PRE-ADIPOCYTE PROLIFERATION AND DIFFERENTIATION IN RESPONSE TO HORMONE SUPPLEMENTATION OF DECAPITATED FETAL PIG SERA

T. G. Ramsay, G. J. Hausman and R. J. Martin

University of Georgia, Athens 30602
and
U.S. Department of Agriculture, Athens, Georgia 30613

ABSTRACT

Growth hormone and thyroid hormones have been implicated as important serum factors for adipocyte development in cell culture. Fetal decapitation removes these factors from serum of the growing fetal pig and results in development of fewer adipocytes than in intact fetuses. These experiments examined the effects of growth hormone or thyroxine supplementation to decapitated fetal pig sera upon pre-adipocyte proliferation and differentiation. Hormones were supplemented to concentrations present in sera from intact pig littermates (reference). Sera + hormones were analyzed for their effects upon pre-adipocyte proliferation as determined by \(^{3}H\)-thymidine incorporation; enzyme expression as determined by sn-glycerol-3-phosphate dehydrogenase activity; and induction of complete differentiation into lipid-filled adipocytes as based upon a pre-adipocyte differentiation assay. Sera from decapitated fetuses (decap sera) promoted less pre-adipocyte proliferation and enzyme expression than reference sera. Growth hormone had no effect in decap sera upon these parameters. Decap sera permitted detection of 54% more lipid-accumulating, newly formed adipocytes on percol gradients than reference sera, but growth hormone reduced detection to 29% of reference sera. Thyroxine specifically stimulated pre-adipocyte proliferation more than decap sera, but not to the level of reference sera. Complete differentiation, a formation of lipid-accumulating adipocytes was promoted also by thyroxine in comparison to basal decap sera. The results of these experiments indicate thyroid hormones are an important component of fetal sera for regulation of adipocyte development, whereas growth hormone may only affect cellular metabolism and not promote pre-adipocyte growth and development.

(Key Words: Pigs, Adipose Tissues, Hormones, Cell Culture, Growth, Development.)

Introduction

Histological analysis has indicated that adipocytes develop rapidly between 60 and 110 d of gestation in subcutaneous adipose tissue of the fetal pig (Hausman and Kaufman, 1986). Vodovar and Desnoyers (1978) estimated that most subcutaneous adipocytes are present at birth in swine. Those experiments have suggested primary factors that regulate adipocyte precursor (pre-adipocyte) proliferation and differentiation must have their major actions during fetal development.

Fetal decapitation experiments have indicated that the fetal hypothalamic-pituitary axis is important for regulation of this pre-adipocyte proliferation and differentiation. Fetal decapitation at 45 d of gestation reduced adipocyte hyperplasia of porcine adipose tissue when examined at 110 d of gestation (Hausman et al., 1981). In vitro cell culture experiments have suggested that these in vivo tissue effects of fetal decapitation were a result of changes in serum factors (Ramsay et al., 1984). Identification of these serum factors in fetal pigs that regulate pre-adipocyte proliferation differentiation and then lipid accretion will provide essential knowledge for development of techniques to regulate adipose tissue growth during the important fetal phase of growth.

Many factors influenced by the hypothalamic-pituitary axis are affected by fetal decapitation. Growth hormone and thyroid hormones have been implicated as serum factors of major importance for adipocyte development in

culture. Nixon and Green (1984) have estimated that growth hormone contributes approximately 50% of the adipogenic activity to fetal bovine serum. Ailhaud et al. (1983) have shown that thyroid hormones are important for adipocyte development in culture. Growth hormone and thyroid hormones were undetectable by radio-immunoassay (RIA; rat primary antibody) in decapitated fetal pig sera (Martin et al., 1985), which suggests that these proposed adipogenic hormones may be important for normal adipose tissue formation in the fetal pig. The present experiments were designed to determine if physiological concentrations of growth hormone or thyroxine present in fetal pig sera are essential for pre-adipocyte proliferation and differentiation with resultant formation of lipid accumulating adipocytes.

