Adipocyte Conversion of CHEF Cells in Serum-free Medium

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ABSTRACT When grown in the presence of serum with added insulin, Chinese hamster embryonic fibroblasts (CHEF/18) cells can be induced to become preadipocytes that are committed to the adipocyte pathway of terminal differentiation (Sager, R., and P. Kovac, 1982, *Proc. Natl. Acad. Sci. USA*, 79:480–484). We found that commitment to the adipocyte pathway, as well as terminal differentiation to form mature adipocytes, can occur in a defined serum-free medium containing insulin. When CHEF/18 cells are plated in serum-containing medium, only 5–10% of cells in each colony undergo terminal differentiation, whereas in serum-free medium, >90% of the cells became adipocytes. These and other results show that CHEF/18 cells require no adipogenic factors in addition to insulin and the other components of the serum-free medium (transferrin, epithelial growth factor, thrombin) to form adipocytes, and furthermore, that serum inhibits the rate of terminal adipocyte differentiation of these cells. As little as 10 ng/ml insulin added to serum-containing medium can induce adipogenesis, suggesting that insulin rather than an insulinlike growth factor is the active agent. The results further demonstrate that virtually every CHEF/18 cell can be induced into the adipocyte pathway.

In embryonic development, secondary stem cells of mesenchymal origin give rise to a number of differentiated cell types including adipocytes, myoblasts, myotubes, chondrocytes, osteocytes, and adult fibroblasts. This process has been mimicked in cell culture by treating fibroblastic cells of embryonic origin with 5-azacytidine for as short a time as a single cell cycle (1–3). Indeed, the response of embryonic fibroblastic cell lines to this drug has provided the basis for their identification as secondary mesenchymal stem cells. Since 5-azacytidine decreases the extent of cytosine methylation in DNA, its effect on stem cells has suggested that decreased DNA methylation is involved in the differentiation process. The results reported here are part of an ongoing investigation of molecular mechanisms underlying specific steps in development and the distribution of stem cells into alternative pathways of terminal differentiation.

In a previous report, we described the ability of 5-azacytidine to induce Chinese hamster embryonic fibroblastic (CHEF/18)[1] stem cells to become preadipocytes, myoblasts, or chondrocytes, and showed further that the same clonal CHEF/18 cell populations could be directed exclusively into the adipocyte pathway if the cells were grown in the presence of added insulin without 5-azacytidine (1). Since insulin was added to a medium containing 10% fetal bovine serum (FBS), it was possible that the added insulin was interacting with some other adipogenic serum factor in eliciting the adipogenic response. The action of adipogenic factors in serum has been described in the maturation of adipocytes from 3T3 preadipocytes (4).

In this paper, we describe the differentiation of CHEF/18 stem cells into adipocytes in serum-free medium. This medium, originally described by Cherington et al. (5), and subsequently improved by Sager et al. (6), supports continuous growth of CHEF/18 cells at a rate similar to that in serum (6). The medium contains epithelial growth factor, transferrin, and thrombin, in addition to 10 μg/ml insulin, a concentration required for optimal growth. The high concentration of insulin that is required suggests that it may be substituting for an insulinlike growth factor (7, 8) for the growth response, but this possibility has not been tested due to the unavailability of purified somatomedins or multiplication stimulating activity for growth studies. It is likely that insulin rather than an insulinlike growth factor is required for the adipocyte conversion in view of the fact that adipocyte differentiation can be induced by as little as 10 ng/ml insulin in serum-containing medium as described in this paper. Commercial serum itself contains very low levels of insulin, and does not induce adipogenesis in CHEF/18 cells.

[1] *Abbreviations used in this paper:* CHEF cells, Chinese hamster embryonic fibroblastic cells; FBS, fetal bovine serum; GPDH, sn-glycerol-3-phosphate dehydrogenase.

