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# Effects of an antiestrogen and estradiol on food intake, body composition, and metabolism in ovariectomized rats.

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EFFECTS OF AN ANTIESTROGEN AND ESTRADIOL ON FOOD INTAKE,
BODY COMPOSITION, AND METABOLISM IN OVARIECTOMIZED RATS

A Thesis Presented

By

HENRY WALTER HELLER

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
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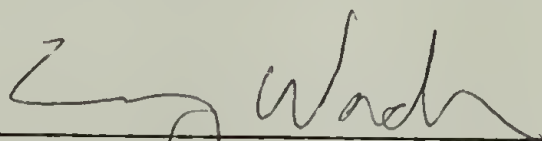Department of Psychology

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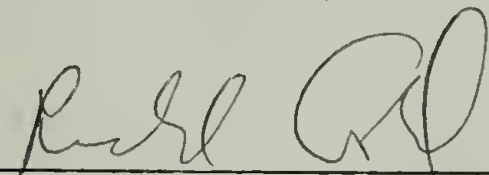By

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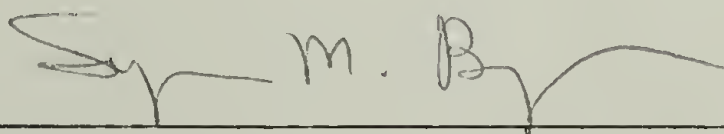Approved as to style and content by:

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## ACKNOWLEDGEMENT

ABSTRACT

Effects of an Antiestrogen and Estradiol on Food Intake,
Body Composition, and Metabolism in Ovariectomized Rats

February 1984

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In ovariectomized rats receiving daily injections for
35 days the effects of oil or of 10 ug of TAM did not
differ significantly, but 50 ug, 250 ug, and 1000 ug of
TAM all produced significant reductions of total food
intake, percent body weight, and carcass fat.

Ovariectomized rats deprived of food for five hours
daily and receiving daily injections for 35 days of oil or
of 2 ug of estradiol benzoate (EB) showed no difference in
total food intake, although animals receiving 250 ug of
TAM or 2 ug of EB and 250 ug of TAM showed significant
reductions. Animals receiving EB, TAM or TAM+EB showed
significant reductions of percent body weight, carcass
fat, and parametrial white adipose tissue weight and lipo-
protein lipase activity. In interscapular brown adipose
tissue EB did not have an affect, but TAM produced in-
creases in weight and protein which were reversed by EB.
EB and TAM+EB increased oxygen consumption on the first
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day after the initial injection, but subsequently oxygen consumption decreased. TAM produced an initial increase in oxygen consumption which then remained relatively elevated.

In ovariectomized rats receiving oil injections a five-hour daily food deprivation produced a dramatic reduction in total food intake and a decrease in body weight compared to animals receiving oil on ad lib. food. Five-hour daily deprivation attenuated the EB-induced reduction of total food intake and body weight. Five-hour daily food deprivation had no effect on food intake or body weight in rats receiving TAM. Drugs and food manipulations produced no significant effects on oxygen consumption, but the patterns shown by TAM and by EB closely resembled those shown in the previous experiment. In animals receiving ad lib. food EB produced a continued elevation, while .oil and TAM did not differ.

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CHAPTER I

INTRODUCTION

Seventy years ago Stotsenburg (1913) showed that bilateral ovariectomy increased food intake, body weight and body length in rats. He ascertained that the increased length was not sufficient to account for the increased weight and attributed the additional increase to fat (although he did not measure the body fat). He also found that unilateral ovariectomy had no effect on food intake, body weight, or body length but did produce hypertrophy of the remaining ovary. Subsequently Wang, Richter, and Guttmacher (1925) established that implanting ovaries in castrate male rats led to a decrease in food intake. These studies suggested that a substance secreted by the ovaries could produce a reduction in food intake and body weight. Bugbee and Simond (1926) demonstrated that an extract made from ovaries reduced weight gain in ovariectomized rats, and Bogart, Lasley, and Mayer (1944) established that giving estrone to ovariectomized rats reversed the increase in food intake and body weight. These findings have been replicated frequently with both peripheral and hypothalamic applications of estrogens (for review see Wade, 1976).

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Stotsenburg (1913) speculated that the weight gain from ovariectomy was partly attributable to an increase of fat deposition. Holt, Keeton, and Vennesland (1936) found that there was an increase of bone length following ovariectomy but failed to find any changes in the chemical composition of the body. Leshner and Collier (1973) ascertained that ovariectomy produced increases in fat content of female rats but did not have an effect on protein content. Several subsequent studies have produced similar findings and indicated that estradiol counteracts this increase (see, for example, Gray & Wade, 1981; Roy & Wade, 1977). Mueller and Hsaio (1981) used the Lee index--a measure of obesity which uses body weight and body length--to confirm that ovariectomized rats became more obese and that estradiol treatment, while not returning already heavy rats to the weights of controls, returned them to weights that were consistent with their increased length. They estimated that about 40% of the increased weight was due to increased skeletal and associated growth, and the remainder was due to increased fat. Simson, Jones, Schwarz, and Gold (1982) reported that one group of ovariectomized rats showed nonsignificant increases in fat compared to intact animals, but that three other groups of rats all showed increases in fat compared to intact controls. Other studies suggest that the

weight gain is attributable to growth only, not an increase in fat deposition. Clark and Tarttelin (1982) found no difference in fat content of ovariectomized and intact rats, but housed the animals in groups, a situation which can in itself alter food intake and body weight. Despite some findings to the contrary the preponderance of evidence suggests that ovariectomy does increase fat content of the rat.

Roy and Wade (1977) demonstrated that ovariectomized rats not receiving estradiol, which were pair-fed with ovariectomized rats receiving estradiol, did not lose as much weight as their pair-feeding partners. Mueller and Hsaio (1980) reported that ovariectomized rats which were restricted to preovariectomy food intake still gained large amounts of weight. These findings indicate that the change in food intake alone is not sufficient to produce the change in body weight. Changes in metabolic rate may account for changes in body weight that are not attributable to altered food intake. Carlisle, Wilkinson, Laudenslager, and Keith (1979) reported a nonsignificant seven percent increase of metabolic rate as measured by oxygen consumption in response to estradiol. Laudenslager, Wilkinson, Carlisle, and Hammel (1980) reported significant increases in heat production as measured by oxygen

consumption in ovariectomized rats with estrogen treatment and decreases following removal of estrogen. They also determined that restricting activity did not affect this increase. Laudenslager, Carlisle, and Calvano (1982) found increases in heat production measured by oxygen consumption at 15°C and at -5°C in estradiol-treated ovariectomized rats. These reported increases in metabolic rate in response to estradiol could partially account for the findings of Roy and Wade (1977) and of Mueller and Hsaio (1980).

Changes in body weight due to changes in metabolism may be due to differences in heat production. Brown adipose tissue (BAT) contributes significantly to diet-induced thermogenesis (Rothwell & Stock, 1979) and to non-shivering thermogenesis in cold-adapted animals (Foster & Frydman, 1979). Tulp (1981) has shown that removal of interscapular BAT can lead to increased weight gain and fat deposition in rats and Connolly, Morrisey, & Carnie (1982) have shown this in mice. Fatty acids are the primary fuel for BAT thermogenesis (Nichols, 1979). Increases in lipogenesis in BAT are caused by exposure to cold (Trayhurn, 1979) and by sucrose overfeeding (Granneman & Wade, 1983) in rats. Bertin, Goudern, and Portet (1978) established that the activity of lipoprotein lipase (LPL)--an enzyme which hydrolyzes triglycerides and per-

mits fatty acid uptake by the cell-- increases in BAT of rats in response to cold but Granneman and Wade (1983) showed that it does not increase in response to sucrose overfeeding.

Wade and Gray (1978) established that cytoplasmic estrogen receptors are present in BAT. Edens and Wade (1983) demonstrated that ovariectomized rats receiving estradiol increase incorporation of tritium into fatty acids in interscapular BAT. They found no change in BAT LPL activity with estradiol treatment. These results suggest that increased BAT thermogenesis may contribute to the weight loss due to estradiol treatment. The source of the increased tritium incorporation into BAT is unclear. It could be due to in situ synthesis or to increased uptake of fatty acids synthesized elsewhere. Although Edens and Wade (1983) found no increase in BAT LPL activity to explain increased uptake, de Gasquet, Planche, and Boulange (1981) found increases in weight of white adipose tissue (WAT) in high-fat fed rats along with a decrease in WAT LPL activity. This suggests that uptake of fatty acids from triglycerides by tissues can occur by mechanisms other than LPL.

