administration of GH to rats stimulated in vitro oxidation of palmitate to CO₂ in epididymal fat pads. Increased rates of oxidation of glucose to CO₂ occurred when GH was added to incubation media (Leal and Greenbaum, 1961; Goodman, 1963; Goodman and Coiro, 1981). In contrast, using tissue culture techniques, Nyberg et al., (1980) found that chronic exposure of human adipose tissue to GH had no effect on oxidation of glucose to CO₂. Furthermore, Goldman (1973) observed no effect of GH on rates of fatty acid oxidation in vitro in epididymal fat pads from rats. No differences from control values in rates of oxidation of acetate to CO₂, when either GH or GH plus organophosphates were added to incubation media, were evident in the present study. Goodman and Schwartz (1974) suggested that, rather than exerting a direct effect
on rates of fatty acid oxidation, GH increases availability of fatty acids to tissues by enhancing triacylglycerol hydrolysis in adipose tissue; increases in rates of oxidation of fatty acids as a result of GH, then, could be considered indirect effects. Growth hormone, in combination with organophosphates, had no effect on rates of oxidation of acetate to CO₂ in steer adipose tissue.

Reports in the literature have indicated that GH administration results in inhibition of lipogenesis (Goodman, 1963; Goodman and Schwartz, 1974) and in decreased carcass fat content (Young, 1945; Wagner and Veenhuizen, 1978). Furthermore, Goodman and Schwartz (1974) suggest that the inhibitory effects of GH on lipogenesis are related more closely to decreased fat deposition than to increased fat mobilization. Rates of fatty acid synthesis from acetate were not affected significantly (P > .05) when GH was added to incubation media in the present study. Goodman (1968) and Goodman and Schwartz (1974) observed that even after prolonged incubation with GH, no inhibition of fatty acid synthesis occurred and that in vivo conditions are necessary to demonstrate inhibitory effects of GH on fatty acid synthesis; a possible explanation is that formation and secretion of a second, endogenous, circulating substance may be required for the inhibition of fatty acid synthesis by GH (Daughaday, 1971; Daughaday et al., 1972). This explanation could account for the inability of GH to depress fatty acid synthesis in adipose tissue in the present study.

Combining GH with organophosphates resulted in rates of fatty acid synthesis from acetate that were similar to control rates, a finding that was unexpected considering the depressive effect of organophosphates on fatty acid synthesis rates in steer adipose tissue observed in previous
experiments with ronnel and Dursban®. Addition of growth hormone plus Dursban® to incubation media produced a depression in rates of fatty acid synthesis versus control rates in the present study; because of high standard errors of the means, however, the depressive effect did not carry statistical significance (P>.05).

Some reports have indicated that glucose utilization is depressed as a result of GH administration (Goodman, 1963, 1968). Furthermore, GH administration promotes lipolysis in adipose tissue (Galbraith and Topps, 1981). Both of these observations indicate that GH may be more involved in antagonism of glucose utilization and in provision of fatty acids as substrates for energy utilization, thus sparing glucose, rather than in regulation of anabolism (Wallace and Basset, 1966). The effect of GH and organophosphates on lipolytic rates in adipose tissue have not been dealt with in the present investigation. The significance of alterations in lipolysis should not be overlooked, however, and additional experiments designed to test the effects of combinations of GH and organophosphate growth-promotants deserve consideration.
DISCUSSION AND SUMMARY

The necessity for improvements in world food production has become an urgent problem. One level at which this problem is being dealt with is in the development of more efficient practices in livestock production. The advent of growth-promoting compounds, such as the anabolic, synthetic estrogen, DES, led to significant improvements in livestock growth and feed efficiency (Burroughs et al., 1954). In the last 10 years, organophosphate pesticides have been shown to promote growth in cattle (Rumsey et al., 1975; Rumsey, 1979) but, unlike the anabolic agents, the mechanism whereby organophosphates improve growth performance is poorly understood (Rumsey et al., 1981a). Furthermore, postnatal somatic growth consists of two major components: accretion of body fat and skeletal muscle protein deposition. With respect to animals used for human consumption, manipulation of body growth to favor growth of lean tissue is ideal, thereby decreasing accretion of trimmable fat. Growth-manipulating agents that selectively partition dietary nutrients toward proliferation of more lean and less adipose tissue continuously are sought after. No studies have been conducted to determine how organophosphates might affect metabolism in adipose tissue and skeletal muscle. With these ideas in mind, the work described in this dissertation was carried out with the following objectives: 1) to determine the effects of two organophosphate pesticides with similar chemical structures, ronnel and Dursban®, on metabolic activity in adipose tissue and skeletal muscle from steers and 2) to examine possible interactions of ronnel and Dursban® with two hormones involved in growth, i.e., thyroxine and growth hormone.
Effects of Organophosphates on Metabolic Activity in Adipose Tissue and Skeletal Muscle from Steers