Methodology

Two separate experiments were performed in this study. Experiment 1 examined the effects of growth hormone supplementation of 110-d decapitated fetal pig (decap) serum on stromal-vascular cell cultures. Experiment 2 examined the effects of thyroxine supplementation of 100-d decapitated fetal pig serum on stromal-vascular cell cultures. Experiment 2 did not use 110-d fetal pig serum because this serum was no longer available.

Both experiments used crossbred gilts with known breeding dates. Experiments 1 and 2 used five and three gilts, respectively. All animals were fed the same stock diet throughout gestation.

Surgical Procedures. At 45 d of gestation gilts were placed under halothane anesthesia and a midventral laparotomy was performed. The uterus was exteriorized and one of the two uterine horns was randomly selected for fetal decapitation according to the procedures of Stryker and Dziuk (1975). The fetuses in the other uterine horn were palpated and thus served as sham-operated controls. The uterus was replaced within the abdominal cavity and then the incision was closed. The pregnant gilts underwent a laparotomy again at the selected time of 110 d in Exp. 1 and 100 in Exp. 2.

Blood from individual fetuses was collected from the uterine vein and arteries. Blood was kept on ice for 4 h prior to centrifugation and sterile filtration of the derived sera. Sera from Exp. 1 were analyzed for growth hormone content by a homologous double-antibody radio-immunoassay for porcine growth hormone (pGH). Growth hormone was assayed using guinea pig pGH antibody prepared and characterized by Marple (1972). Sera from Exp. 2 were analyzed for total thyroxine (T₄) and total triiodothyronine (T₃) by commercial RIA kits⁵.

Cell Culture Techniques. Cells were obtained from 40- to 60-g male Sprague-Dawley rats for evaluation of sera differences. Animals were anesthetized with sodium pentobarbital (50 mg/kg ip), freed of hair and disinfected with iodine and alcohol. Inguinal adipose tissue from two to four rats were pooled, finely minced and incubated with 5 ml/g of tissue of the digestion buffer described by Bjorntorp et al. (1979). A fivefold excess of buffer (room temperature) without enzymes was added to the digestion flask after 1 h of digestion at 37 C in a shaking water bath. Flask contents were mixed and filtered through nylon screens⁶ with 275- and 20-µm mesh openings to remove undigested tissue and large cell aggregates. The filtered cells were suspended in medium 199 and centrifuged at 600 x g for 10 min to separate the floating fat cells from the pellet of stromal-vascular cells. The stromal-vascular cell pellet was washed with plating medium, centrifuged and resuspended.

Aliquots of the stromal-vascular fraction were removed, stained with Rappaport’s stain and counted on a hemocytometer. Stromal-vascular cells were seeded on 25-cm² flasks in 4 ml of plating medium at a density of 1.0 x 10⁴ cells/cm². Cells were seeded also at the same density in 2 ml of medium on 35-mm petri dishes.

Cells were cultured at 37 C in a humidified 5% CO₂ atmosphere. Plating medium consisted of medium 199 with Earle’s salts⁷, 5 mM glucose, 40 mg/liter gentamicin sulfate, 50 mg/liter cephalothin, 2 mg/liter Fungizone⁷ and 10% fetal bovine serum. Plating medium was replaced with test medium after 24 h of incubation. Test media were identical to plating medium except for the serum component and hormone supplementation.

Test Media. Test media contained decapitated fetal pig (decap) sera or intact fetal pig (reference) sera at 5% medium concentration. Por-

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⁴ Cambridge Medical Diagnostics Inc., Billerica, MA.
⁵ Tetko, Elmsford, NY.
⁶ Gibco Grand Island, NY.
cine growth hormone (activity = 2 IU/mg) for sera supplementation was a gift*. Thyroxine was purchased9.

**Proliferation Assay** (figure 1). Proliferation or replication of pre-adipocytes and stromal-vascular cells in response to the various test media was examined by [3H]-thymidine incorporation according to the procedures of Novakofski (1983). Duplicate flasks were labeled for 24 h on d 3 of culture with .2 μCi [3H]-thymidine in 4 ml test media per flask for both experiments. Cultures were rinsed twice with 5 ml of Earle’s salt solution after labeling and refed with 4 ml of fresh, unlabeled test media. No other labeling time points were examined due to the limited availability of these fetal sera.

