Studies on Cell Proliferation in Inguinal Adipose Tissue during Early Development in the Rat

A.M. GABEN-COGNEVILLE[1] and E. SWIERCZEWSKI, U. 29 I.N.S.E.R.M., Centre de Recherches de Biologie due Développement Foetal et Néonatal, 123 Bd de Port-Royal – 75014 Paris (France)

ABSTRACT

[2-^{14}C] Thymidine was injected into rats aged 3, 5 and 10 days, and incorporation of the precursor into deoxyribonucleic acid (DNA) of the inguinal fat tissue was measured for short time periods. Using chromatographic procedures to measure the distribution of thymidine and its metabolites in the soluble fraction of the tissue, degradation of the precursor was found to be similar at all ages. The data indicate that thymidine was more rapidly utilized for DNA synthesis in 3-day-old rats than in older animals. When ^{14}C-thymidine was injected in vivo and adipocytes and stromal cells were then separated from the inguinal tissue of 3-and 5-day-old rats, the incorporation into DNA was significant in both types of cells already 30 min after pulse labeling. Stromal cells took up twice as much of label as the adipocytes. Furthermore, real incorporation into DNA was found in the adipocytes when incubated in vitro in a culture medium supplemented with ^{14}C-thymidine. The possibility is discussed that early in postnatal life adipocytes might synthesize DNA for further cell division.

INTRODUCTION

Two questions are of particular interest to the investigator of adipose tissue development: is the number of adipose cells determined very early in the developing fat deposit and which cell types have the potential to multiply?

Some evidence suggests that cell number becomes fixed early in life both in obese humans and animals. The possibility of affecting cell multiplication in animals either by manupulating dietary intake or by hypothalamic lesions decreases as the animals grow older (1-3). The most interesting report on man is that early onset of obesity is associated with a greater increase in cell number than obesity beginning later in life (4,5). Most of these investigations on the cellularity of different adipose sites used counting and sizing techniques (6) which resulted in misinterpretation of the cell number in very young animals (7). Experiments using thymidine incorporation into (DNA) deoxyribonucleic acid as an index of cellular multiplication either were carried out with adult animals (8,9) or else labeling periods were too long to determine the real number and site of cells in the proliferative process (10). In addition, these experiments were performed with epididymal fat, which is not representative of the other adipose sites. Differences are observed in cellularity, in fat cell size, and in response to several stimuli between one adipose site and another (3,11, 12).

In the present study, we used the inguinal fat pad of newborn rats, which develops early and becomes macroscopically visible 12 hr after birth (13). With this tissue, it was possible to follow the different steps of development using the incorporation of ^{14}C-thymidine into DNA during short labeling periods. The kinetics of incorporation of thymidine vary with age and tissues and are influenced by the rate of degradation of the precursor after injection (14,15). Since no information was available on this point in the tissues of newborn rats, we also measured changes in labeled thymidine and its metabolites in the non-precipitable fraction of the whole tissue. Other experiments were performed on isolated adipocytes and stromal cells in vitro or after injection of thymidine in vivo to investigate whether cell multiplication is restricted to the stromal cells or whether adipocytes participate in the proliferative process during the early stages of development.

MATERIALS AND METHODS

Chemicals

Radioactive biochemicals: [2-^{14}C] thymidine (50 mCi/mmole) and [*methyl*-^{14}C] thymidine (50 mCi/mmole) were purchased from the Commissariat à l'Energie Atomique (Saclay – France); tissue culture medium 199 was obtained from Institut Pasteur (Paris – France). Collagenase and DNase I were obtained from Worthington Biochemical Corp. (Eurobio – France). Unlabeled thymidine, thymine, thymidine triphosphate (TTP), thymidine diphosphate (TDP), thymidine monophosphate (TMP) and β-Aminoisobutyric acid (AIBA) were obtained from Sigma (Eurobio – France). Other chemicals were obtained from Merck (Socolab – France).

[1] To whom correspondence should be addressed.

Animals

Three-, 5- and 10-day-old Sherman rats obtained from Janvier Breeding Laboratory (Le Genest – France) were used. In another set of experiments, adult animals (6 mo. old, weighing ca. 400 g) were used as controls. Animals were chosen which corresponded to average age and weight relationships determined from animals within the colony. These adult rats were fed ad libitum with a standard diet made up by "Extralabo," Pietrement Lab., Ste. Colombe, Provins, France. The composition of that diet was: Protein 25%; Fat 4%; Cellulose 6%; Minelras 9%; H_2O 12%; Vit. E 1.500 mg/100 kg; Vit. A 1.000 000 UI/100 kg; Vit. D_3 200.000 UI/100 kg. Radioactive compounds were administered by subcutaneous injection at a dose of 0.2 μCi per g body weight in 0.1 ml saline.