The increased synthesis or uptake of fatty acids by BAT suggests that some redistribution of metabolic fuels

may occur (Edens & Wade, 1983). One source of these may be fatty acids from WAT which are released in response to estradiol. Wade and Gray (1978) demonstrated that Estrogen receptors also occur in WAT so effects on these tissues may be direct. Ovariectomy has been found to increase WAT LPL activity in rats, an effect which is reversed by estradiol treatment (Edens & Wade, 1983; Gray & Greenwood, 1982; Gray & Wade, 1981; Hamosh & Hamosh, 1975; Ramirez, 1981). Edens and Wade (1983) also demonstrated that estradiol treatment decreased the incorporation of tritium into WAT fatty acids in rats. Application of estradiol to the hypothalamus--which does influence food intake--does not produce changes in WAT LPL activity (Nunez, Gray, & Wade, 1980). Estradiol also affects hepatic acetyl coenzyme A carboxylase (ACC) and fatty acid synthetase (FAS) (Gray & Greenwood, 1982), two rate limiting enzymes of lipogenisis, in WAT. Initially both enzymes decrease, but after one week return to control levels, and after two weeks are significantly elevated.

Wade and Gray (1979) and Ramirez (1980) have suggested that decreases in fat in WAT and increases in blood levels of triglycerides following estradiol treatment (Edens & Wade, 1983; Gray & Greenwood, 1982; Gray & Wade, 1981; Ramirez, 1980, 1981) may represent redistribution of fatty acid fuels. This suggestion is supported by the

findings of Ramirez (1980, 1981) that estradiol does not alter the absolute clearance rates of triglycerides but does increase the rate of triglyceride clearance by muscle compared to that by WAT. Other substances which may serve as fuels are also affected by ovariectomy and estradiol. Ahmed-Sorour and Baily (1981) reported increased gluconeogenisis and reduced glycogen deposition in liver, uterus, and muscle following ovariectomy. These effects were reversed with estradiol treatment. Gray and Greenwood (1982) did not find changes in levels of blood glucose, however. Gray and Greenwood (1982) and Ramirez (1980) established that estradiol treatment of ovariectomized rats results in an increase of plasma free fatty acids.

Several nonsteroidal "antiestrogens" have been shown to have estrogenic effects on food intake and body weight in rats. Roy and Wade (1976, 1977) found reduced food intake and body weight in ovariectomized rats in response to the administration of MER-25. The administration of MER-25 to the hypothalamus by Roy, Maass, and Wade (1977) produced similar results. King and Cox (1976) administered CI-628 peripherally, and Donohoe and Stevens (1982) administered it to the hypothalamus of ovariectomized rats. Both treatments produced reductions in food intake and body weight. Several groups have shown reduc-

tions in food intake and body weight in ovariectomized rats in response to peripheral (Gray and Wade, 1981; Ramirez, 1980; Wade and Blaustein, 1978) or central (T. Bartness, personal communication) administration of naf-oxidine. Bowman, Leake, Miller, and Morris (1981) and Bowman, Leake and Morris (1981) produced reductions in food intake and body weight in ovariectomized rats with peripheral administration of en-clomiphene. Martin (1981) produced body weight losses in rats with tamoxifen.

Roy and Wade (1977) used pair feeding to demonstrate that MER-25 produces weight losses which, like those pro-duced by estradiol, cannot be explained solely by changes in food intake. Gray and Wade (1981) established that nafoxidine reduces the amount of fat in ovariectomized rats. Nafoxidine has no effect on plasma free fatty acids (Ramirez, 1980) but, unlike estradiol, reduces levels of triglycerides (Gray & Wade, 1981; Ramirez, 1980). Like estradiol, nafoxidine does produce decreases in WAT LPL activity (Gray & Wade, 1981).

Tamoxifen is a triphenylethylene which is a potent estrogen antagonist, but which also shows some estrogenic effects (for review see Furr, et al, 1979). Wilking, Appelgren, Carlstrom, Pousette, and Theve (1982) found by autoradiography that tamoxifen concentrates in several tissues including BAT shortly after injection in mice.

Given this and the presence of estrogen receptors in BAT (Wade and Gray, 1978) it is possible that tamoxifen may affect BAT growth and thermogenesis. Martin (1981) showed that a high dose (5 mg/kg) of tamoxifen suppresses weight gain in ovariectomized rats, but unfortunately did not report food intake.

Different antiestrogens may produce different effects (see for example, Jordan, Clark, & Allen, 1981). At the time this study was begun there were indications that tamoxifen had only antiestrogenic effects (for example, Döhler, van zer Mühlen, and Döhler, 1977). It was thus possible that tamoxifen would not act like other anti-estrogens on food intake and body weight. Also, because tamoxifen is becoming commonly used in humans for treatment of cancer, effects of tamoxifen are of considerable interest.

The purpose of the present studies was to describe the effects of tamoxifen on food intake and various factors related to or affecting body weight and composition, to compare the effects of tamoxifen with those of estradiol, and to examine the effects of concurrent administration of tamoxifen and estradiol.

It is worth noting that tamoxifen and several other antiestrogens bind to a high-affinity, saturable binding

site (the nature and function of which is not yet known) in many estrogen target tissues (Sutherland, Murphy, Foo, Green, Whybourne, & Krozowski, 1980) and in many tissues which do not contain estrogen receptors (Sudo, Monsma, & Katzenellenbogen, 1983). This site has an extremely low affinity for estrogens (Sudo, et. al., 1983). Thus interpretation of results when antiestrogens are used may be difficult.

EXPERIMENT 1

The initial question asked was what effect, if any, tamoxifen alone has on food intake, body weight, and body composition in ovariectomized rats.

## Methods

### Animals

Thirty six female CD rats (Charles Rivers Breeding Laboratories, Wilmington, MA) were singly housed in hanging wire-bottom cages with room temperature approximately 22°C and with 12 hours of light per day. Purina Rodent Chow pellets (#5001) and tap water were available ad libitum. The rats were bilaterally ovariectomized under methoxyflurane anaesthesia (Metofane, Pitman-Moore) via two lateral incisions. Following ovariectomy the animals were divided into five groups (n=7 or 8) which were balanced for body weight and food intake and the groups were randomly assigned to experimental conditions. The animals weighed between 227 and 330 grams at the start of injections 25 days following ovariectomy.

### Drugs

Tamoxifen citrate (TAM)(Stuart Pharmaceutical, Wil-

mington, DE) was dissolved in sesame oil to produce a full dose in .1 ml.

## Procedure

The rats received daily, subcutaneous injections of TAM at doses of 0 ug, 10 ug, 50 ug, 250 ug, and 1000 ug per animal approximately six hours before lights out. At the time of injections body weight and food intake were measured. Spillage was collected on papers placed beneath the cages and weighed daily. Injections and measures of body weight and food intake continued for 35 days. On the morning following the last day of injections the animals were given an overdose of sodium pentobarbital (Nembutal), shaved, eviscerated, placed in preweighed aluminum foil baking pans, and weighed in preparation for carcass analysis. A modification (Gray & Wade, 1981) of the method of Leshner, Litwin, and Squibb (1972) was used to determine carcass composition. Water and fat were determined gravimetrically and protein content was determined by the method of Lowry, Rosebrough, Farr, and Randall (1951). The data from one animal receiving 250 ug of tamoxifen which died were dropped.

## Data analysis

Food intake was summed across the experimental period and the final body weight was calculated as the per cent

of the initial body weight. All data were analyzed by one-way analysis of variance followed by the Duncan's multiple range test using the Interactive Data Analysis Package (IDAP)(Wiedman & Hosmer, 1983). Results were considered significant for $p<.05$, two-tailed.

## Results

### Body weight

TAM significantly decreased the final body weight expressed as the percent of the initial body weight, $F(4,30)=28.49$, $p<.0001$, an effect which is similar to that reported for other antiestrogens and for estradiol. Percent weights of rats receiving 50 ug or 250 ug of TAM were lower than those of rats receiving oil or 10 ug of TAM (which did not differ) and per cent weights of rats receiving 1000 ug of TAM were lower than those of animals receiving oil, 10 ug, or 50 ug of TAM (see Figure 1A).