All test media were replaced with a differentiation promoting medium at d 5 of culture. Differentiation medium contained 10% adult pig (175 to 200 kg) serum, 1.0 nM procine insulin and 10 U/ml heparin in medium 199 with Earle’s salts7, 5 mM glucose, 40 ng/liter gentamicin sulfate, 50 mg/liter cephalothin, 2 mg/liter Fungizone®. Differentiation medium was changed on alternate days until d 14.

Cells were harvested from the culture flasks on d 14 with Hank’s salt solution containing .22% crystalline trypsin, .02% collagenase and .5% bovine serum albumin. Differentiated pre-adipocytes were separated from undifferentiated pre-adipocytes and stromal-vascular cells by centrifugation on a solution of Percol and Hank’s salt solution with a density of 1.02 according to the procedures of Novakofski (1983). The isolated cell fractions were placed in vials with scintillation cocktail10 for determination of tritium incorporation. Differentiated pre-adipocytes are adipocytes that formed in culture from 3H-thymidine labeled pre-adipocytes (adipocyte precursors). The 9-d period following exposure to differentiation medium allows labeled pre-adipocytes to differentiate and accumulate sufficient lipid for separation from undifferentiated cells on Percol density gradients. Passage of cells isolated from the adipocyte fraction yields secondary cultures containing less than 5% stromal cells which cannot be induced to form adipocytes.

**Complete Differentiation Assay** (figure 1). Differentiation of the pre-adipocyte is the process of cellular conversion by which this precursor cell becomes growth-arrested and develops structural and functional markers of the adipocyte (see Cryer, 1985, for review). This assay measures relative ability of a test serum to stimulate pre-adipocytes to convert into lipid accumulating adipocytes. This assay does not detect cells that do not accumulate lipid.

Cell cultures were incubated with 5% adult pig serum from d 1 to 5 of culture. Flasks were labeled for 24 h on d 3 of culture with .2 μCi [3H]-thymidine per flask. Cultures were rinsed twice with 5 ml of Earle’s salt solution7 after labeling and refed with fresh, unlabeled 5% adult pig serum. This medium was replaced on d 5 at confluency with the various test media. Incubation and labeling of all cultures derived from the same digest with the same medium results in all cultures containing similar populations of marked pre-adipocytes (less than 4% variation in total incorporation) available for induction of differentiation and lipid accretion following exposure to the various test media.

Duplicate flasks were exposed to the test media from d 5 until d 14. Test media were changed on alternate days during this period. Cells were harvested on d 14 by the procedures described for the proliferation assay.

**Enzyme Analysis.** Sn-glycerol-3-phosphate dehydrogenase (E.C. 1.1.1.8) activity was determined on cultures that were exposed to test media for 15 d. Media were replaced with fresh media on alternate days. Cultures were grown in 35-mm petri dishes to limit media utilization. Enzyme activity was determined by a modification of the procedures of Wise and Green (1979).

Cultures were homogenized by sonic dismem branation in 1.0 ml of an ice cold homogenizing buffer containing .25 M sucrose, 1 mM EDTA, 5 mM Tris base and 1 mM dithiothreitol at pH 7.4. This buffer was found to stabilize enzyme activity better than previously recommended buffers (Kozak and Jensen, 1974; Wise and Green, 1979). Homogenates were centrifuged at 12,500 x g for 5 min at 4 C in Eppendorf tubes. The supernatant from the centrifugate was used for analysis.

The sn-glycerol-3-phosphate dehydrogenase assay mixture contained 100 mM triethanolamine-HCl (pH 7.5), 2.5 mM EDTA, .1 mM β-mercaptopetanol, .16 mM NADH and .8 mM

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* Eli Lilly and Co., Indianapolis, IN.
9 Sigma Chemical Co., St. Louis, MO.
10 Scintiverse I, Fisher Scientific, Pittsburgh, PA.
Day of culture | Protocol
---|---
0 | Plate all cells in 10% FBS