THE JOURNAL OF CELL BIOLOGY · VOLUME 100 FEBRUARY 1985 429–434

We report here that serum-free medium was effective for the conversion of CHEF/18 stem cells to preadipocytes (Stage I), and for terminal differentiation as defined by cell shape and lipid content (Stage II). We also report that >90% of CHEF/18 cells in each colony became adipocytes when grown in serum-free medium. The similarity of preadipocytes induced in serum-free medium to those induced in serum is further documented by the similarities in activity and heat-stability of the enzyme sn-glycerol-3-phosphate dehydrogenase (GPDH) (9), assayed here in both types of preadipocytes.

MATERIALS AND METHODS

The origin and maintenance in culture of CHEF/18 cells (10) and of the preadipocyte cell lines II/2-2, 18BIISF, and 18ABIISF (11) were previously described. These lines have been maintained in the absence of added insulin and tested repeatedly. They have shown consistent patterns of differentiation as described here. Cultures are grown in alpha-MEM medium (K.C. Biologicals, Lenexa, KS) supplemented with 5 or 10% FBS (M.A. Bioproducts, Walkersville, MD), 100 μg/ml streptomycin (Gibco Laboratories, Grand Island, NY), and 100 U/ml penicillin (Gibco Laboratories) at 37°C with humidified 6.5% CO_2 in air. Serum-free cultures are grown in a mixture of alpha-MEM and Ham's F12 medium (Flow Laboratories, Inc., McLean, VA) in a 1:1 ratio supplemented with 10 ng/ml epithelial growth factor (Collaborative Research Inc., Lexington, MA), 0.1 U/ml human thrombin (gift of B. Zetter, Children's Hospital Medical Center, Boston, MA), 5 μg/ml human plasma transferrin (United States Biochemical Corp., Cleveland, OH), 10 μg/ml bovine pancreatic insulin (24 IU/mg), 100 μg/ml streptomycin, and 100 U/ml penicillin at 37°C with humidified 10% CO_2 in air (6). To detect and identify adipocytes, we fixed cultures with buffered formalin, stained them with lipophilic Oil Red O, and counterstained them with hematoxylin as previously described (1). All reagents were obtained from Sigma Chemical Co., St. Louis, MO, unless otherwise indicated. Domestic cat serum was purchased from the Colorado Serum Co., Denver, CO.

Enzyme Extracts: Subconfluent cultures grown in 850 cm^2 roller bottles were washed twice with cold (4°C) sodium phosphate-buffered saline. Subsequent steps were carried out at 0–4°C. The cells were scraped from the surface into 5 ml of homogenization buffer containing 1 mM EDTA, 1 mM beta-mercaptoethanol, 50 mM Tris-HCl (pH 7.5). The cell suspensions were homogenized with 20 strokes in a Ten-Broeck homogenizer (Corning Medical and Scientific, Medfield, MA) and subjected to centrifugation at 8,000 *g* for 5 min. The pellets were discarded, and the subsequent solutions were centrifuged at 100,000 *g* for 1 h. The final supernatant was assayed for GPDH (EC1.1.1.8) activity as described below.

GPDH Assay: Oxidation of 0.13 mM beta-nicotinamide adenine dinucleotide as a result of reduction of 0.2 mM dihydroxyacetone phosphate was assayed at 25°C as described by Wise and Green (9). We define 1 U of enzyme activity as the oxidation of 1 nmol of nicotinamide adenine dinucleotide per minutes. Protein determinations were done using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA).

Heat Inactivation of GPDH: Enzyme extracts were diluted 1:1 with homogenization buffer at 50°C. At various times during incubation at 50°C, samples were chilled at 0°C and then stored at 4°C until assayed within 48 h. Storage of up to 48 h before assay caused no detectable change in results.