### Food intake

Total food intake was also significantly decreased by TAM, $F(4,30)=5.65$, $p<.002$. Rats receiving 250 ug or 1000 ug of TAM ate significantly less than those receiving oil or 10 ug (which did not differ). Rats receiving 50 ug of TAM ate less than those receiving oil but did not differ significantly from those receiving 10 ug of TAM (See
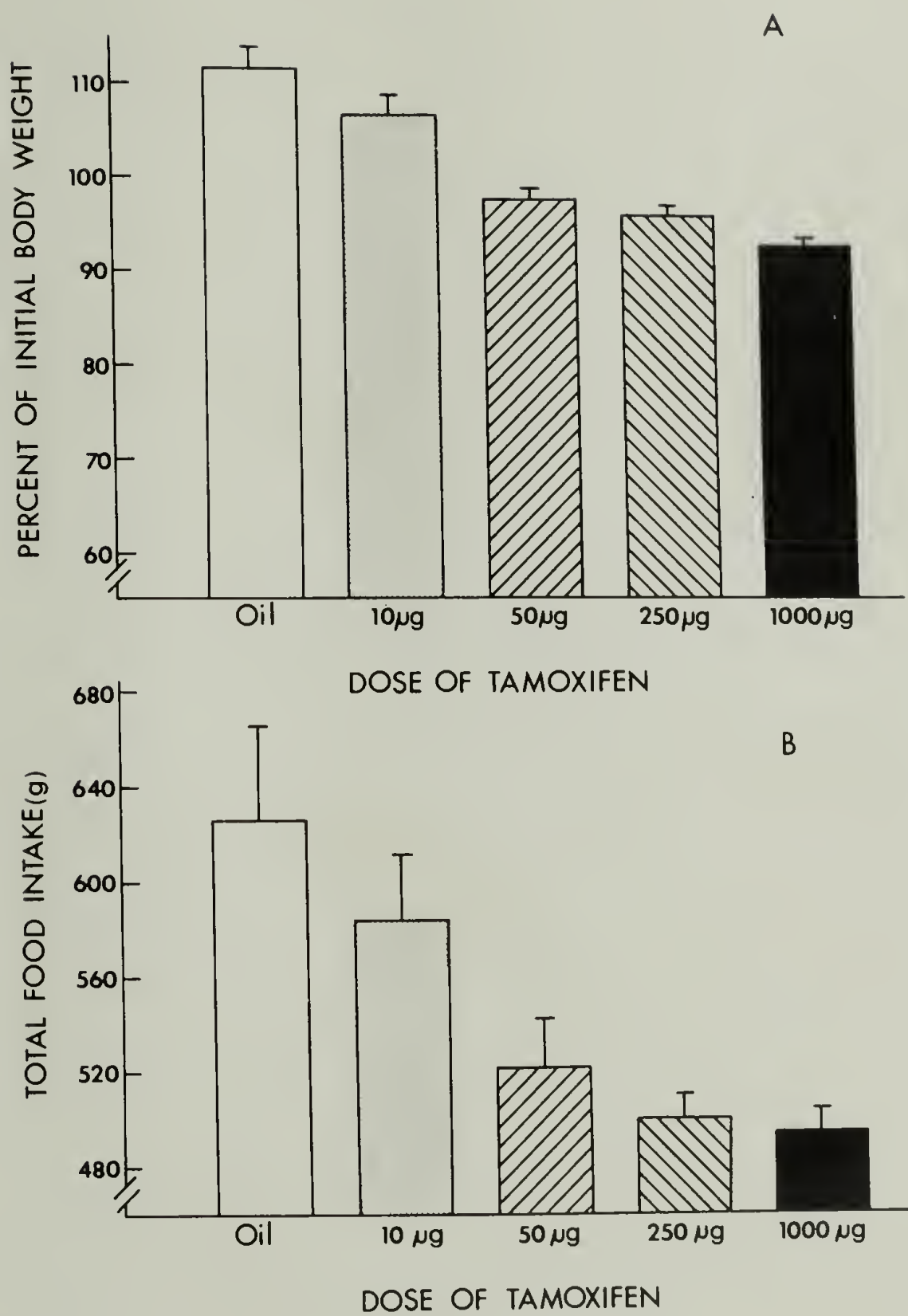
Figure 1. A. Effect of TAM on final body weight as percent of initial body weight (mean+standard error).  B. Effect of TAM on total food intake (mean+standard error).

Figure 1B).

## Carcass composition

Because of the differences in body weight among groups different ways of expressing the body components produced slightly different results. With the exception of protein the effects of TAM on carcass composition were similar to those previously reported for estrogens (See Table 1).

The effect of TAM on water expressed in grams was short of significance, $F(4,30)=2.06$, $p=.11$, although 1000 ug of TAM reduced grams of water relative to oil. The effect of TAM on water as a percent of wet weight was significant, $F(4,30)=3.92$, $p<.02$ with TAM at 50 ug, 250 ug, or 1000 ug producing an increase in percent water compared to oil or 10 ug of TAM (which did not differ significantly). TAM had a significant effect on fat whether expressed as grams, $F(4,30)=7.95$, $p<.001$, as per-cent of wet weight, $F(4,30)=5.08$, $p<.01$, or as percent of dry weight. $F(4,30)=6.08$, $p<.001$. In each case the oil and the 10 ug TAM groups had more fat than the other three groups.

The effect of TAM on protein was not significant regardless of how protein was expressed, although 1000 ug of TAM did produce an increase of protein expressed as a

percent of dry weight relative to the oil group.

Table 1. Rat carcass composition from Experiment 1 showing weight in grams, percent of carcass wet weight (wet percent), and percent of carcass dry weight (dry percent)(mean±standard error).

| | Dose of Tamoxifen | | | | |
| --- | --- | --- | --- | --- | --- |
| | 0 ug (n=7) | 10 ug (n=7) | 50 ug (n=7) | 250 ug (n=7) | 1000 ug (n=7) |
| Water | | | | | |
| grams | 174.7 ±5.3 | 168.0 ±4.2 | 161.9 ±3.2 | 157.8 ±3.0 | [a]150.8 ±1.7 |
| wet percent | 58.4 ±1.2 | 59.3 ±1.3 | [a]61.8 ±0.9 | [a]62.2 ±0.9 | [b]62.6 ±0.4 |
| Fat | | | | | |
| grams | 53.9 ±5.3 | 47.5 ±4.2 | [b]35.0 ±3.2 | [b]32.7 ±3.0 | [b]30.6 ±1.7 |
| wet percent | 18.1 ±1.4 | 16.7 ±1.3 | [b]13.3 ±1.0 | [b]12.9 ±1.1 | [b]12.7 ±0.6 |
| dry percent | 43.1 ±2.4 | 40.7 ±1.7 | [b]34.5 ±1.8 | [b]33.9 ±2.2 | [b]32.6 ±1.5 |
| Protein | | | | | |
| grams | 37.8 ±2.4 | 37.0 ±2.7 | 32.7 ±2.2 | 31.0 ±1.7 | 33.0 ±1.6 |
| wet percent | 12.7 ±0.3 | 13.0 ±0.6 | 12.5 ±0.8 | 12.2 ±0.7 | 13.7 ±0.6 |
| dry percent | 30.7 ±1.1 | 32.1 ±2.0 | 32.8 ±1.9 | 32.6 ±2.2 | [a]36.7 ±1.6 |

a. differs from 0 ug, $p<.05$.
b. differs from 0 ug and 10 ug, $p<.05$.

# CHAPTER III

## EXPERIMENT 2

Experiment 1 showed that TAM produced effects on food intake and on body weight and composition which are similar to those produced by other antiestrogens and by EB. The next step in describing the effects of TAM was to compare the effects of TAM with those of estradiol benzoate (EB) and to explore for antagonistic effects by the concurrent administration of TAM and EB. Because of reported effects of ovariectomy and estrogens on energy balance (Laudenslager, et al, 1980) resting oxygen consumption was measured as were the effects of TAM and EB on WAT and on BAT. Uterine weight was used as a bioassay for the antiestrogenic activity of TAM as TAM alone produces only slight increases in uterine weight and TAM antagonizes EB-induced increases in uterine weight (Harper & Walpole, 1967).

## Methods

### Animals

Animals were treated as in Experiment 1 except as noted below. Following ovariectomy the animals were divided into four groups (n=9) which were balanced for

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body weight and food intake, and the groups were randomly assigned to experimental conditions. The animals weighed between 221 and 282 grams at the start of injections 16 days following ovariectomy.

## Drugs

Tamoxifen citrate (Stuart Pharmaceutical, Wilmington, DE) and estradiol benzoate (EB, Schering Corporation, Bloomfield, NJ) were dissolved in sesame oil to produce a full dose in .1 ml.

## Procedure

The animals received daily, subcutaneous injections of oil, 2 ug EB, 250 ug TAM, or 250 ug TAM plus 2 ug EB (TAM+EB) per animal approximately six hours before lights-out. Daily measures of food intake and body weight were made at this time.

The animals were divided into three groups composed of equal numbers of animals from each experimental group. Injections began on three consecutive days and all measures were recorded in terms of number of days of injections.

Prior to initiation of injections and throughout this experiment food was removed from the animals approximately six hours prior to lights-out and returned one hour prior to lights-out. Food was available ad libitum at all other

times.   Water was available ad libitum at all times

throughout the experiment.

Prior to initiation of the injections all animals

were adapted to the Plexiglas chambers used for measuring

oxygen consumption for 30 minutes per day for seven days.

Oxygen consumption was measured between one and three

hours prior to lights out.   Resting oxygen consumption

was measured by placing a conscious animal in an airtight

Plexiglas chamber  connected by tubing to an Applied Elec-

trochemistry S3-A oxygen analyzer.  Air passed through the

chamber, through soda lime to remove carbon dioxide, and

through calcium sulfate (Dririte) to remove water before

entering the analyzer.  A flow rate of 750 ml/min was used

and the analyzer was calibrated to 20.94% oxygen for room

air.  The percent of oxygen in the chamber air was read

from the oxygen analyzer.  The animal was in the chamber

for five minutes before readings were taken to prevent

interference by room air.  The values used for calcula-

tions were the two highest values obtained (minimum con-

sumption) with the animals resting with the requirements

that a period of activity occurred between the readings

and that they did not differ by more than .05 units.  The

mean of these two values was subtracted from 20.94 and the

result multiplied by 7.5 to determine absolute consumption

in ml oxygen/min. This value was divided by the body weight in kg raised to the .75 power.