1 | **Proliferation assay**
   Incubate cells with 5% test sera

| **Complete differentiation assay**
   Incubate cells with 5% adult pig serum

3 | Label cells with 
   $^3$H-thymidine in 
   5% test sera

| Label cells with 
   $^3$H-thymidine 
   in 5% adult pig serum

5 | Incubate all cultures with 
   Differentiation medium 
   (10% adult pig serum, 1 nM 
   insulin, 10 U/ml heparin)

| Incubate cultures with 
   5% test sera

14 | Harvest cultures and 
   separate cell fractions

| Harvest cultures and separate 
   cell fractions

- **Measure** $^3$H-thymidine 
  incorporation
- **Measure** $^3$H-thymidine 
  incorporation

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Figure 1. Diagram of proliferation and complete differentiation assays. FBS = fetal bovine serum. Adult pig serum from 175- to 200-kg pigs.

Dihydroxyacetone phosphate in a total volume of 2 ml. Changes in absorption at 340 nm were measured in a Gilford Model 240 recording spectrophotometer at 25 C. One unit of activity corresponded to oxidation of 1 nmol of NADH·min$^{-1}$·mg$^{-1}$ protein. The assay was linear for sample concentration and time. Variation between duplicate determinations never exceeded 5.0%. Cytosolic protein concentrations of cell culture homogenates were determined according to Lowry et al. (1951).

**Statistical Analysis.** The paired t-test (Steel and Torrie, 1980) was used to analyze differ-
ences between cultures incubated with decap sera and hormone supplemented decap sera. The paired t-test was used to determine significant differences between cultures incubated with sera from decapitated and reference fetuses for each pair within a litter. This same analysis was used for comparing hormone supplemented sera and reference sera. Significant effects were defined at the 95% confidence level.

Results

Growth hormone was present in 110-d reference sera at a concentration of 54.7 ± 7.4 ng/ml as determined by pGH-RIA. Fetal decapitation reduced sera growth hormone concentrations to 4.6 ± 1.4 ng/ml. Supplementation of growth hormone to each decap serum to produce growth hormone concentrations equivalent to each paired reference serum had no effect on pre-adipocyte proliferation (table 1). Decap sera elicited less pre-adipocyte proliferation than reference sera.

Stromal vascular cell proliferation was increased in decap sera although not significantly. Growth hormone supplementation increased this effect on stromal-vascular cells further, but was not different from basal decap sera incubations (P>.05).

Growth hormone addition had no effect on the ability of decap sera to promote enzymatic expression of pre-adipocytes during differentiation (table 2). Decap sera stimulated much less expression than reference sera upon incubation with cell cultures.

Decap sera permitted the detection of 54% more lipid-accumulating, differentiated pre-adipocytes than reference sera (table 3). The addition of physiological concentrations of growth hormone to decap sera reduced detection of differentiated pre-adipocytes to a level far below reference sera.

Initially, triiodothyronine (T₃) was to be supplemented to decap sera in the second experiment of this study. Unfortunately, T₃ concentrations in reference sera were below detectability of our RIA (<30 ng/dl). Thyroxine (T₄) was detectable at 100 d of gestation in fetal pig sera. Reference sera contained a mean T₄ concentration of 6.1 ± .3 μg/dl. Thyroxine was undetectable in decap sera, as has been reported previously.

Fetal decapitation reduced the ability of sera to promote proliferation (table 4), as in the first experiment of this study. Addition of T₄ to decap sera caused significant stimulation of pre-adipocyte proliferation. Thyroxine had no effect on stromal-vascular cell proliferation.

Thyroxine had a slight but nonsignificant effect on enzyme expression (table 5). Thyroxine had a much greater effect in decap sera on promoting lipid accretion in differentiated pre-adipocytes (table 6). Decap sera stimulated the development of more lipid-accumulating, differentiated pre-adipocytes than reference sera, as in the first experiment in this study. Thyroxine addition to decap sera caused a further 30% increase in differentiated pre-adipocytes above basal decap sera.