To carry out the first objective of the present investigation, incubation experiments were used to determine effects of ronnel or Dursban® on substrate oxidation and lipogenesis in subcutaneous adipose tissue and skeletal muscle from 6-, 12- and 18-month-old steers. Results of incubation experiments indicate that metabolic activity of steer adipose tissue, as reflected by rates of oxidation of acetate to CO₂, tends to be more suppressed by ronnel at 6 than at 12 or 18 months of age. Effects of ronnel may be more clearly observable in adipose tissue from younger, more metabolically active steers and may be less easily detected with advancing age. The capacity for oxidative phosphorylation with subsequent generation of ATP continues throughout an animal's life; with advancing age, however, specific requirements for ATP to support growth decrease (Guyton, 1976; Atkins, 1981). When Dursban® was added to incubation media, oxidation rates tended to increase in adipose tissue from 12-month-old steers and decreased in adipose tissue from 18-month-old steers. Both ronnel and Dursban® produced similar effects on rates of oxidation of acetate to CO₂ in adipose tissue from 12-month-old steers. Decreases in oxidation rates with advancing age of steers occurred in studies with both ronnel and Dursban®, thus lending support to the general observation that metabolic activity decreases with age.

The capacity for fatty acid synthesis from acetate when either ronnel or Dursban® was present in incubation media was determined to provide a second index of metabolic activity in steer adipose tissue. Ronnel depressed rates of fatty acid synthesis significantly (P<.05) in adipose
tissue from 6- and 12-month-old steers but had no effect on fatty acid synthesis rates in adipose tissue from 18-month-old steers. Similarly, addition of Dursban® to incubation media significantly (P<.05) depressed rates of fatty acid synthesis in adipose tissue from 12-month-old steers but had no effect on rates in adipose tissue from 18-month-old steers. The apparent refractoriness to organophosphates of adipose tissue from older steers again may be a reflection of decreased metabolic activity with advancing age. With respect to rates of fatty acid synthesis, expressed on the basis of tissue weight, results of experiments with ronnel and Dursban® are in agreement with other reports indicating that rates of fatty acid synthesis decrease with increasing age (Ingle et al., 1973; Pothoven and Beitz, 1973). Based on overall rates of fatty acid synthesis, therefore, adipose tissue from younger steers was more metabolically active than adipose tissue from older steers, thus providing further manifestation of the general decline in metabolic responsiveness that occurs with age.

Recycling of $^{14}$C from acetate into glycerol by way of the TCA cycle in steer adipose tissue generally decreased when ronnel or Dursban® was present in incubation media. If recycling of $^{14}$C into glycerol is considered a metabolic event in adipose tissue, the decreased capacity of adipose tissue to incorporate acetate carbon into glycerol provides additional support that organophosphates depress metabolic activity in steer adipose tissue.

Effects of organophosphates on metabolic activity in skeletal muscle also were examined. Glucose was chosen as the substrate for energy utilization in skeletal muscle; glucose can be oxidized to produce ATP and is a precursor for glyceride-glycerol synthesis necessary for triacylglycerol
formation, a storage form of energy in skeletal muscle (Lehninger, 1975; Atkins, 1981). Additionally, glucose is a source of glycerol for cell membrane phospholipid biosynthesis (Lehninger, 1975). On the basis of glucose oxidation to CO₂, ronnel decreased metabolic activity in skeletal muscle from 6-month-old steers but increased metabolic activity as steers grew to 12 months of age. In skeletal muscle from 12-month-old steers, Dursban® addition to incubation media tended to increase rates of oxidation of glucose to CO₂. Patterns of oxidation rates in skeletal muscle, therefore, were similar under the influence of either ronnel or Dursban®.

Rates of fatty acid synthesis from glucose in skeletal muscle in experiments involving both ronnel and Dursban® were low relative to rates in adipose tissue. Because the majority of fatty acids required for triacylglycerol synthesis in skeletal muscle are derived primarily from blood plasma, low rates of fatty acid synthesis in skeletal muscle were expected. Addition of organophosphates to incubation media decreased fatty acid synthesis rates in skeletal muscle from 12-month-old steers but had no consistently observed effects in skeletal muscle from 6- and 18-month-old steers. The depression in fatty acid synthesis rates in skeletal muscle from 12-month-old steers when either ronnel or Dursban® was added to incubation media may indicate direction of glucose away from fatty acid synthesis and toward oxidation with subsequent ATP generation; rates of oxidation of glucose to CO₂ were 4- to 16-fold greater than fatty acid synthesis rates in skeletal muscle in experiments with both ronnel and Dursban®.