On the day before injections began and on the final day of injections the animals were anaesthetized (Metofane, Pitman-Moore) and the naso-anal length was determined with the animals stretched by a 135 g weight. On the morning following the last day of injections, the animals were given an overdose of sodium pentobarbital (Nembutal) and prepared for carcass analysis as described in Experiment 1. At this time parametrial white adipose tissue, intrascapular brown adipose tissue, and uterus were removed and weighed to the nearest .1 mg. The weight of the WAT was added to the carcass wet and dry weights and to the lipid weight for each animal. One animal in the EB group died during the night following the last injection. Data from this animal for food intake, body weight, naso-anal length, and oxygen consumption are included in the results, but data on body components were not obtained.

WAT and BAT were homogenized and frozen for later analysis of LPL activity by a modification (Hietanen & Greenwood, 1977; Gray & Wade, 1981) of the method of Schotz, Garfinkel, Huebotter, and Stewart (1970). An aliquot of homogenized BAT solution was placed in 5 ml ethanol for subsequent protein and DNA determination. Protein

was measured by the method of Lowry, et. al. (1951) and DNA by the method of Burton (1956).

## Data analysis

Total food intake, final body weight expressed as per cent of initial body weight, and carcass composition were calculated and expressed as in Experiment 1. The data were analyzed by one-way analysis of variance followed by the Duncan's multiple range test using IDAP (Wiedman & Hosmer, 1983), except oxygen consumption which was analyzed with a repeated measures ANOVA using BMDP4V, and Lee index which was analyzed with the Kruskal-Wallis test using IDAP. A value of $p < .05$, two-tailed, was considered significant.

## Results

## Body weight

As had been expected treatment significantly reduced the final body weight expressed as percent of initial body weight, $F(3,32) = 77.06$, $p < .0001$. Percent weights of rats receiving TAM or TAM+EB were lower than those of rats receiving EB or oil. Percent weights of rats receiving EB were significantly lower than were those of rats receiving oil only (Figure 2A). The weights of all three groups receiving drugs were reduced by the fourth day of injec
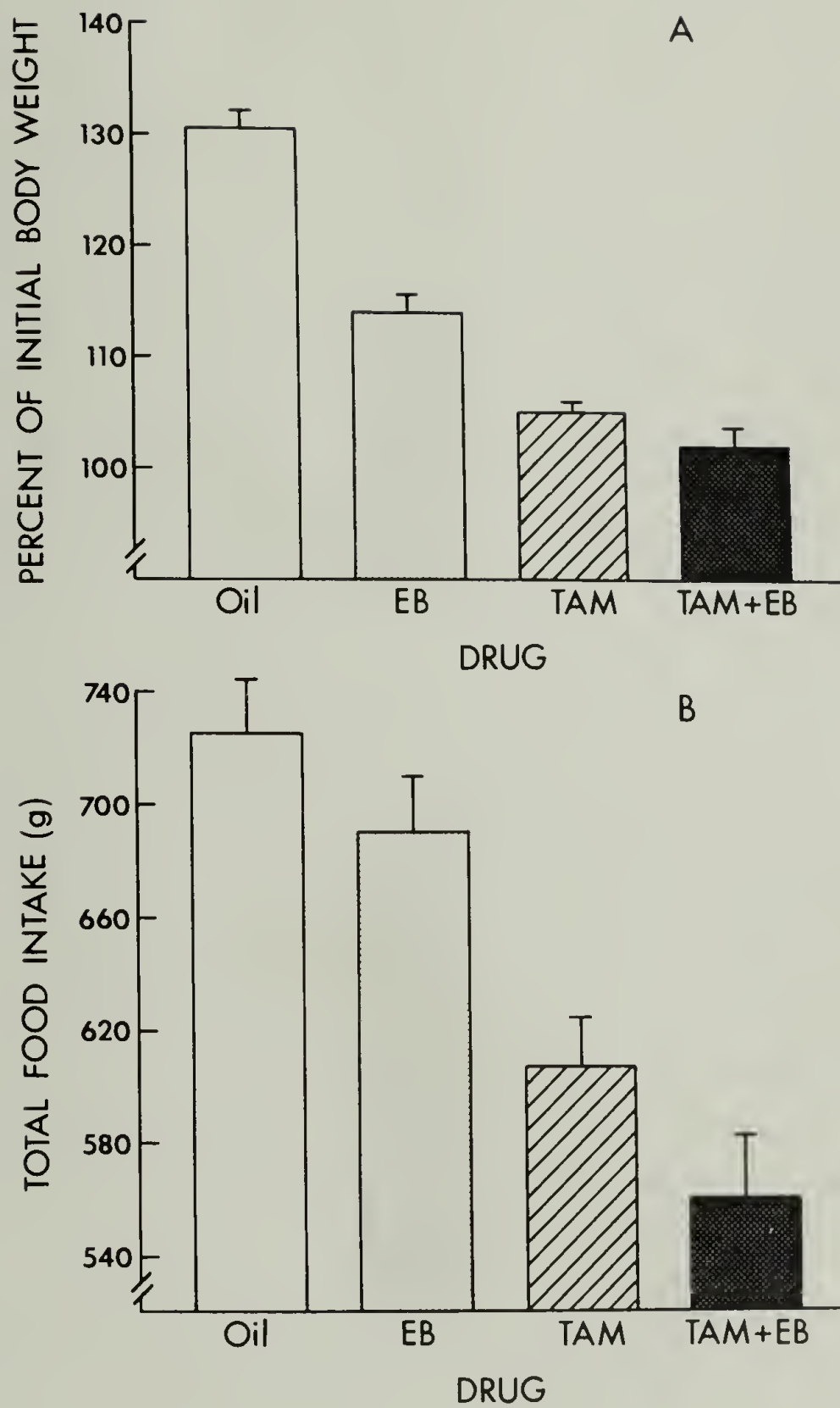
Figure 2. A. Effects of drugs on final body weight as percent of initial body weight (mean+standard error). B. Effects of drugs on total food intake (mean+standard error).
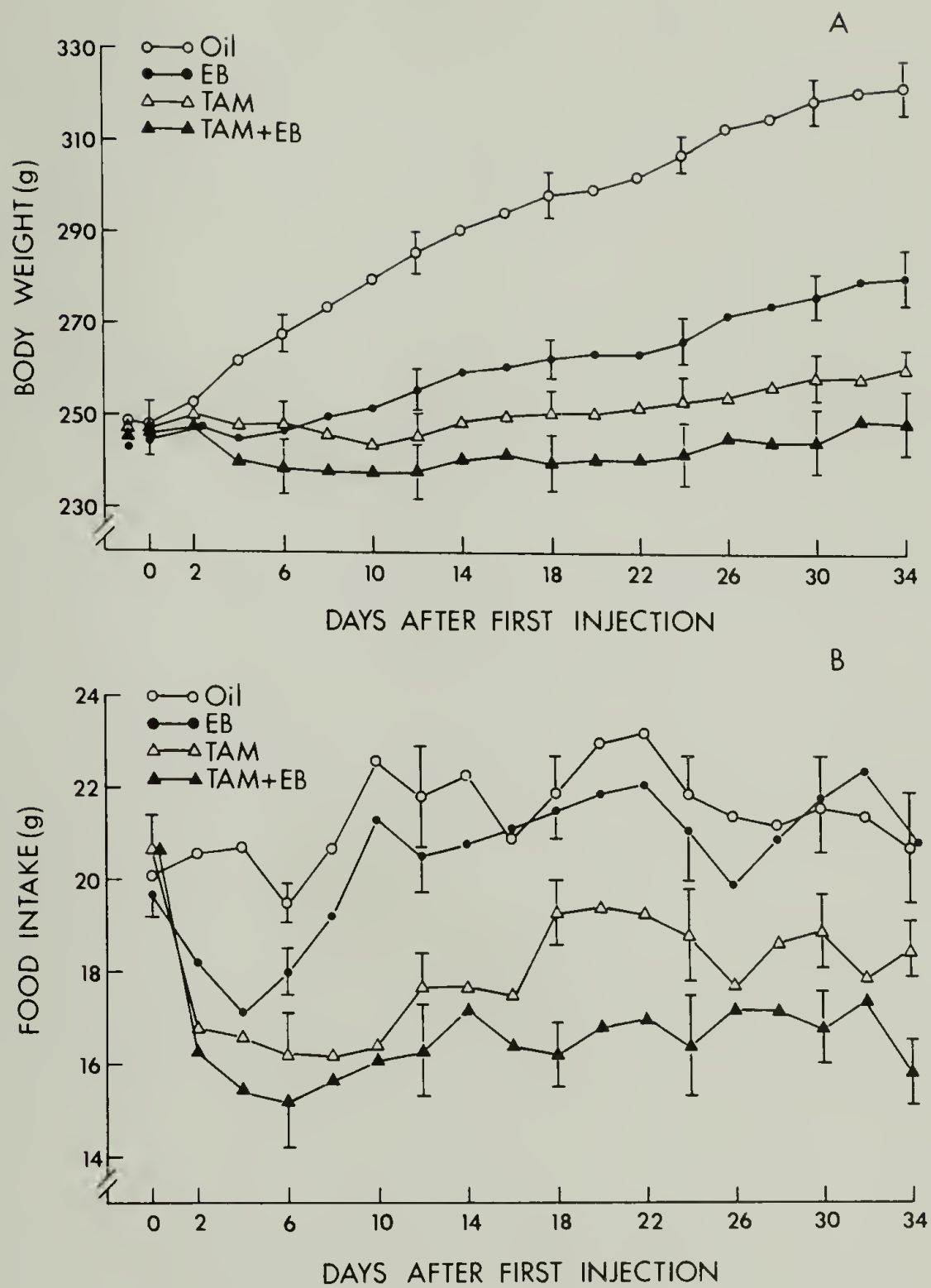
Figure 3. A. Effects of drugs on body weight (mean±standard error). B. Effects of drugs on 24 hour food intake (mean±standard error).