Discussion

Fetal decapitation reduced the ability of serum to stimulate pre-adipocyte proliferation and expression of sn-glycerol-3-phosphate dehy-

TABLE 1. EFFECT OF PORCINE GROWTH HORMONE (pGH) SUPPLEMENTATION OF 110-D DECAPITATED FETAL PIG (DECAP) SERA ON PRE-ADIPOCYTE AND STROMAL-VASCULAR CELL [³H]-THYMIDINE INCORPORATION IN VITROab

<table>
<thead>
<tr>
<th>Sera</th>
<th>Pre-adipocyte</th>
<th>Stromal-vascular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decap</td>
<td>10,059 ± 470d</td>
<td>126,435 ± 13,756</td>
</tr>
<tr>
<td>Decap + pGHc</td>
<td>10,805 ± 702d</td>
<td>144,272 ± 11,382</td>
</tr>
<tr>
<td>Reference</td>
<td>13,602 ± 710</td>
<td>97,049 ± 3,732</td>
</tr>
</tbody>
</table>

aMean ± SE for six sera; values are expressed as [³H]-thymidine dpm incorporated per flask.
bCells were labeled on d 3 of culture for 24 h with .2 μCi [³H]-thymidine per flask.
cGrowth hormone was supplemented to each decap serum to the concentration in the respective reference serum.
dDifferent from reference (P<.05).
### TABLE 2. EFFECT OF PORCINE GROWTH HORMONE (pGH) SUPPLEMENTATION OF 110-D DECAPITATED FETAL PIG (DECAP) SERA ON SN-GLYCEROL-3-PHOSPHATE DEHYDROGENASE ACTIVITY IN VITRO

<table>
<thead>
<tr>
<th>Sera</th>
<th>Enzyme activity</th>
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<tbody>
<tr>
<td>Decap</td>
<td>265 ± 35&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Decap + pGH&lt;sup&gt;b&lt;/sup&gt;</td>
<td>256 ± 122&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Reference</td>
<td>887 ± 118&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± SE for six sera; values are expressed as units per mg protein. Measurements were made on d 15 of culture.

<sup>b</sup>Growth hormone was supplemented to each decap serum to the concentration in the respective reference serum.

<sup>c</sup>Different from reference (P<.05).

drognase activity as a result of differentiation. The hypothalamic-pituitary axis has been thought to be a source of factors and to be a regulator of peripheral factors that affect adipose tissue growth and development (Martin et al., 1981; Grimaldi et al., 1982; Lau et al., 1983; Novakofski et al., 1983). Pre-adipocyte proliferation and differentiation (Hayashi et al., 1981) have been stimulated by pituitary and hypothalamix extracts in support of this concept. Purification of extracts from bovine pituitary has demonstrated growth hormone to be a major adipogenic factor for 3T3 pre-adipocytes in fetal bovine serum (Morikawa et al., 1982; Bowen-Pope and Ross, 1984). A GH effect on pre-adipocytes has not been shown with any other cell culture system. Growth hormone did not promote enzymatic enzyme expression during differentiation in 0b17 cells (Gaillard et al., 1984) or in the present experiment. Neither did growth hormone effect pre-adipocyte proliferation in secondary culture (Roncari, 1981) or primary culture (Novakofski et al., 1983).

The effect of growth hormone on 3T3 pre-adipocyte differentiation may be due to the use of fetal bovine sera to induce enzyme expression as the selection criteria for pre-adipocyte clones. Fetal bovine serum contains high concentrations of growth hormone (>100 ng/ml by rat GH-RIA, personal observation). Therefore, indirect

### TABLE 3. EFFECT OF PORCINE GROWTH HORMONE (pGH) SUPPLEMENTATION OF 110-D DECAPITATED FETAL PIG (DECAP) SERA ON STIMULATION OF COMPLETE PRE-ADIPOCYTE DIFFERENTIATION

<table>
<thead>
<tr>
<th>Sera</th>
<th>Relative differentiation promotion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decap</td>
<td>113.9 ± 4.7&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Decap + pGH&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.6 ± 2.4&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>Reference</td>
<td>74.2 ± 5.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± SE for six sera; values are expressed as percent of [<sup>3</sup>H]-thymidine dpm incorporated into cells exposed to 5% mature pig sera.

<sup>b</sup>Cells were labeled on d 3 of culture for 24 h with .2 μCi [<sup>3</sup>H]-thymidine in 4 ml of 5% mature pig sera per flask. Cells were maintained on test media from d 5 to 14.