Glycerol production from glucose in skeletal muscle, as influenced by ronnel and Dursban®, also was examined. In general, addition of ronnel
to incubation media was associated with greater rates of glycerol synthesis than when no ronnel was present. The potential for increased capacity to synthesize glycerol may reflect increased capacity of growing skeletal muscle to synthesize cell membranes. Rates of glycerol production were 4- to 8-fold greater in skeletal muscle from 6-month-old steers than in skeletal muscle from either 12- or 18-month-old steers, perhaps reflecting a decreased capacity for muscle cell membrane synthesis and, in turn, a decrease in muscle growth that occurs during the postnatal period (Swatland, 1976). In contrast, however, no effects of Dursban® on glycerol production in skeletal muscle were evident.

In summary, results of the present investigation indicate that ronnel and Dursban® depress metabolic activity of adipose tissue from 6- and 12-month-old growing steers without concomitantly decreasing metabolic activity of skeletal muscle. Furthermore, rates of substrate oxidation and fatty acid synthesis in adipose tissue and skeletal muscle in older (18-month-old) steers were less affected by ronnel. The latter result may be a reflection of decline in metabolic activity with age. Additionally, tissues from older animals may become refractory toward potential manipulators of metabolism. Similarities in effects of ronnel and Dursban® in adipose tissue and skeletal muscle suggest that organophosphate pesticides possessing growth-promoting activity may act by way of alterations in partitioning of acetate and glucose between major metabolic processes in adipose tissue and skeletal muscle. Although ronnel and Dursban® are structurally similar, any differences in effects of ronnel and Dursban® in adipose tissue and skeletal muscle may be related to subtle differences in their molecular properties.
Interactions of Organophosphates with Thyroxine and Growth Hormone

The mechanisms of action of some growth promotants, e.g., the anabolic agents, are related closely to alterations in hormonal status (Trenkle, 1970; Borger et al., 1973; Trenkle and Burroughs, 1978). One of the manifestations of estrogen administration to ruminants is increased plasma concentrations of growth hormone (GH; Trenkle, 1970). Furthermore, feeding ronnel to cattle results in significant increases in plasma thyroxine (T₄) concentrations in steers (Rumsey et al., 1981a). The second objective of the present investigation, therefore, was to examine possible interactions of organophosphate compounds with two hormones, T₄ and GH, known to influence growth. As in the studies described above, rates of oxidation of acetate to CO₂ and rates of fatty acid synthesis from acetate were used to assess effects of hormones and organophosphate-hormone combinations on metabolic processes in steer adipose tissue.

Significantly (P<.05) increased rates of oxidation of acetate to CO₂ in comparison with control rates were observed when T₄ was added to incubation media. This result is consistent with general increases in metabolic rate induced by T₄ (Hoch, 1974; Guyton, 1976). Combination of T₄ with either organophosphate resulted in rates of oxidation of acetate to CO₂ that were less than rates occurring with T₄ alone; organophosphates, when added to incubation media along with T₄, depressed the T₄-induced increase in oxidation rates in adipose tissue. These results suggest that organophosphates decrease metabolic activity, even in the presence of a known stimulator of metabolic processes.

A significant (P<.05) decrease in rates of fatty acid synthesis compared with control rates occurred when T₄ was added to incubation media.
Thyroxine is a stimulator of lipolysis; T₄ administration results in mobilization of free fatty acids from adipose tissue for oxidation in other body tissues (Goodman and Bray, 1966; Guyton, 1976). Decreased rates of fatty acid synthesis, therefore, would be expected under the influence of T₄. Addition of T₄ plus either ronnel or Dursban® to incubation media resulted in fatty acid synthesis rates that were similar to control rates but greater than rates produced when only T₄ was present in media. Results from earlier studies described in this dissertation strongly suggest that either ronnel or Dursban®, when added to incubation media, depresses fatty acid synthesis in adipose tissue. The reason why depressions in fatty acid synthesis occur when T₄ or organophosphates are added separately to incubation media but not when T₄ and organophosphates are added together is unclear.