tions and the groups showed similar patterns of change although the EB group began to gain weight several days before the TAM or TAM+EB groups began to regain weight (Figure 3A).

## Food intake

Treatment also significantly reduced total food intake, $F(3,32)=14.34$, $p<.001$. TAM and TAM+EB both reduced food intake relative to EB or oil. EB did not produce the expected significant effect on total food intake relative to oil (Figure 2B). TAM and TAM+EB produced immediate reductions of food intake which continued for the duration of the injections. Animals receiving EB showed an immediate, transient reduction of food intake which lasted about six days and then increased food intake to that of controls (Figure 3B).

## Carcass composition

Because of the differences of body weights among the various groups the way in which the carcass components were expressed affected the results. Both TAM and EB produced nonsignificant effects on water and fat in the expected direction. Concurrent administration of these drugs increased these effects (Table 2).

Treatment produced a significant effect on grams of water, $F(3,31)=20.79$, $p<.0001$, with the EB group reduced

Table 2.  Rat carcass composition from Experiment 2 showing weight in grams, percent of carcass wet weight (wet percent), and percent of carcass dry weight (dry percent)(mean±standard error).

|  | Drug | | | |
|---|---|---|---|---|
|  | Oil (n=9) | EB (n=8) | TAM (n=9) | TAM+EB (n=9) |
| **Water** | | | | |
| grams | 169.7 ±2.9 | 151.3 ±3.2 | [b]140.1 ±2.7 | [b]137.4 ±4.0 |
| wet percent | 60.6 ±0.7 | 62.0 ±0.9 | 62.4 ±0.6 | [a]63.9 ±0.4 |
| **Fat** | | | | |
| grams | 39.1 ±2.2 | [a]29.1 ±3.2 | [a]26.8 ±2.2 | [b]21.0 ±1.7 |
| wet percent | 13.9 ±0.7 | 11.8 ±1.2 | 11.9 ±1.0 | [a] 9.7 ±0.7 |
| dry percent | 35.2 ±1.2 | 30.8 ±2.2 | 32.6 ±2.4 | [a]28.2 ±2.4 |
| **Protein** | | | | |
| grams | 54.6 ±1.9 | [a]46.6 ±1.0 | [a]45.3 ±1.4 | [c]38.2 ±2.7 |
| wet percent | 19.5 ±0.5 | 19.1 +0.2 | 20.2 ±0.5 | 22.7 ±3.7 |
| dry per cent | 49.5 ±1.1 | 50.4 ±1.3 | 53.7 ±1.5 | 50.4 ±2.9 |

a. differs from oil, $p<.05$.
b. differs from oil and EB, $p<.05$.
c. differs from oil, EB, and Tam, $p<.05$.

compared to the oil group and with the TAM and TAM+EB groups reduced relative to both the EB and the oil groups. When water was expressed as per cent of carcass wet weight treatment produced a significant effect, $F(3,31)=4.54$, $p<.01$, with only the TAM+EB group showing an increase in water compared to the oil group.

Treatment also produced significant effects on fat expressed as grams, $F(3,31)=10.71$, $p<.0001$, and as per cent of carcass wet weight, $F(3,31)=3.87$, $p<.02$, but not on fat expressed as per cent of dry weight. Grams of fat were reduced relative to oil rats in those receiving EB or TAM, and were reduced relative to oil or EB rats in those receiving TAM+EB. The TAM+EB group also showed reductions relative to the oil group in fat expressed as per cent of carcass wet weight and of dry weight.

Protein was significantly affected by treatment only when expressed in grams, $F(3,31)=12.98$, $p<.0001$. Rats receiving TAM or EB showed decreases relative to the oil group while those receiving TAM+EB showed reductions relative to each of the other groups.

Body length and Lee index

There were no differences in initial body lengths among treatments, but all drug treatment groups showed reduced final body lengths compared to the oil group,

Table 3. Body length, Lee index, and uterine weight of rats from Experiment 2 (mean±standard error).

| | Drug | | | |
|---|---|---|---|---|
| | Oil (n=9) | EB (n=9) | TAM (n=9) | TAM+EB (n=9) |
| Body length | | | | |
| initial (mm) | 212.3 ±1.1 | 210.1 ±2.0 | 210.4 ±1.1 | 209.8 ±2.2 |
| final (mm) | [a]226.2 ±0.8 | 220.0 ±1.1 | 218.1 ±2.0 | 217.0 ±2.3 |
| Lee index | | | | |
| initial | 295.3 ±2.8 | 297.8 ±2.3 | 297.7 ±1.2 | 297.5 ±2.1 |
| final | [a]302.7 ±1.4 | [b]297.0 ±1.7 | 292.3 ±1.8 | 289.3 ±1.6 |
| Uterine weight[e] (mg) | 572.3 ±58.4 | [c]1195.0 ±51.7 | 611.2 ±22.1 | [d]759.8 ±40.0 |

a. greater than EB, TAM, and TAM+EB, p<.05.
b. greater than TAM and TAM+EB, p<.05.
c. greater than oil, TAM, and TAM+EB, p<.05.
d. greater than oil and TAM, p<.05.
e. n=8 for EB group.

$F(3,32)=6.04$, p<.01 (Table 3). Lee indices also were not different before injections began but were different fcllowing treatment, Kruskal-Wallis $H(3)=20.32$, p<.0001, with the oil group greater than the three groups receiving drugs and the EB group greater than the TAM or TAm+EB groups (Table 3). These results indicate that both TAM and EB tend to reverse the ovariectomy-induced obesity.

## Uterine weight

Uterine weight was significantly affected by treat-

ment, $F(3,31)=38.32$, $p<.0001$. The TAM group and the oil group did not differ significantly, the TAM+EB group was heavier than both, and the EB group was considerably heavier than the other three groups (Table 3). These results indicate that TAM was active as an antiestrogen in at least one tissue.

## White adipose tissue

The effects of TAM and of EB on parametrial WAT were similar. Concurrent administration of the drugs increased these effects. The weight of parametrial WAT was significantly affected, $F(3,31)=3.91$, $p<.02$, with the weights in both the TAM and the TAM+EB groups reduced compared to the oil group (Table 4). WAT expressed as per cent of

Table 4. Parametrial white adipose tissue results for rats in Experiment 2 (mean±standard error).

| | Oil (n=9) | EB (n=8) | TAM (n=9) | TAM+EB (n=9) |
|---|---|---|---|---|
| White Adipose Tissue | | | | |
| weight (grams) | 3293.9 ±276.9 | [a]1862.6 ±224.5 | [a]1532.6 ±315.0 | [b] 918.1 ±315.0 |
| per cent of body weight | 1.016 ±0.080 | [a]0.662 ±0.084 | [a]0.583 ±0.112 | [b]0.366 ±0.034 |
| LPL activity (umol FFA/h/mg protein) | 0.519 ±0.109 | [a]0.194 ±0.042 | 0.255 ±0.151 | [a]0.075 ±0.025 |

a. differs from oil, $p<.05$.
b. differs from oil and EB, $p<.05$.

body weight did not show significant differences. WAT LPL activity was significantly affected, $F_{(3,31)}=3.69$, $p<.03$, with both the EB group and the TAM+EB group significantly reduced   compared to the oil group and the TAM group showing a nonsignificant reduction.