<sup>c</sup>Growth hormone was supplemented to each decap serum to the concentration in the respective reference serum.

<sup>d</sup>Different from reference (P<.05).

<sup>e</sup>Different from basal decap (P<.05).
DECAPITATED PIG SERA AND PRE-ADIPOCYTES

TABLE 4. EFFECT OF THYROXINE (T4) SUPPLEMENTATION OF 100-D DECAPITATED FETAL PIG (DECAP) SERA ON PRE-ADIPOCYTE AND STROMAL-VASCULAR [3H]-THYMIDINE INCORPORATION IN VITRO ab

<table>
<thead>
<tr>
<th></th>
<th>Pre-adipocytes</th>
<th>Stromal-vascular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decap</td>
<td>9,676 ± 429d</td>
<td>76,270 ± 1,116</td>
</tr>
<tr>
<td>Decap + T4c</td>
<td>10,851 ± 637de</td>
<td>77,234 ± 4,187</td>
</tr>
<tr>
<td>Reference</td>
<td>13,821 ± 945</td>
<td>77,565 ± 4,716</td>
</tr>
</tbody>
</table>

a Mean ± SE for five sera; values are expressed as [3H]-thymidine dpm incorporated per flask.

b Cells were labeled on d 3 of culture for 24 h with .2 μCi [3H]-thymidine per flask.

TThyroxine was supplemented to each decap serum to the concentration in the respective reference serum.

d Different from reference (P<.05).

e Different from basal decap (P<.05).

Selection pressure for a growth hormone response has been utilized in 3T3 pre-adipocyte clone selection. Much less selection pressure occurs in primary culture in comparison to cell line culture. Thus, examination of cells in primary culture may more truly reflect the in vivo situation than cell line culture.

Early hypopituitary and in vivo growth hormone injection studies correlated growth hormone supplementation and adipocyte number. Growth hormone injection produced significant increases in cell number in human dwarfs (Knittle et al., 1972; Brook, 1973; Bonner et al., 1974). The disagreement by the cell culture experiment suggests that the in vivo growth hormone effect is through the action of other factors, or requires the removal of inhibitors of growth hormone action.

Many of the growth-promoting actions of growth hormone are through insulin-like growth factor (IGF) activity (Daughaday, 1981). Insulin-like growth factor I did not promote 3T3 pre-adipocyte differentiation in vitro, but was suspected to be mitogenic (Morikawa et al., 1984). Experiments with 3T3 fibroblasts have indicated that these cells may be sensitive to the proliferative actions of IGF (Nissley et al., 1976). Insulin-like growth factor I has been conclusively shown to be mitogenic for 0b17 pre-adipocytes and to inhibit their differentiation (Grimaldi et al., 1983). Insulin-like growth factor may act on pre-adipocytes in vivo to stimulate a proliferative response, while other factors induce differentiation to produce increases in adipocyte number during development. The recent development of purified IGF and assays for their detection will permit exploration of the role that these polypeptides have in adipose tissue development.

Porcine growth hormone only had an inhibitory action upon lipid accumulation in this culture system. Decap sera without growth hor-

TABLE 5. EFFECT OF THYROXINE (T4) SUPPLEMENTATION OF 100-D DECAPITATED FETAL PIG (DECAP) SERA ON SN-GLYCEROL-3-PHOSPHATE DEHYDROGENASE ACTIVITY IN VITRO a

<table>
<thead>
<tr>
<th></th>
<th>Enzyme activity</th>
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<tbody>
<tr>
<td>Decap</td>
<td>706 ± 48c</td>
</tr>
<tr>
<td>Decap + T4b</td>
<td>783 ± 69</td>
</tr>
<tr>
<td>Reference</td>
<td>850 ± 51</td>
</tr>
</tbody>
</table>

a Mean ± SE for four sera; values are expressed as units per mg protein. Measurements were made on d 15 of culture.

b Thyroxine was supplemented to each decap serum to the concentration in the respective reference serum.