RESULTS

Adipocyte Conversion in Serum-free Medium

As previously reported, when CHEF/18 cells are grown in basal medium, supplemented with 5 or 10% FBS, they form a confluent monolayer, and when 10 μg/ml insulin is added at confluence, <5% of the cells become mature adipocytes. The adipocytes are arrayed in distinctive narrow sectors as shown in a confluent culture in Fig. 1 *a* and previously reported (1). During terminal differentiation of adipocytes, the elongate fibroblastic cells develop into spherical cells and often contain Oil Red O-staining fat droplets even before becoming round. Adipocyte-containing colonies include cells in various stages of terminal differentiation, including spherical cells containing one or two large fat droplets that push the nucleus off center and exhibit the classic signet-ring adipocyte morphology.

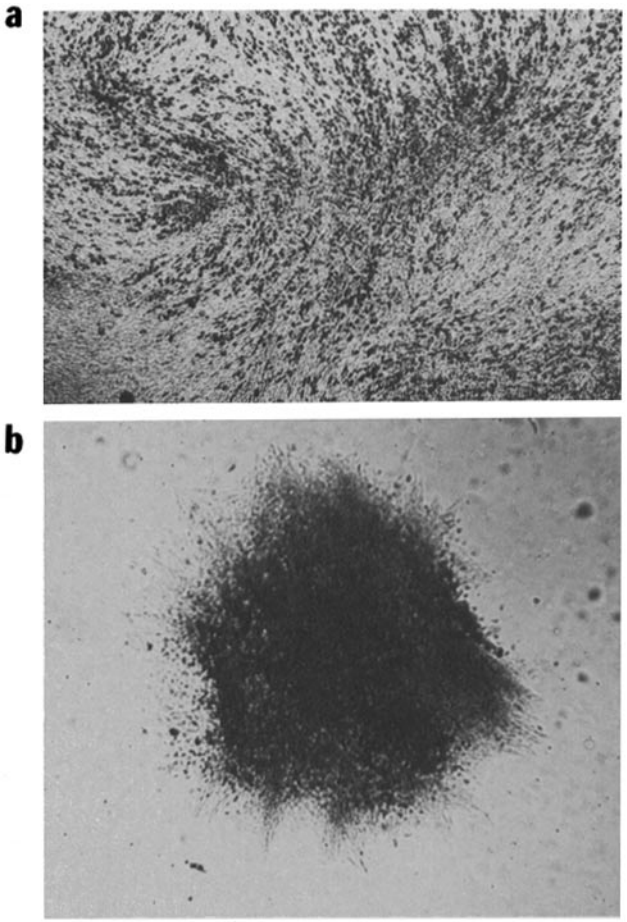

FIGURE 1 Adipocyte colony morphology of CHEF/18 cells in serum-free or serum-containing medium. CHEF/18 cells were plated on 60-mm dishes and grown to confluence in medium containing 5% FBS and 10 μg/ml insulin (*a*) or in serum-free medium containing insulin (*b*). Cultures were fixed and stained as described in Materials and Methods. Oil Red O–stained cells appear darker than cells stained with hematoxylin alone. × 35.

On the other hand, in serum-free cultures, CHEF/18 cells tended to aggregate rather than forming a confluent monolayer. As shown in Fig. 1 *b*, the cells formed focuslike clusters consisting of round Oil Red O-staining adipocytes. These adipocytes were densely packed at the centers of colonies whereas non-Oil Red O-staining fibroblastic cells appeared in monolayers at the periphery. The aggregation probably results from membrane changes induced by thrombin which is present in the serum-free medium. The aggregation is somewhat reversible since adding serum results in "melting down" of clusters (6).

It is apparent from qualitative observation that adipocyte formation within each colony is much greater in serum-free medium than in medium supplemented with serum. Direct counts under the microscope indicated that at least 90% of cells were converted to adipocytes in colonies grown in serum-free medium.