Studies by Rumsey et al., (1981a) suggested that the growth-promoting effect of ronnel in steers may be related to alterations in thyroid function. The present study has dealt with specific metabolic consequences of T₄ or T₄ plus organophosphates at the level of body tissues. Effects of T₄ and combinations of T₄ and organophosphates on metabolic activity in skeletal muscle were not tested in the present investigation. Trends toward decreased metabolic activity in adipose tissue and increased metabolic activity in skeletal muscle observed when either ronnel or Dursban® was added to incubation media (see Parts I and II of this dissertation) and evidence that ronnel administration to steers results in increased plasma T₄ concentrations may indicate that organophosphates act by increasing T₄ concentrations in plasma while simultaneously influencing tissue response to T₄ stimulation.
Neither GH nor GH plus organophosphates produced rates of oxidation of acetate to CO₂ or rates of fatty acid synthesis that were different from control values in steer adipose tissue. Growth hormone may increase availability of fatty acids to tissues by stimulating triacylglycerol hydrolysis in adipose tissue rather than exerting a direct stimulatory effect on rates of fatty acid oxidation (Goodman and Schwartz, 1974). Furthermore, GH administration results in inhibition of lipogenesis (Goodman, 1963; Goodman and Schwartz, 1974); decreased rates of fatty acid synthesis with GH addition to incubation media were expected in the present study. Goodman (1968) and Goodman and Schwartz (1974) observed that in vivo conditions were necessary to demonstrate inhibitory effects of GH on fatty acid synthesis and that even after prolonged incubation with GH, inhibition of fatty acid synthesis did not occur. A second, endogenous, circulating substance may be required for inhibition of fatty acid synthesis by GH (Daughaday et al., 1972). This explanation may be applicable to the failure of GH to depress fatty acid synthesis in adipose tissue in the present study. No effect of GH plus either organophosphate on fatty acid synthesis rates was observed in the present investigation. Other reports indicate that GH may be more involved in provision of fatty acids as substrates for energy utilization by way of enhanced rates of lipolysis, thus sparing glucose (Goodman, 1963, 1968; Galbraith and Topps, 1981). Effects of T₄ or GH in combination with organophosphates on lipolytic rates in adipose tissue have not been tested in the present investigation. Experiments to determine effects of T₄, GH, and organophosphates on lipolysis rates merit consideration.
Because no previous studies are available relating effects of growth promotants to substrate oxidation and fatty acid synthesis in adipose tissue and skeletal muscle, results of the present investigation are an introductory contribution to the study of effects of growth-promoting compounds at the level of body tissues.
LITERATURE CITED


Eddy, G. W. 1961. Laboratory tests of residues of organophosphorus compounds against house flies. J. Econ. Entomol. 54:386-388.


ACKNOWLEDGEMENTS

I wish to express my sincere and heartfelt appreciation to the members of my committee: to my major professor, Dr. Donald C. Beitz, a man of many talents and complete humility, for his inexhaustible supply of patience and unselfish willingness to listen, to provide sound and valuable advice, and to talk with me about baseball; to Dr. Jerry W. Young for having the answers to so many of my questions and for his editorial wisdom; and to Dr. Joel R. Coats, Dr. Richard C. Ewan, and Dr. Allen H. Trenkle for their individual contributions to my graduate career by way of their courses, their expertise, and the personal advice they frequently have been generous with. I also would like to thank Dr. David F. Cox for always knowing what to do and for making statistics something no one should be afraid of. I truly have appreciated the opportunity to work in close association with these scientists and gentlemen.

What can I say about my fellow graduate students and the staff in Nutritional Physiology? Just this-you are the best, people. You've succeeded in making something of an animal scientist out of a city kid. I shall always remember the warmth of Iowa.

I am especially grateful to Dow Chemical, U.S.A. for supporting this work.

I would like to give special thanks to Dr. Ross L. Hood for the inspiration he has been to me, for showing me so much in so little time, and for making me learn, the hard way, about life's ups and downs; and to my dear friend, Hester Fassel, who always has been there when I have needed support-You're one in a million, Lady.
Finally, I would like to say a very special thank you to my father and brothers for their constant encouragement and complete faith. You guys make it all worthwhile.
Table A1. Rates of oxidation of acetate to CO₂, fatty acid synthesis, and recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 6-month-old steers as influenced by ronnel

<table>
<thead>
<tr>
<th>Steer</th>
<th>Ronnel dose</th>
<th>CO₂</th>
<th>Fatty acids</th>
<th>Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>-   -</td>
<td>nmoles/2 hr x g</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>873.2</td>
<td>7877.9</td>
<td>108.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>326.7</td>
<td>1891.6</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>417.6</td>
<td>1195.6</td>
<td>30.7</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>891.4</td>
<td>6217.2</td>
<td>80.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>391.5</td>
<td>2076.6</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>344.7</td>
<td>902.0</td>
<td>23.4</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>802.9</td>
<td>5559.4</td>
<td>101.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>331.8</td>
<td>2135.6</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>212.4</td>
<td>641.2</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>525.4</td>
<td>6814.2</td>
<td>55.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>350.0</td>
<td>1793.5</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>248.0</td>
<td>748.1</td>
<td>18.0</td>
<td></td>
</tr>
</tbody>
</table>

*aValues are means of triplicate incubations.*
Table A2. Rates of oxidation of acetate to CO₂, fatty acid synthesis, and recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 12-month-old steers as influenced by ronnel