## Brown adipose tissue

EB produced no effects in BAT. TAM produced increases in BAT weight and protein which were blocked or

Table 5. Brown adipose tissue values for rats in Experiment 2 (mean±standard error).

| | Drug | | | |
|---|---|---|---|---|
| | Oil (n=9) | EB (n=8) | Tam (n=9) | Tam+EB (n=9) |
| **Brown Adipose Tissue** | | | | |
| weight (grams) | 403.3 ±51.7 | 382.8 ±42.6 | 426.3 ±37.0 | 298.2 ±35.4 |
| per cent of body weight | 0.124 ±0.015 | 0.134 ±0.013 | b0.164 ±0.015 | 0.118 ±0.011 |
| protein (mg/pad) | 38.1 ±4.8 | 39.4 ±5.5 | b47.9 ±4.3 | 32.3 ±4.2 |
| protein (ug/mg BAT) | 95.6 ±4.8 | 101.0 ±5.0 | a112.0 ±5.0 | 108.2 ±4.3 |
| DNA (ug/pad) | 834.5 ±135.3 | 894.9 ± 95.6 | 968.9 ±141.7 | 827.0 ±100.1 |
| DNA (ug/mg BAT) | 2.000 ±0.125 | 2.379 ±0.190 | 2.279 ±0.262 | a2.866 ±0.243 |
| LPL activity (umol FFA/h/mg protein) | 0.131 ±0.023 | 0.153 ±0.008 | 0.113 ±0.009 | 0.139 ±0.015 |

a. differs from oil, $p<.05$.
b. differs from Tam+EB, $p<.05$.

attenuated by EB. The weight of BAT did not differ signi-
ficantly among groups and the effect of treatment on the
weight of BAT expressed as per cent of body weight fell
slightly short of significance, $F(3,31)=2.33$, $p=.09$, al-
though the TAM group was elevated compared to the TAM+EB
group in BAT as     per cent of body weight (Table 5).
Similar results occurred for mg of protein per BAT pad,
$F(3,31)=1.87$, $p=.16$, with the TAM group elevated relative
to the TAM+EB group. Protein as ug per mg of BAT was also
short of significance, $F(3,31)=2.41$, $p=.09$ with the TAM
group elevated relative to the oil group. The ug of DNA
per pad did not differ significantly among groups, however
the ug of DNA per mg BAT did, $F(3,31)=3.12$, $p<.05$, with
the TAM+EB group elevated compared to the oil group.
There were no significant differences in LPL activity
among the groups.

## Oxygen consumption

While EB produced the expected increase in oxygen
consumption on the first day after injections began this
group then showed reduced oxygen consumption. This result
is quite different from those of Laudenslager, et al.
(1980, 1982) who found continued elevation of oxygen con-
sumption in response to estradiol. Drug treatment did not
have a significant effect on oxygen consumption as a
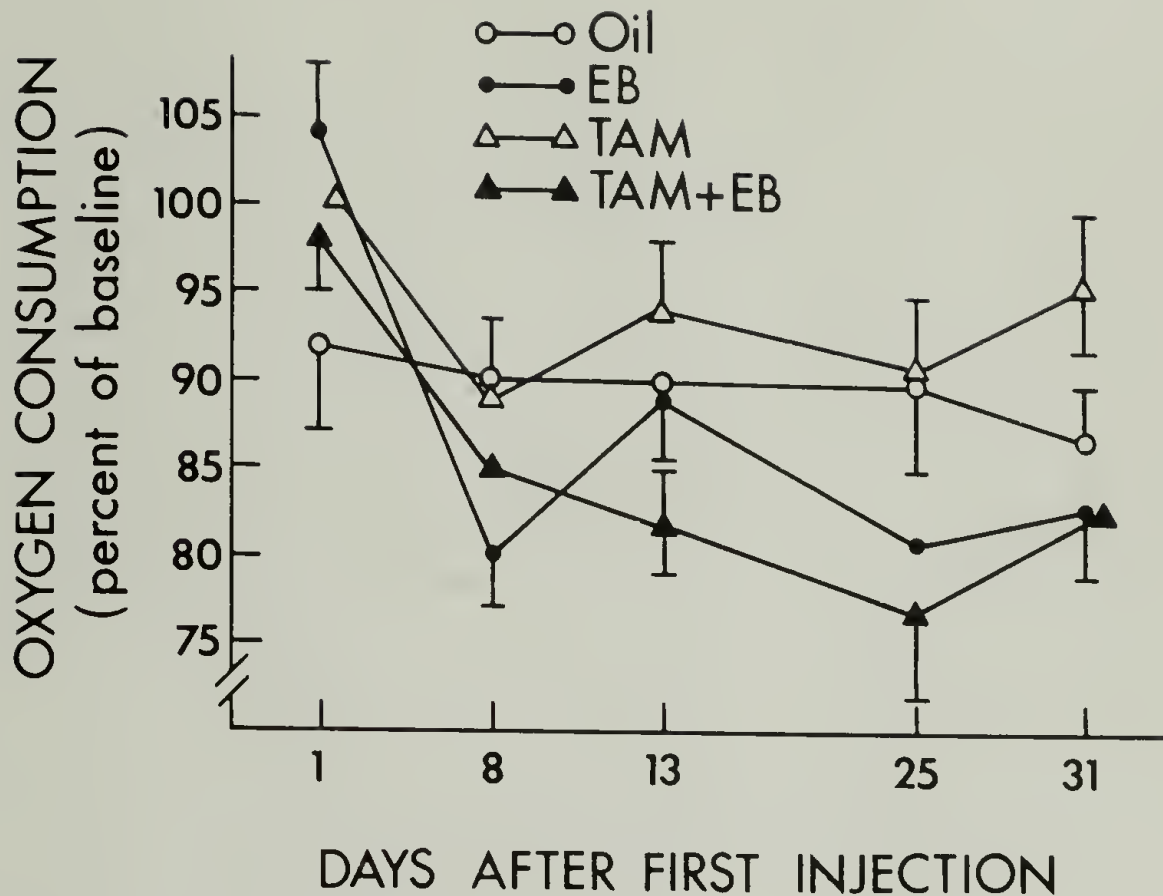
Figure 4. Effect of drugs on oxygen consumption
(ml $O_2$/min/kg$^{.75}$) as percent of baseline
(mean±standard error).

percent of baseline values (using ml $O_2$/min/kg$^{.75}$), but

there was a decrease in oxygen consumption over days,

$F(4,128)=17.00$, $p<.0001$. The time by drug interaction was

significant, $F(12,128)=2.76$, $p<.01$. The interaction was

due to an increase on the first day after injections began

in the EB, TAM, and TAM+EB groups

relative to the oil group with both the EB and the TAM+EB

groups decreasing subsequently. The TAM group stayed at

or above the level of the oil group throughout (Figure 4.)

CHAPTER IV

EXPERIMENT 3

Because of the unexpected effect of EB on oxygen consumption in Experiment 2 it was decided to compare the effects of brief daily food deprivation with those of ad libitum feeding on various measures in ovariectomized animals receiving oil, EB, or TAM.  Because both EB and antiestrogens have been shown to affect utilizable fuels in blood, blood was collected for analysis.

## Methods

### Animals

Animals weere treated as in Experiments 1 and 2 except as noted below.  Following ovariectomy the animals were divided into six groups (n=5 or 6) which were balanced for body weight and food intake and the groups were randomly assigned to experimental conditions.  The animals weighed between 351 and 468 grams at the start of injections 90 days after ovariectomy.

### Drugs

Tamoxifen citrate (Stuart Pharmaceutical, Wilmington, DE) and estradiol benzoate (EB, Shering Corporation,

Bloomfield, NJ) were dissolved in sesame oil to produce a full dose in .1 ml.

## Procedure

Animals each received either oil, 2 ug EB, or 250 ug TAM. Three groups, each receiving one of the above treatments, received food ad libitum throughout the study, while the other three groups had food removed approximately six hours before lights-out and returned approximately one hour before lights-out starting on the first day of injections. Water was available ad libitum at all times throughout the experiment. Injections were given daily approximately six hours before lights-out, and every third day body weight and food intake were measured at this time.

The animals were divided into three groups composed of equal numbers of animals from each experimental group. Injections began on three consecutive days and all measures were recorded in terms of number of days of injections.

Prior to initiation of the injections, all animals were adapted to the Plexiglas chambers used for measuring oxygen for 30 minutes per day for seven days.

Resting oxygen consumption was measured as described in Experiment 2 every third day beginning two days before

the start of injections. Three days after the last oxygen measurement and at the same time of day as testing the animals were decapitated and blood was collected. The blood was centrifuged and the serum prepared appropriately for the various analyses and then frozen for later analysis.

Plasma glucose was analyzed colorimetrically using Sigma Kit #635. Total glycerides were assayed fluoro-metrically by the method of Buccolo and David (1973). Free fatty acids were measured colorimetrically by the method of Soloni and Sardinia (1973). Serum albumin was measured colorimetrically using a modification of the method of Rodkey (1964).

Data analysis

Total food intake and final body weight expressed as a percent of initial body weight were calculated and expressed as in Experiment 1. The above data and the blood measures were analysed by two-way analysis of variance (drug (3) x feeding (2)) followed by the Duncan's multiple range test with IDAP (Wiedman & Hosmer, 1983). Oxygen consumption expressed as per cent of baseline was analyzed by a mixed analysis of variance (drug (3) x feeding (2) x repeated measure (4)) using BMDP4V. A value of $p < .05$, two-tailed, was considered significant.