The rate and extent of adipocyte conversion of growing cells in serum-free medium was compared with that in medium containing 5 or 10% FBS as shown in Table I. Plates were fixed at the indicated intervals after plating, stained with Oil Red O to detect adipocytes, and scored for the percent of colonies containing at least 20 adipocytes. Control plates receiving no insulin had a background of 2% or fewer lipid-containing cells for up to 4 wk. In cultures grown in serum-

TABLE I

*Development of Adipocyte Colonies in CHEF/18 Cells Grown in Serum-free Medium**

Additions to alpha medium	Weeks after plating		
	1	2	3
Serum-free (10 μg/ml insulin)‡	87.5	100	100
5% FBS§	0	2	7
5% FBS‖	0	0	0.6
5% FBS + 10 μg/ml insulin§	0	62	97
5% FBS + 10 μg/ml insulin‖	0	85	98

* Percentage of total colonies that contain at least 20 adipocytes.
‡ 10^3 cells/60-mm dish (plating efficiency [PE] = 1–3%).
§ 50 cells/60-mm dish (PE ≥ 70%).
‖ 100 cells/60-mm dish (PE ≥ 65%).

free medium, adipocytes became apparent after 1 wk in almost every colony. In contrast, in medium supplemented with 5 or 10% serum plus insulin, adipocytes appeared later than in conditions of serum-free growth.

These results indicate that postulated serum adipogenic factors (4) are unnecessary for adipocyte differentiation of CHEF/18 cells. To confirm this conclusion, we tested terminal differentiation in domestic cat serum which contains negligible amounts of serum adipogenic factors; 3T3-derived preadipocytes do not differentiate to adipocytes in basal medium supplemented with domestic cat serum and insulin (4). We grew CHEF/18-derived preadipocytes in alpha-MEM medium supplemented with 10% domestic cat serum and insulin (10 μg/ml). Although the cells did not grow well in this medium, the cells were capable of rounding up and producing large quantities of lipid as judged by Oil Red O staining.

Stage I Determination

As previously discussed (1), the adipocyte conversion of CHEF/18 cells can be operationally divided into two stages: Stage I, in which stem cells become committed to the adipocyte pathway by becoming preadipocytes, and Stage II, in which preadipocytes undergo terminal differentiation. Do the stages occur in serum-free medium with similar or different kinetics than in serum?

To answer this question, we need to examine the two stages independently rather than together as in Table I. To assay the conversion of stem cells to preadipocytes, we need to identify preadipocytes. The method used in this paper is based upon our finding that Stage I is inhibited in confluent cultures compared with growing cultures, whereas Stage II is not inhibited. This point is illustrated by the experiment shown in Table II.

The rate of development of lipid globules was compared in growing and confluent cultures of stem cells and of a stable preadipocyte cell line, 18ABIISF, originally derived by growth of CHEF/18 stem cells for one passage in serum-free medium (6), and subsequently used in numerous experiments (see reference 11). Stem cell colonies growing in serum plus insulin developed adipocytes rapidly and extensively, whereas the same cultures, to which insulin was added at confluence but not during growth, were inhibited in the adipose conversion. On the contrary, the preadipocytes developed into adipocytes whether insulin was added during growth or after confluence had been reached. This result, seen reproducibly in many experiments, provides a simple assay of a cell population or cloned cell line to determine whether it is committed to the

TABLE II

*Effect of Confluency on Development of Adipocytes in CHEF/18 and Insulin-induced Preadipocytes Cultures**

Cell line	Weeks of feeding added insulin		
	½	1	2
Growing CHEF/18‡	0	0	57 ± 13
Confluent CHEF/18§	<1	<1	4 ± 1
Growing preadipocytes‡‖	0	0	57
Confluent preadipocytes§	8	20	62

* Values are the numbers of colonies with at least 20 Oil Red O-stained adipocytes (10^3 cells/60-mm dish). Medium contained 5% FBS and insulin (10 μg/ml).
‡ Insulin was added ½ wk after plating and confluency was reached 2 wk later.
§ Insulin was added when cell reached confluence.
‖ 18ABIISF is a preadipocyte line derived by one passage of CHEF/18 cells in serum-free medium.

adipocyte pathway. (The cells discussed in Table II were plated at 10^3 [rather than 10^2 cells/dish as in Table I] to compare growing and confluent cultures under similar conditions. Since colony formation is inhibited by crowding, the values obtained at 2 wk cannot be compared quantitatively with those in Table I. A similar number of colonies or "colony centers" are observed whether 10^2 or 10^3 cells are plated, as described previously [1].)