<table>
<thead>
<tr>
<th>Steer</th>
<th>Ronnel dose</th>
<th>CO₂</th>
<th>Fatty acids</th>
<th>Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>nmoles/2 hr x g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2406.4</td>
<td>1256.8</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>608.7</td>
<td>3463.0</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>469.5</td>
<td>19.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>290.5</td>
<td>1138.1</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>494.7</td>
<td>1291.9</td>
<td>53.2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3106.8</td>
<td>1193.0</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>799.2</td>
<td>1600.8</td>
<td>20.3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>452.2</td>
<td>340.0</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>383.1</td>
<td>771.8</td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>396.7</td>
<td>700.1</td>
<td>27.8</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2839.7</td>
<td>1130.3</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>638.8</td>
<td>620.4</td>
<td>16.9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>398.4</td>
<td>515.6</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>339.1</td>
<td>450.4</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>322.3</td>
<td>930.1</td>
<td>39.2</td>
<td></td>
</tr>
</tbody>
</table>

Values are means of triplicate incubations.
Table A3. Rates of oxidation of acetate to CO₂, fatty acid synthesis, and recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 18-month-old steers as influenced by ronnel.³

<table>
<thead>
<tr>
<th>Steer</th>
<th>Ronnel dose</th>
<th>CO₂</th>
<th>Fatty acids</th>
<th>Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>--- nmoles/2 hr x g ---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>263.1</td>
<td>624.1</td>
<td></td>
<td>35.8</td>
</tr>
<tr>
<td>2</td>
<td>1249.6</td>
<td>706.4</td>
<td></td>
<td>2.8</td>
</tr>
<tr>
<td>3</td>
<td>607.4</td>
<td>986.6</td>
<td></td>
<td>8.1</td>
</tr>
<tr>
<td>4</td>
<td>410.8</td>
<td>193.3</td>
<td></td>
<td>7.5</td>
</tr>
<tr>
<td>5</td>
<td>174.9</td>
<td>227.6</td>
<td></td>
<td>3.4</td>
</tr>
<tr>
<td>1</td>
<td>308.3</td>
<td>277.5</td>
<td></td>
<td>9.1</td>
</tr>
<tr>
<td>2</td>
<td>464.6</td>
<td>1271.3</td>
<td></td>
<td>6.0</td>
</tr>
<tr>
<td>3</td>
<td>1207.9</td>
<td>1177.8</td>
<td></td>
<td>7.8</td>
</tr>
<tr>
<td>4</td>
<td>278.4</td>
<td>169.6</td>
<td></td>
<td>19.1</td>
</tr>
<tr>
<td>5</td>
<td>292.8</td>
<td>93.9</td>
<td></td>
<td>5.2</td>
</tr>
<tr>
<td>1</td>
<td>228.6</td>
<td>286.1</td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>.774.5</td>
<td>1397.6</td>
<td></td>
<td>7.2</td>
</tr>
<tr>
<td>3</td>
<td>1490.0</td>
<td>1002.8</td>
<td></td>
<td>3.1</td>
</tr>
<tr>
<td>4</td>
<td>369.2</td>
<td>180.1</td>
<td></td>
<td>4.2</td>
</tr>
<tr>
<td>5</td>
<td>832.0</td>
<td>138.8</td>
<td></td>
<td>6.0</td>
</tr>
</tbody>
</table>

³Values are means of triplicate incubations.
Table A4. Rates of oxidation of glucose to CO₂, fatty acid synthesis, and glycerol production in skeletal muscle from 6-month-old steers as influenced by ronnel

<table>
<thead>
<tr>
<th>Steer</th>
<th>Ronnel dose</th>
<th>CO₂</th>
<th>Fatty acids</th>
<th>Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>nmoles/2 hr x g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>251.4</td>
<td>75.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>212.2</td>
<td>75.4</td>
<td></td>
<td>214.0</td>
</tr>
<tr>
<td>3</td>
<td>181.8</td>
<td>--</td>
<td></td>
<td>39.9</td>
</tr>
<tr>
<td>4</td>
<td>107.0</td>
<td>--</td>
<td></td>
<td>3.8</td>
</tr>
<tr>
<td>1</td>
<td>254.5</td>
<td>58.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>224.9</td>
<td>67.3</td>
<td></td>
<td>242.5</td>
</tr>
<tr>
<td>3</td>
<td>140.0</td>
<td>--</td>
<td></td>
<td>43.4</td>
</tr>
<tr>
<td>4</td>
<td>88.1</td>
<td>--</td>
<td></td>
<td>4.2</td>
</tr>
<tr>
<td>1</td>
<td>215.7</td>
<td>44.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>194.4</td>
<td>62.2</td>
<td></td>
<td>169.8</td>
</tr>
<tr>
<td>3</td>
<td>178.0</td>
<td>--</td>
<td></td>
<td>109.4</td>
</tr>
<tr>
<td>4</td>
<td>106.3</td>
<td>--</td>
<td></td>
<td>9.4</td>
</tr>
<tr>
<td>1</td>
<td>194.6</td>
<td>59.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>207.6</td>
<td>87.5</td>
<td></td>
<td>257.7</td>
</tr>
<tr>
<td>3</td>
<td>119.2</td>
<td>--</td>
<td></td>
<td>63.6</td>
</tr>
<tr>
<td>4</td>
<td>64.7</td>
<td>--</td>
<td></td>
<td>13.0</td>
</tr>
</tbody>
</table>