c Different from reference (P<.05).
TABLE 6. EFFECT OF THYROXINE (T4) SUPPLEMENTATION OF 100-D DECAPITATED FETAL PIG (DECAP) SERA ON STIMULATION OF COMPLETE PRE-ADIPOCYTE DIFFERENTIATIONab

<table>
<thead>
<tr>
<th>Sera</th>
<th>Relativedifferentiation promotion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decap</td>
<td>69.3 ± 2.7d</td>
</tr>
<tr>
<td>Decap + T4C</td>
<td>90.4 ± 4.1de</td>
</tr>
<tr>
<td>Reference</td>
<td>33.0 ± 1.7</td>
</tr>
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</table>

aMean ± SE for five sera; values are expressed as percent on [3 H]-thymidine dpm incorporated into cells exposed to 5% adult pig sera.
bCells were labeled on d 3 of culture for 24 h with .2 µCi [3 H]-thymidine in 4 ml of 5% mature pig sera per flask. Cells were maintained on test media from d 5 to 14.
cThyroxine was supplemented to each decap serum to the concentration in the respective reference serum.
dDifferent from reference (P<.05).
eDifferent from basal decap (P<.05).

Thyroxine supplementation promoted detection of more adipocytes that had differentiated from 3 H-thymidine-labeled pre-adipocytes than reference sera. This effect was probably due to the elevated lipid concentrations and deficiencies in insulin-antagonistic or lipolytic hormone in decap sera compared with reference sera (Martin et al., 1985). Growth hormone addition inhibited lipid accumulation and thus reduced the ability of percol gradients to separate effectively newly formed adipocytes from the stromal-vascular fraction. Physiological concentrations of growth hormone in decap sera were more effective in limiting lipid accumulation than growth hormone in reference sera. This suggests that fetal decapitation alters the concentration of serum factors that are antagonistic to the metabolic actions of growth hormone.

In vivo experiments with fetal genetically lean and obese pigs have indicated a potential role for thyroid hormones in adipose tissue development. Martin et al. (1982) have reported that serum thyroid hormone levels are elevated in fetal genetically obese pigs prior to detectable differences in adipocyte differentiation. These elevated thyroid hormone levels occur during the rapid development phase of the tissue structure (Hausman and Kauffman, 1986). The thyroid hormones may function to promote preadipocyte proliferation during the third trimester in fetal pig adipose tissue. Physiological thyroxine concentrations were responsible for 27% of the difference in the proliferative capacity of decap and reference sera at 100 d of gestation. Serum-free culture conditions must be developed for this culture system before the proliferative actions of T4 can be determined to be of a direct or indirect nature.

The slight effect (although not significant) of T4 to promote sn-glycerol-3-phosphate dehydrogenase differentiation was expected. Thyroxine has been shown to promote acetate incorporation into lipid (Gharbi-Chihi et al., 1981) by binding to nuclear T3 receptors (Anselmet et al., 1984) in 0b17 cells. Thyroxine has a much lower affinity for these T3 receptors than T3, thus its effect on enzyme differentiation was not expected to be great.

Thyroxine was very effective in stimulating complete morphological differentiation of preadipocytes. The high lipid concentrations of decap sera (Martin et al., 1985) coupled with thyroxine treatment tripled the detection of differentiated, lipid-filled cells in comparison to reference sera. Serum lipids provided the necessary substrates for lipid accretion. The concentration of triglyceride in plasma has been highly correlated (.92) with the amount of lipid accumulation in developing rat adipocytes in culture (Bjorntorp et al., 1985). Thyroxine may have been more potent in amplifying the expression of enzymes other than sn-glycerol-3-phosphate dehydrogenase that influence lipid accretion in primary culture. Further examination of the effects of thyroid hormone on enzyme differentiation in primary culture are necessary.
The results of these experiments indicate that thyroid hormones are an important component of fetal sera for regulation of adipocyte development. On the other hand, growth hormone does not promote the growth and development of pre-adipocytes. The ability of physiological concentrations of growth hormone and thyroid hormones to compensate only partially for differences in pre-adipocyte proliferation and differentiation between decap and reference sera indicates that other important regulatory factors still need to be identified in fetal pig serum.

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