This method was then used to compare the effects of serum and serum-free medium on the Stage I commitment of CHEF/18 stem cells. The results are shown in Fig. 2. Cells were preincubated with insulin during growth for 1, 2, 3, or 4 d, either with serum (Fig. 2*A*) or in serum-free medium (Fig. 2*B*). They were then grown to confluence in serum, without insulin. Confluence was reached at ~2 wk from the start of the experiment, and insulin was added to assay their differentiating ability. The results in Fig. 2, *A* and *B* show that the Stage I commitment process occurs with similar kinetics in both serum and serum-free media. In both conditions, some induction occurs even after 1 d of exposure to insulin, and reaches its maximum after 3–4 d.

Effect of Serum-free Medium on Terminal Differentiation (Stage II)

To examine Stage II, we grew two preadipocyte cell lines, 18ABIISF and 18BIISF, in either serum-free medium or medium with 5% FBS and 10 μg/ml insulin. The results with the two cell lines were similar (Table III). Serum-free medium facilitated differentiation more efficiently than did serum plus insulin. With serum-free medium, adipocytes arose earlier and more extensively than with serum. Thus, no adipogenic factors were required for the terminal differentiation of these preadipocytes.

Insulin Concentration Required for Adipogenesis

Insulin at 10 μg/ml is a required component for growth supported by the serum-free medium. Consequently, serum-free conditions cannot be used to determine the insulin concentration required for adipose conversion. An estimate of the required concentration has been made by adding insulin at various concentrations to cells growing in serum. The results of one such experiment are shown in Table IV. CHEF/18 stem cells were plated at 10^3 cells/60-mm dish in medium containing 5% serum. At the first refeeding, 3 d later, insulin was added to aliquot dishes at a series of concentrations from