*Values are means of triplicate incubations.*
Table A5. Rates of oxidation of glucose to CO₂, fatty acid synthesis, and glycerol production in skeletal muscle from 12-month-old steers as influenced by ronnel¹

<table>
<thead>
<tr>
<th>Steer</th>
<th>Ronnel dose</th>
<th>CO₂</th>
<th>Fatty acids</th>
<th>Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>-</td>
<td>nmoles/2 hr x g</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>125.0</td>
<td>38.3</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>66.2</td>
<td>--</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>45.6</td>
<td>27.1</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>59.2</td>
<td>23.9</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>37.4</td>
<td>13.8</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>119.2</td>
<td>10.1</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>99.9</td>
<td>--</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>44.4</td>
<td>4.7</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>100.2</td>
<td>13.0</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>40.5</td>
<td>8.7</td>
<td>13.2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>95.4</td>
<td>3.1</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>109.5</td>
<td>1.4</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>53.0</td>
<td>6.5</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>85.5</td>
<td>4.6</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>36.9</td>
<td>6.7</td>
<td>12.9</td>
<td></td>
</tr>
</tbody>
</table>

¹Values are means of triplicate incubations.
Table A6. Rates of oxidation of glucose to CO₂, fatty acid synthesis, and glycerol production in skeletal muscle from 18-month-old steers as influenced by Ronnel\(^a\)

<table>
<thead>
<tr>
<th>Steer</th>
<th>Ronnel dose</th>
<th>CO₂</th>
<th>Fatty acids</th>
<th>Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>- - - nmoles/2 hr x g - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>253.4</td>
<td>0.9</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>149.5</td>
<td>1.7</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>168.5</td>
<td>0.1</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>112.9</td>
<td>0.4</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>81.5</td>
<td>0.6</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>146.5</td>
<td>0.8</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>135.1</td>
<td>0.8</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>123.2</td>
<td>4.1</td>
<td>7.5</td>
</tr>
<tr>
<td>4</td>
<td>125.2</td>
<td>0.5</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>106.0</td>
<td>0.1</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>120.3</td>
<td>0.6</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>109.4</td>
<td>1.1</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>140.5</td>
<td>---</td>
<td>4.6</td>
</tr>
<tr>
<td>4</td>
<td>167.7</td>
<td>0.8</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>82.7</td>
<td>0.1</td>
<td>2.6</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Values are means of triplicate incubations.
Table A7. Rates of oxidation of acetate to CO$_2$, fatty acid synthesis, and recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 12-month-old steers as influenced by Dursban®

<table>
<thead>
<tr>
<th>Steer</th>
<th>Dursban® dose</th>
<th>CO$_2$</th>
<th>Fatty acids</th>
<th>Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>- - - nmoles/2 hr x g - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2406.4</td>
<td>1256.8</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>608.7</td>
<td>3463.0</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>385.0</td>
<td>19.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>290.5</td>
<td>545.8</td>
<td>99.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>494.7</td>
<td>1291.9</td>
<td>53.2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2674.7</td>
<td>1027.6</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>670.4</td>
<td>454.1</td>
<td>19.8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>372.6</td>
<td>962.0</td>
<td>35.7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>387.4</td>
<td>320.6</td>
<td>510.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>449.7</td>
<td>867.7</td>
<td>44.1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2591.8</td>
<td>1116.0</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>833.4</td>
<td>461.9</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>472.6</td>
<td>356.1</td>
<td>76.3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>647.9</td>
<td>84.6</td>
<td>192.9</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>414.2</td>
<td>651.8</td>
<td>68.3</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2748.3</td>
<td>2308.7</td>
<td>213.6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>687.0</td>
<td>1421.4</td>
<td>184.9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>679.7</td>
<td>898.9</td>
<td>82.6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>288.2</td>
<td>833.2</td>
<td>217.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>493.1</td>
<td>1878.4</td>
<td>261.4</td>
<td></td>
</tr>
</tbody>
</table>

Values are means of triplicate incubations.
Table A8. Rates of oxidation of acetate to CO₂, fatty acid synthesis, and recycling of acetate carbon into glycerol in subcutaneous adipose tissue from 18-month-old steers as influenced by Dursban®