<div align="center"><u>Results</u></div>

## <u>Body weight</u>

Both oil and EB combined with the five-hour food deprivation to produce unexpected results. Deprivation slightly reduced body weight in the oil group and attenuated the EB-induced reduction of weight. A significant main effect of drug on final body weight expressed as percent of initial body weight was found, $F(2,28)=20.00$, $p<.0001$ with TAM reducing percent body weight compared to EB and to oil and with EB reducing per cent body weight compared to oil. There was no effect of feeding condition. The drug x feeding condition interaction approached significance, $F(2,28)=2.80$, $p<.08$. Both TAM groups were lighter than both oil groups and the EB restricted group, the EB <u>ad lib</u> groups was lighter than both oil groups, and the EB restricted group was lighter than the oil <u>ad lib</u> group (Figure 5A).

## <u>Food intake</u>

Five-hour food deprivation produced a striking and inexplicable reduction of food intake in the oil group and eliminated or attenuated the reduction expected in the EB group. It did not affect feeding in the TAM group. There was a significant main effect of drug on total food intake
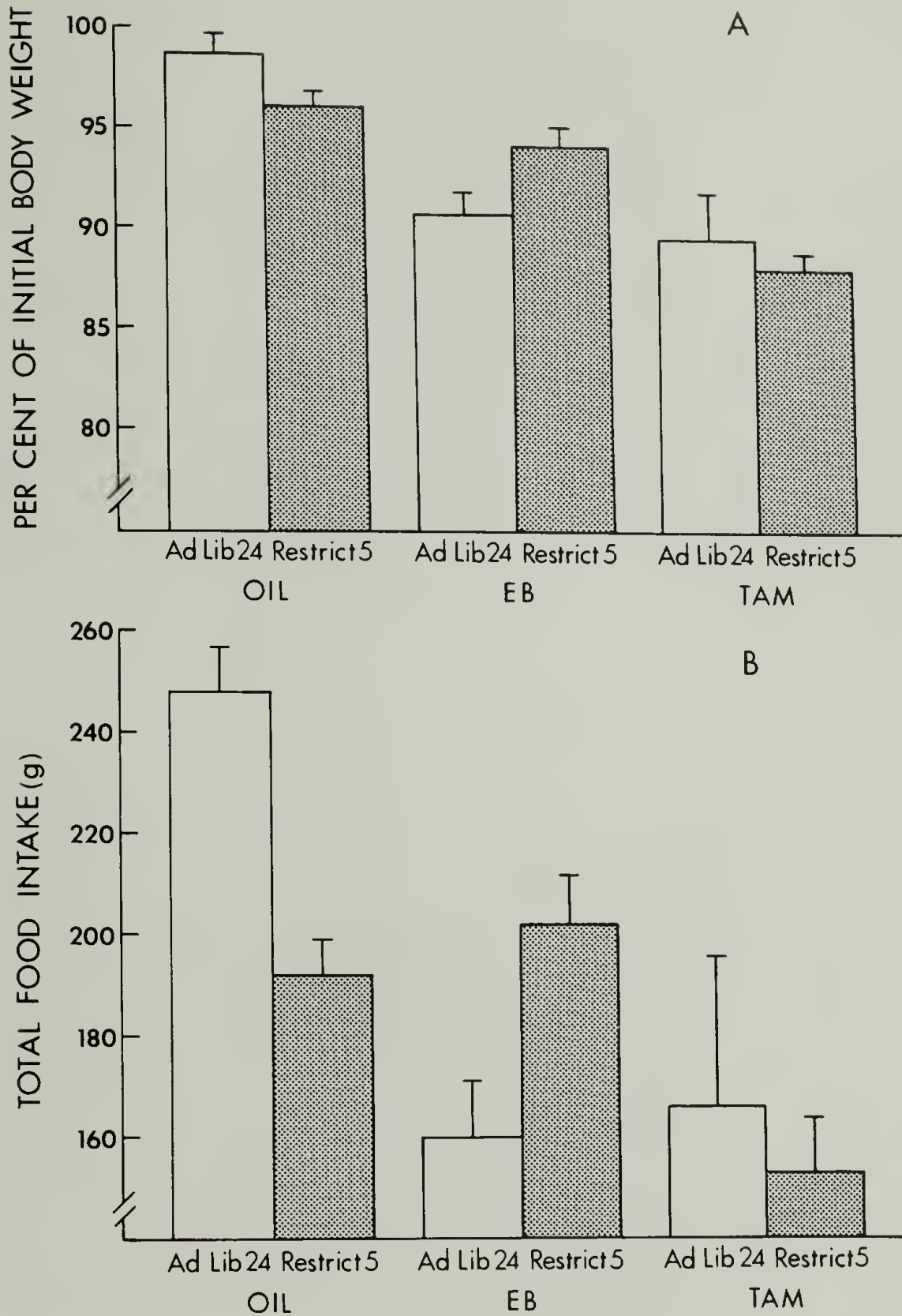
Figure 5. A. Effects of drug and feeding condition on final body weight as percent of initial body weight (mean+standard error). B. Effects of drug and feeding condition on total food intake (mean+standard error).

$F(2,28)=7.08$, $p<.01$, with both TAM and EB reducing food intake relative to oil, but not differing significantly from each other. There was no effect of feeding condition. There was a significant drug x food interaction, $F(2,28)=4.72$, $p<.02$ with the oil _ad lib_ group eating significantly more than all other groups except the EB restricted group which did not differ significantly from any of the groups (see Figure 5B).

## Oxygen consumption

There was no main effect of either drug or feeding condition on oxygen consumption expressed as a per cent of baseline and the drug x feeding condition interaction also was not significant. There was a significant effect of time, $F(3,84)=4.80$, $p<.004$, but none of the possible interactions with drug or feeding condition were significant. The patterns of oxygen consumption of the food restricted groups did resemble those seen in Experiment 2 (Figure 6) while the pattern shown by the EB _ad lib_ group more closely resembled the results found by Laudenslager, et. al. (1980, 1982).

## Blood analysis

The main effect of drug on glucose fell short of significance, $F(2,28)=2.60$, $p=.09$. The main effect of feeding condition on glucose and the drug x feeding condi-
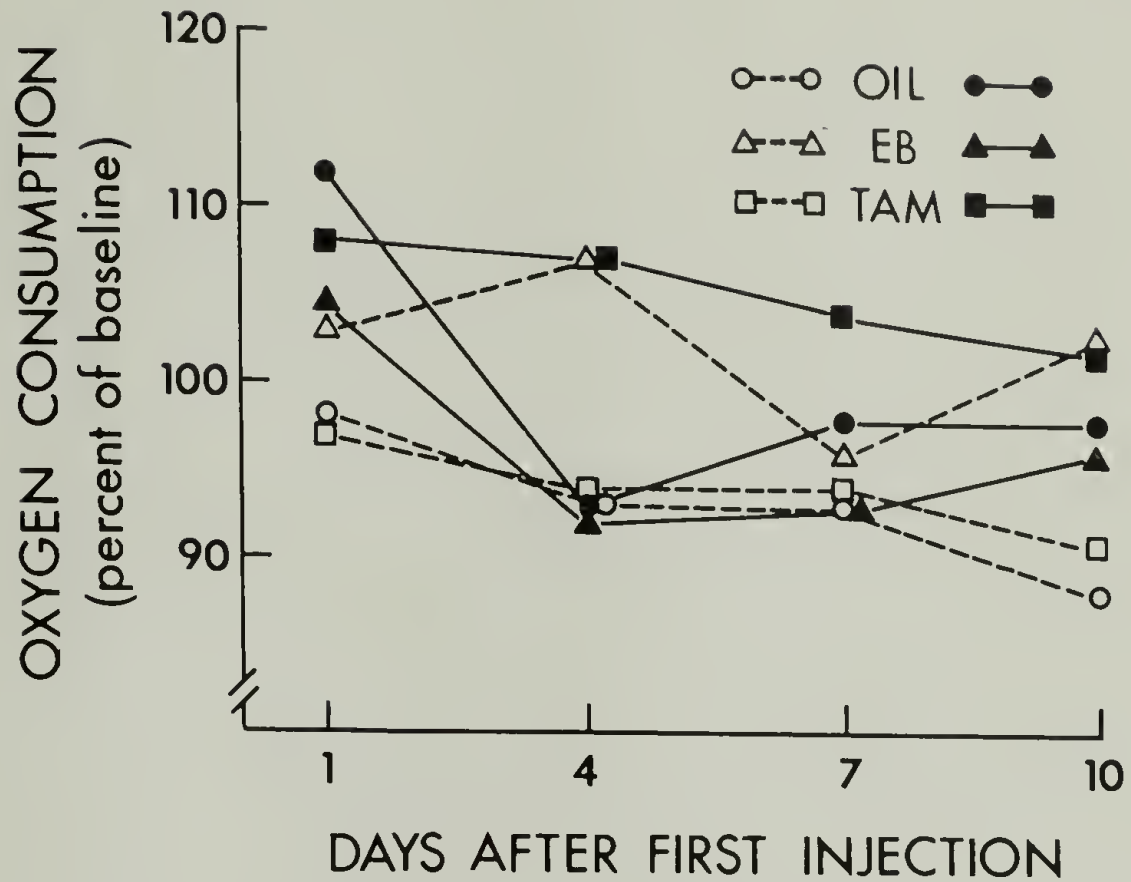
Figure 6. Effects of drugs and feeding condition on oxygen consumption (ml $O_2$/min/kg$\cdot^{75}$) as percent of baseline (mean)(standard errors omitted for clarity). Solid symbols represent food-deprived groups and open symbols represent ad lib. groups.