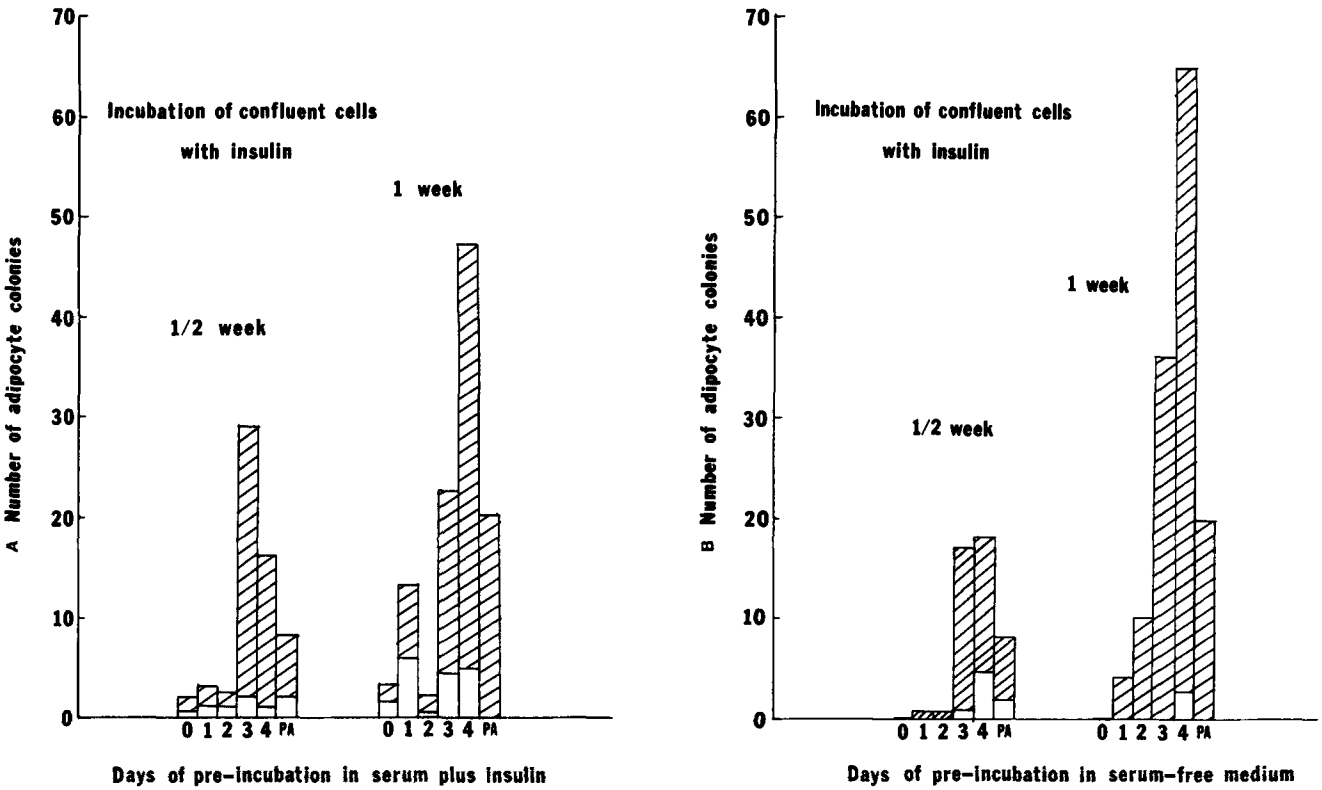

FIGURE 2 Time course of Stage I induction by insulin in serum-free or serum-containing medium. 10^3 CHEF/18 cells were plated in 60-mm dishes and grown for 0, 1, 2, 3, or 4 d with 10 μg/ml insulin in medium containing 5% FBS (a) or in serum-free medium (*b*). The cultures were grown to confluence in alpha-MEM medium with 5% FBS and then fed continuously with alpha-MEM alone or supplemented with insulin. Plates were fixed after ½ or 1 wk at confluence, stained, and colonies containing at least 20 adipocytes were counted. An insulin-induced preadipocyte line 18ABIISF derived by one passage of CHEF/18 cells in serum-free medium is included as a positive control. Bars with diagonal lines represent the number of adipocyte colonies that developed when cultures received insulin at confluence. Open bars represent the background adipocyte development when cells received only normal alpha-MEM medium at confluence.

TABLE III

*Development of Adipocyte Colonies in Preadipocyte Cultures Derived in Serum-free Medium**

Cell line‡	Weeks of continuous feeding				
	1	1½	2	2½	3
18ABIISF In serum§	0	0	45	48	63
18ABIISF Serum-free medium	—	100	100	100	—
18BIISF In serum§	0	0	23	—	61
18BIISF Serum-free medium	43	73	100	—	100

* Values shown are the percentage of colonies containing Oil Red O-stained adipocytes (10^3 preadipocytes/60-mm dish).
‡ 18ABIISF and 18BIISF are preadipocytes derived by passage of CHEF/18 cells one or three times, respectively, in serum-free medium.
§ Medium containing 5% FBS and insulin (10 μg/ml).

TABLE IV

*Effect of Insulin Concentration on the Development of Adipocyte Colonies in Medium Containing 5% Serum**

Insulin concentration	Weeks of continuous treatment‡				
	1½	2	2½	3	4
μg/ml					
0	0, 0	0, 0	0, 0	1, 0	0, 4
0.001	0, 0	0, 0	0, 0	5	9
0.003	0, 0	0, 0	0, 0	6	—
0.01	0, 0	0, 0	5, 4	11, 15	26
0.1	19, 28	27, 34	33	18, 23	35, 45
1	66, 40	50, 32	54	67, 87	83
10	63, 51	82, 72	81	91, 88	92, 93

* Values shown are number of colony centers with at least 20 Oil Red O-staining adipocytes (10^3 CHEF/18 cells/60-mm dish) in duplicate dishes.
‡ Cells were fed twice weekly starting 3 d after plating. Cells reached confluence at 2 wk after initiation of treatment.