<table>
<thead>
<tr>
<th>Steer</th>
<th>Dursban® dose (mg/kg)</th>
<th>CO₂ (nmoles/2 hr x g)</th>
<th>Fatty acids (nmoles/2 hr x g)</th>
<th>Glycerol (nmoles/2 hr x g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>263.1</td>
<td>624.1</td>
<td>35.6</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1249.6</td>
<td>706.4</td>
<td>2.8</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>607.4</td>
<td>986.6</td>
<td>8.1</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>410.8</td>
<td>193.3</td>
<td>7.5</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>174.9</td>
<td>227.6</td>
<td>3.4</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>405.2</td>
<td>99.8</td>
<td>4.5</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>456.4</td>
<td>1065.9</td>
<td>6.5</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>2786.5</td>
<td>972.3</td>
<td>2.3</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>346.0</td>
<td>131.2</td>
<td>7.4</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>160.2</td>
<td>76.0</td>
<td>4.4</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>531.7</td>
<td>142.7</td>
<td>1.7</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>306.6</td>
<td>1243.2</td>
<td>4.6</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>422.3</td>
<td>675.5</td>
<td>3.7</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>284.8</td>
<td>217.7</td>
<td>3.4</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>88.6</td>
<td>73.3</td>
<td>3.0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>213.8</td>
<td>316.7</td>
<td>2.8</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>295.0</td>
<td>1949.1</td>
<td>7.1</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>619.1</td>
<td>793.2</td>
<td>1.1</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>149.1</td>
<td>115.7</td>
<td>1.9</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>269.1</td>
<td>54.3</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Values are means of triplicate incubations.
Table A9. Rates of oxidation of glucose to CO$_2$, fatty acid synthesis, and glycerol production in skeletal muscle from 12-month-old steers as influenced by Dursban®

<table>
<thead>
<tr>
<th>Steer</th>
<th>Dursban® dose</th>
<th>CO$_2$</th>
<th>Fatty acids</th>
<th>Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>nmoles/2 hr x g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>125.0</td>
<td>38.3</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>66.2</td>
<td>--</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>45.6</td>
<td>27.1</td>
<td>1.3</td>
</tr>
<tr>
<td>4</td>
<td>59.2</td>
<td>23.9</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>37.4</td>
<td>13.8</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>120.9</td>
<td>34.0</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>116.0</td>
<td>2.8</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>52.7</td>
<td>6.5</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>58.5</td>
<td>10.0</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>41.3</td>
<td>8.1</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>118.4</td>
<td>3.1</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>88.4</td>
<td>--</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>80.2</td>
<td>2.8</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>66.7</td>
<td>3.6</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>36.3</td>
<td>6.0</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>102.8</td>
<td>3.7</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>103.1</td>
<td>5.1</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>64.5</td>
<td>8.8</td>
<td>2.4</td>
</tr>
<tr>
<td>4</td>
<td>63.8</td>
<td>6.4</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>51.6</td>
<td>7.5</td>
<td>8.4</td>
<td></td>
</tr>
</tbody>
</table>

Values are means of triplicate incubations.
Table A10. Rates of oxidation of glucose to CO₂, fatty acid synthesis, and glycerol production in skeletal muscle from 18-month-old steers as influenced by Dursban®

<table>
<thead>
<tr>
<th>Steer</th>
<th>Dursban® dose mg/kg</th>
<th>CO₂ nmoles/2 hr x g</th>
<th>Fatty acids nmoles/2 hr x g</th>
<th>Glycerol nmoles/2 hr x g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>253.4</td>
<td>0.9</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>149.5</td>
<td>1.7</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>168.5</td>
<td>0.7</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>112.9</td>
<td>0.4</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>81.5</td>
<td>0.6</td>
<td>2.5</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Steer</th>
<th>Dursban® dose mg/kg</th>
<th>CO₂ nmoles/2 hr x g</th>
<th>Fatty acids nmoles/2 hr x g</th>
<th>Glycerol nmoles/2 hr x g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99.2</td>
<td>2.1</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>151.8</td>
<td>0.6</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>101.7</td>
<td>1.1</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>130.0</td>
<td>0.5</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>76.5</td>
<td>0.3</td>
<td>3.6</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Steer</th>
<th>Dursban® dose mg/kg</th>
<th>CO₂ nmoles/2 hr x g</th>
<th>Fatty acids nmoles/2 hr x g</th>
<th>Glycerol nmoles/2 hr x g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>136.5</td>
<td>0.3</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>105.5</td>
<td>1.0</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>231.3</td>
<td>1.5</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>165.0</td>
<td>0.4</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>93.4</td>
<td>0.0</td>
<td>3.7</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Steer</th>
<th>Dursban® dose mg/kg</th>
<th>CO₂ nmoles/2 hr x g</th>
<th>Fatty acids nmoles/2 hr x g</th>
<th>Glycerol nmoles/2 hr x g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>134.8</td>
<td>--</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>152.0</td>
<td>2.4</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>246.7</td>
<td>0.3</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>147.9</td>
<td>0.7</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>174.0</td>
<td>1.4</td>
<td>3.6</td>
<td></td>
</tr>
</tbody>
</table>