tion interaction were not significant (Table 6). The drug main effect on free fatty acids was not significant, but feeding condition did produce a significant effect, $F(2,28)=7.50$, $p<.02$, with restriction elevating free fatty acids. The drug x feeding condition interaction was not significant (Table 6). There were no significant effects on total glycerides (Table 6). The drug main effect on serum albumin was significant, $F(2,28)=80.00$, $p<.0001$,

Table 6.   Analysis of blood of rats from Experiment 3 showing glucose, free fatty acids (FFA), total glycerides (TG)(triglycerides plus free glycerol), and serum albumin (mean±standard error).

|  | Oil | | EB | | TAM | |
|---|---|---|---|---|---|---|
|  | ad lib (n=5) | rest. (n=5) | ad lib (n=6) | rest. (n=6) | ad lib (n=6) | rest. (n=6) |
| Glucose (mg/100ml) | 135.5 ±9.8 | 132.6 ±10.3 | 109.4 ±6.9 | 118.7 ±10.1 | 109.4 ±6.0 | 123.0 ±11.5 |
| FFA (umol/ml) | 0.441 ±0.070 | 0.535 ±0.078 | 0.449 ±0.078 | a0.708 ±0.071 | 0.478 ±0.086 | 0.607 ±0.050 |
| TG (umol/ml) | 2.29 ±0.49 | 1.80 ±0.30 | 1.86 ±0.24 | 2.26 ±0.36 | 1.96 ±0.43 | 1.80 ±0.32 |
| Albumin (mg/ml) | 26.9 ±1.3 | 28.9 ±0.5 | b37.7 ±0.9 | b39.1 ±0.8 | 28.0 ±1.0 | 27.4 ±1.0 |

a.   greater than all _ad lib_ groups, p<.05.
b.   greater than both oil and both TAM groups, p<.05.

with estradiol increasing albumin above the levels of the oil and TAM groups.  The main effect of feeding condition was not significant and the drug x feeding condition interaction was not significant (Table 6).

# CHAPTER V

## DISCUSSION

TAM when given alone produced effects on food intake, body weight, and body composition which are similar to those produced by EB as has been reported for other anti-estrogens (for example, Gray & Wade, 1981). The 50 ug dose of TAM produced nearly maximal effects with the administration of 5 times (250 ug) or 20 times (1000 ug) this dose producing only small additional effects.

The data from these experiments are consistent with TAM's acting via the estrogen receptor. Since both TAM and EB bind to the estrogen receptor, when both produce similar effects the simplest explanation is that both are acting via the estrogen receptor. However, with the exception of effects in which concurrent application of TAM and EB blocked effects of TAM, the role of a specific antiestrogen binding substance cannot be ruled out by these experiments. In Experiment 2 concurrent administration of EB and TAM blocked a slight increase in protein as a percent of carcass dry weight produced by TAM, blocked the increase of BAT weight as percent of body weight produced by TAM, and attenuated the increase in ug protein per mg BAT produced by TAM.

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Concurrent administration of TAM and EB produced nearly additive effects on food intake, water as percent of body weight, fat as percent of body weight, and Lee index. If both TAM and EB were maximally potent alone, this would imply that they were operating through separate mechanisms. The food restriction in Experiment 2 virtually eliminated the EB effect on food intake. All the measures just mentioned appear to be affected by intake, the additivity may be a result of the attenuation of the EB effects. Several measures--naso-anal length, parametrial WAT weight in grams and as percent of body weight, and WAT LPL activity--which appear to be independent of food intake did not show this additivity.

In the ad lib. conditions of Experiment 3, TAM and EB had virtually identical effects on food intake and on body weight. The five hour food restriction attenuated (Experiment 2, Figure 2) or eliminated (Experiment 3, Figure 5) the reductions in food intake and body weight by EB but did not alter the decreases produced by TAM. The five hour food restriction did not eliminate the effects of EB on various measures relating to body weight and composition (e.g. body weight, parametrial WAT weight, WAT LPL activity, body length). Bowman, Leake, and Morris (1982) found that .7 mg/kg of TAM (about 250 ug in a 350 g rat) produced no increase in cell nuclear estrogen receptors in

the hypothalamus of rats although this dose did produce increases in estrogen receptors in pituitary and in uterus. One possible explanation of the differences in EB effects is that the five-hour food restriction altered effects in the hypothalamus but did not directly affect the periphery of the body. This possibility is strengthened by the finding by Nunez, et al. (1980) that hypothalamic implants of EB--which do decrease food intake--did not affect WAT LPL activity, essentially the obverse of the results in Experiment 2.

The results of Experiments 2 and 3 can aid in dissociating some effects of EB from its effect on food intake. In Experiment 3 the food intake and body weight of the ad lib. EB group were significantly reduced compared to the ad lib. oil group and were essentially the same as those of the ad lib. TAM group. In Experiment 2 the food intake of the EB group did not differ significantly from the oil group, but the body weight of the EB group was significantly reduced. This suggests that factors other than food intake were involved in the EB-induced reduction of body weight as has been previously demonstrated by Roy and Wade (1977) and Mueller and Hsaio (1980).

The effects of EB on parametrial WAT weight and WAT

LPL activity were very similar to those of TAM which suggests that they are independent of food intake. The effect of EB on naso-anal length was also similar to TAM as well as TAM+EB suggesting independence from food intake. Mueller and Hsaio (1981) estimated that about 40 percent of the increased weight resulting from ovariectomy was associated with skeletal growth. Thus effect on WAT and on body growth may account for much of the weight change not related to food intake.

The effects of EB and TAM on resting oxygen consumption are quite different. Animals which were food restricted in Experiments 2 and 3 and received EB showed and initial increase in oxygen consumption followed by a decrease, while those receiving TAM showed the initial increase followed by continued high levels. In Experiment 3 the ad lib. EB group showed continued high levels of oxygen consumption as had been found by Laudenslager, et al. (1980,1982), while the ad lib. TAM group was indistinguishable from the ad lib. oil group (an effect which would be expected of a competitive estrogen antagonist in the absence of estrogens). Concurrent administration of TAM and EB did not appear to produce the effect expected with antagonists. While the continued increase produced by TAM was eliminated, the TAM+EB group was very similar to the EB group rather than the oil group. This may be

explained by EB's blocking the TAM effects in BAT plus having an effect on oxygen consumption independently of BAT.

The animals in these experiments were housed a temperatures in the human comfort range so that BAT may play only a small role in resting oxygen consumption. BAT weight in grams did correlate slightly ($r$=.41) with oxygen consumption in ml $O_2$/min. EB had little or no effect on the various measures in BAT in food restricted rats. The lack of effect on BAT LPL activity is consistent with the findings of Edens and Wade (1983). TAM, on the other hand, increased BAT weight and BAT protein in food restricted rats, but had no effect on DNA or BAT LPL activity. The TAM effects were blocked by concurrent administration of TAM and EB. These results could be consistent with a slight role of BAT in the increased oxygen consumption of the food restricted TAM groups in Experiments 2 and 3.

Large variances caused partly by the small number of animals make interpretation of effects on blood measures difficult. Food restriction caused an increase of free fatty acids in all three groups, a result which is consistent with other reports on the effects of food restriction (see for example, Steffens, 1969). The various

blood measures showed only slight correlations with food intake, body weight, or oxygen consumption, $r$'s<.30.

One unexpected finding was the significant 35 to 40 percent increase of serum albumin in the two EB groups. One cause of this could be interference in the assay by blood globulins (Webster, 1974), however the samples were read within three seconds of the addition of the bromo-cresol green solution and Webster found that albumin readings were stable for about 10 seconds. Peterson and Spaziani (1971) showed that EB increases uptake of albumin by uterus although it does not cause increases of uptake in other tissues. Clark and Peck (1979) suggest that albumin in intercellular fluid of uterus may serve as a storage mechanism to concentrate estrogens. This could provide a reason for increased production of albumin by EB.

EB and TAM combined with ad lib. food administration or food restriction permit separation of some effects from food intake, and may permit manipulations which will produce opposing effects. These combinations should be of use for further investigation of general factors, as well as estrogen receptor mediated effects, on food intake, body composition, and metabolic rate.

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