1 ng/ml to 10 μg/ml. Samples were counted at intervals during growth. Even the lowest concentration, 1 ng/ml, induced a few colonies to form adipocytes in 3 wk. A 10-fold higher concentration induced a few adipocytes in 2½ wk, and another 10-fold increase, to 0.1 μg/ml, induced a dramatic increase in adipogenesis seen at 1½ wk. Effects of 1 and 10 μg/ml were indistinguishable from each other and somewhat higher than those induced by 0.1 μg/ml.

Thus, the results show that the rate of appearance and number of differentiating colonies were proportionate to the amount of insulin added to the medium, and that effects of addition of 10 ng/ml were detectable.

GPDH Induction in Serum-free Medium

Stem cells can also be distinguished from preadipocytes by the appearance of a heat-stable form of the enzyme GPDH, in contrast to the heat-labile form of the enzyme present in the stem cells (9). We have assayed the heat stability and

TABLE V

*Activity and Heat Inactivation of GPDH from CHEF/18 Stem Cells and Preadipocytes**

Cell line‡	Total GPDH activity§	$t_{½}$ of GPDH at 50°C	Activity remaining after 2 min at 50°C
	U/mg protein	*min*	*%*
CHEF/18	6.9 ± 0.6	1.1 ± 0.2	16.1 ± 4.9
II/2-2	5.1 ± 0.5	1.7 ± 0.3	41.1 ± 7.9
18ABIISF	4.3 ± 0.4	1.6 ± 0.1	37.4 ± 4.4

* Results are given as the mean of three or more experiments ± standard error.

‡ II/2-2 is a subclone of insulin-induced preadipocytes. 18ABIISF is a preadipocyte line derived by one passage of CHEF/18 cells in serum-free medium.

§ 1 U = 1 nmol nicotinamide adenine dinucleotide oxidized per min.

enzyme activity of GPDH from preadipocytes induced by insulin in serum-free medium for comparison with preadipocytes induced in medium containing serum supplemented with insulin. Cells were grown in roller bottles in medium without insulin and kept subconfluent to prevent spontaneous adipocyte conversion. The results are shown in Table V. The heat-inactivation curves demonstrate that preadipocytes induced by insulin in serum (cell line II/2-2) are indistinguishable by this test from preadipocytes induced in serum-free medium (cell line 18ABIISF), and that both preadipocyte lines contain a more heat-stable enzyme than is present in CHEF/18 stem cells. The total enzyme activities and rates of heat inactivation reported in Table V are very similar to those we have determined for preadipocytes of various origins. The values are clearly distinguishable from those obtained with CHEF/18 adipocytes that have a total activity of 28.5 U/mg and a $t_{½}$ of 5.0 min (Harrison and Sager, manuscript in preparation).

DISCUSSION

This paper presents evidence showing that CHEF/18 stem cells can be induced to differentiate to form adipocytes in serum-free medium containing epithelial growth factor, transferrin, thrombin, and insulin. We find no evidence for the requirement of other adipogenic factors for either the Stage I conversion of stem cells to preadipocytes, or for the Stage II terminal differentiation. Although 10 μg/ml insulin is required for optimal growth in serum-free medium, as little as 0.01 μg/ml of added insulin is sufficient for adipocyte induction in medium that contains serum. Thus, it is likely that insulin, rather than an insulinlike growth factor is required for adipogenesis, as distinct from growth. Whether any other components of the serum-free medium act synergistically with insulin remains an open question.

Our findings differ from the results with 3T3 cells, in which the requirement of an additional serum factor, possibly growth hormone, has been reported (12). Also, a mouse teratocarcinoma-derived fibro-adipogenic line established by Darmon et al. (13) appeared to require serum factors to differentiate, but preincubation in serum-free medium enhanced the potential of these cells to differentiate when serum-containing medium was added. Darmon et al. (13) also isolated from the teratocarcinoma cells a myogenic cell line that differentiated in serum-free medium. The ability to differentiate without exogenous serum factors may be a general property of the myogenic pathway since L6 myoblasts (14) also differentiate in serum-free medium.