*Values are means of triplicate incubations.*
Table A11. Effect of ronnel on rates of oxidation of acetate to CO₂ and rates of fatty acid synthesis expressed on a cellular basis in subcutaneous adipose tissue from 6-month-old steers^A

<table>
<thead>
<tr>
<th>Ronnel dose</th>
<th>CO₂</th>
<th>Fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg</td>
<td>- - - - - nmoles/2 hr x 10⁶ cells- - - -</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>319.7 ± 38.9</td>
<td>2349.0 ± 219.1</td>
</tr>
<tr>
<td>2.5</td>
<td>348.7 ± 36.7</td>
<td>1970.0 ± 219.1</td>
</tr>
<tr>
<td>5</td>
<td>298.1 ± 38.9</td>
<td>1562.5 ± 232.4</td>
</tr>
<tr>
<td>10</td>
<td>238.4 ± 41.6</td>
<td>1325.6 ± 248.4</td>
</tr>
</tbody>
</table>

^AValues are means of triplicate incubations (n = 5 steers).

Table A12. Effect of ronnel on rates of oxidation of acetate to CO₂ and rates of fatty acid synthesis expressed on a cellular basis in subcutaneous adipose tissue from 12-month-old steers^A

<table>
<thead>
<tr>
<th>Ronnel dose</th>
<th>CO₂</th>
<th>Fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg</td>
<td>- - - - - nmoles/2 hr x 10⁶ cells- - - -</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>665.6 ± 94.7</td>
<td>1360.4 ± 158.7</td>
</tr>
<tr>
<td>2.5</td>
<td>915.2 ± 70.9</td>
<td>799.4 ± 114.8</td>
</tr>
<tr>
<td>5</td>
<td>789.0 ± 69.5</td>
<td>649.8 ± 112.0</td>
</tr>
</tbody>
</table>

^AValues are means of triplicate incubations (n = 5 steers).
Table A13. Effect of ronnel on rates of oxidation of acetate to CO₂ and rates of fatty acid synthesis expressed on a cellular basis in subcutaneous adipose tissue from 18-month-old steers

<table>
<thead>
<tr>
<th>Ronnel dose (mg/kg)</th>
<th>CO₂ (nmol/2 hr x 10^6 cells)</th>
<th>Fatty acids (nmol/2 hr x 10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1042.1 ± 211.6</td>
<td>1106.6 ± 181.3</td>
</tr>
<tr>
<td>2.5</td>
<td>775.9 ± 194.3</td>
<td>1131.0 ± 167.9</td>
</tr>
<tr>
<td>5</td>
<td>1208.2 ± 199.5</td>
<td>1355.1 ± 186.6</td>
</tr>
</tbody>
</table>

aValues are means of triplicate incubations (n = 5 steers).
Table A14. Effect of Dursban® on rates of oxidation of acetate to CO₂ and rates of fatty acid synthesis expressed on a cellular basis in subcutaneous adipose tissue from 12-month-old steers

<table>
<thead>
<tr>
<th>Dursban® dose (mg/kg)</th>
<th>CO₂ (nmol/2 hr x 10⁶ cells)</th>
<th>Fatty acids (nmol/2 hr x 10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>665.6 ± 79.4</td>
<td>1109.4 ± 165.2</td>
</tr>
<tr>
<td>0.5</td>
<td>769.1 ± 57.2</td>
<td>574.6 ± 118.9</td>
</tr>
<tr>
<td>1</td>
<td>829.3 ± 58.3</td>
<td>441.7 ± 116.8</td>
</tr>
<tr>
<td>2</td>
<td>843.9 ± 58.3</td>
<td>1183.3 ± 111.0</td>
</tr>
</tbody>
</table>

Values are means of triplicate incubations (n = 5 steers).

Table A15. Effect of Dursban® on rates of oxidation of acetate to CO₂ and rates of fatty acid synthesis expressed on a cellular basis in subcutaneous adipose tissue from 18-month-old steers

<table>
<thead>
<tr>
<th>Dursban® dose (mg/kg)</th>
<th>CO₂ (nmol/2 hr x 10⁶ cells)</th>
<th>Fatty acids (nmol/2 hr x 10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1042.1 ± 262.0</td>
<td>1106.6 ± 196.4</td>
</tr>
<tr>
<td>0.5</td>
<td>1240.1 ± 262.0</td>
<td>1176.8 ± 215.2</td>
</tr>
<tr>
<td>1</td>
<td>641.0 ± 262.0</td>
<td>1068.3 ± 196.4</td>
</tr>
<tr>
<td>2</td>
<td>548.1 ± 254.2</td>
<td>1558.3 ± 196.4</td>
</tr>
</tbody>
</table>

Values are means of triplicate incubations (n = 5 steers).