Rather than inducing CHEF/18 cells to differentiate, FBS inhibited the rate of adipocyte development in these cultures. Kuri-Harcuch and Green (15) reported the existence of an acid-activated inhibitor of adipocyte differentiation in FBS, but the serum we used was not acidified. Inhibitory factors in normal serum may resemble the inhibitor (DI) of myoblast differentiation in normal FBS discovered by Evinger-Hodges et al. (16).

Evidence that the Stage I conversion occurs in a similar manner in serum-containing and in serum-free media is based on two criteria. (*a*) When insulin in serum-containing medium is added to cells at confluence, preadipocytes differentiate into adipocytes rapidly (Table II), whereas stem cells require at least 4 wk as previously reported (1). In contrast, when insulin is added to growing cells in serum-containing or serum-free medium, both stem cells and preadipocytes differentiate at about the same rate. (*b*) CHEF/18 stem cells contain a heat-labile form of the enzyme, GPDH, whereas preadipocytes induced in serum contain a more heat-stable form of the enzyme. As previously described (17), in other cells the two isozymes are probably coded by different but related genes, suggesting that differentiation involves a switch in transcription from the gene that codes the labile form to the gene that codes the stable form. As shown in Table V, preadipocytes induced in either serum-containing or serum-free medium have about the same total activity and level of heat stability of GPDH.

The finding that confluent CHEF/18 cells are inhibited in adipose conversion compared with growing cells was initially surprising, and led to a clarification of the importance of cell growth in the Stage I process. Whereas terminal differentiation, i.e., Stage II, occurs in confluent cultures, provided that cells have already undergone the Stage I commitment and have become preadipocytes, we found that Stage I is inhibited in confluent cultures.

These results could be interpreted to indicate that during embryogenesis stem cells proliferate and produce mixed populations of parental stem cells and preadipocytes in ratios set by demands from appropriate stimuli, such as insulin in adipocyte differentiation. After a particular cell density in a tissue is attained, however, stem cells would cease production of preadipocytes. Terminal differentiation would then be regulated by receipt of the appropriate signal, presumably an increase in insulin.

The role of cell division in differentiation has been addressed by many investigators, considered in detail by Holtzer et al. (18), and recently reexamined by Taylor and Jones (19) in a cell system somewhat similar to ours. Using the heteroploid embryonic mouse fibroblastic cell line 10T 1/2, Taylor and Jones (19) showed that several doublings were required for expression of the stem cell conversion to myotubes following induction by azacytidine. However, the commitment to myoblast differentiation could be achieved by a short treatment with the drug during the S phase of the cell cycle. We found previously that a single round of the cell cycle in the presence of azacytidine was sufficient for adipocyte conversion (1), but we did not examine shorter times. In this report we show that insulin must be present for four to six doublings to induce Stage I commitment in a large fraction of the treated cells, in either serum-containing or serum-free medium. However, an increase over background occurs after a single day of insulin treatment (Fig. 2), suggesting that a short exposure to insulin may indeed induce the Stage I process in a susceptible

fraction of cells. Susceptibility may occur at a specific stage in the cell cycle.

If insulin and azacytidine do induce Stage I during a single S phase of the cell cycle, our earlier proposal (1) that these two agents induce the same molecular event would be strengthened. This molecular event may be the induced expression of a commitment protein. The fact that >90% of the CHEF/18 stem cells become committed to the adipocyte pathway during serum-free growth provides a unique opportunity for molecular studies of the commitment process and the direct search for a postulated commitment protein.

We thank S. James for preparing the manuscript.

This work was supported in part by National Institutes of Health Training Grant CA09361 and CA24828.

Received for publication 15 June 1984, and in revised form 15 August 1984.

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