Experimental Procedures

The animals were decapitated 30, 60 and 120 min after the injection. The left and right inguinal fat pads were rapdily dissected, pooled and weighed. In all experiments, the whole tissue or the isolated cells were homogenized manually in a tight fitting glass grinder (Kontes, Polylabo – France). In experiments with whole inguinal tissue, total lipids were extracted three times with chloroform/methanol (2:1, v/v) according to Folch et al. (16). The methanol-water layer of the chloroform/methanol extracts contained 75% of the total free ^{14}C-thymidine and its phosphorylated derivatives and degradation products. This was shown by paper chromatography after evaporation of the extracts as described below. To assure complete removal of unincorporated thymidine and derivatives, the pellets containing the nucleic acids and proteins were dried with air and washed again three times with 10% cold TCA (no radioactivity was present in the last washing). The dry extracts obtained by the Folch extraction method and the combined TCA supernatants were measured separately for radioactivity. The total amount of label found in these fractions was called hereafter "total radioactivity in soluble fraction."

RNA and DNA were separated from the pellets by the method of Schmidt and Thannhauser (17). Aliquots of the RNA and DNA fractions were taken to measure the radioactivity. No label was found in the RNA fraction, showing that a good separation of the nucleic acids was achieved. The amount of DNA was determined by the colorimetric assay of Burton (18) adapted for low concentrations.

Parallel experiments were done using DNase digestion treatment to check whether the radioactivity of the precipitable fraction was only incorporated into DNA. After extraction by chloroform/methanol and subsequent TCA washing, the tissue residue containing nucleic acids and proteins was digested in 1N KOH for at least 15 hr at 37 C. In these conditions, DNA was resistant to hydrolysis as shown by Steudel and Peiser (19). The reaction was stopped by addition of 6N HCl and the precipitate was centrifuged. The pellets were incubated during 3 hr at 37 C in a medium containing Mg SO_4 (0.9 μmole), sodium acetate buffer (pH = 5, 150 μmoles), DNase (30 U/100 μg DNA) in a total volume of 1.8 ml. The reaction was stopped by 600 μl of 20% TCA. The DNA was determined in the supernatant by the Burton colorimetric method. A standard curve of DNA was treated in the same conditions as above. An aliquot of the DNA supernatant was measured for radioactivity as in former experiments.

In another set of experiments, the animals were killed 30 and 60 min after injection of ^{14}C-thymidine. The tissue was rapidly dissected and adipocytes and stromal cells were isolated from the whole tissue by the collagenase method of Rodbell with minor modifications (20). Pooled white fat was thoroughly minced with scissors, placed in closed plastic vials in 4 ml bicarbonate buffer (pH = 7.4) containing 3 mg/ml of crude collagenase. The bicarbonate buffer contained 2% bovine albumin instead of 4%, and glucose was omitted from the incubation medium. The incubations were carried out at 37 C in a shaking water bath during 40 min. At the end of incubation, the suspension of cells was diluted with fresh buffer, filtered through silk and centrifuged for 3 min at 200 x g. This procedure yielded two distinct cell pools, fat cells floating to the surface and stromal cells which settled. Both cell preparations were washed three times with the buffer without albumin and were homogenized as previously described. Lipids were extracted from the adipocytes with chloroform/methanol. The adipocytes and the stromal cells were washed with 10% TCA, and the nucleic acids were extracted from the pellets by the usual procedure. The [2-^{14}C] thymidine incorporation into DNA was measured in both cellular pools. In additional experiments, after incubation with collagenase, the separated cells were washed with phosphate-buffered saline (PBS), without $CaCl_2$ and $MgCl_2$ (pH = 7.4) and then once more with PBS-EDTA (0.02%) to remove the Ca^{++} ions of the Krebs-Ringer medium and prevent cellular aggregation.

In vitro studies: adipocytes and stromal cells

TABLE I

Various Aspects of Growth and Development of Inguinal Adipose Tissue at 3, 5 and 10 Days Postnatally

Age Days	Inguinal tissue total weight[a] mg	Total DNA[a] mg	Protein[a] mg/gm tissue	Lipids[a] mg/gm tissue
3	86 ± 2[b] (n = 31)[c]	0.128 ± 0.009[b] (n = 31)[c]	24.1 ± 1.0[b] (n = 8)[c]	421 ± 10[b] (n = 54)[c]
5	134 ± 7[b] (n = 24)[c]	0.218 ± 0.015[b] (n = 24)[c]	31.7 ± 1.5[b] (n = 12)[c]	561 ± 13[b] (n = 35)[c]
10	321 ± 11[b] (n = 14)[c]	0.281 ± 0.025 (n = 14)[c]	21.7 ± 0.8[b] (n = 10)[c]	669 ± 18[b] (n = 21)[c]

[a]Results are means ± SE of n rats.
[b]Differences between 3, 5 and 10 days in all data presented here are highly significant ($P<0.01$).
[c]Number of rats used in the experiment.

were isolated from the inguinal fat pads by the collagenase method, but a PBS (pH = 7.4) was used instead of the bicarbonate buffer. The two cell pools were washed four times with PBS and then were suspended in Medium 199 in Hanks BSS containing 10% fetal calf serum, penicillin (100 U/ml) and streptomycin (100 μg/ml) at pH = 7.4. Before incubation, the medium was gassed with 95% air 5% CO_2. The cell suspensions were incubated for 1 hr at 37 C in closed plastic vials containing 10 μCi of [2-^{14}C]thymidine in 8 ml culture medium. The vials were gently shaken during the incubation period. The reaction was stopped by cooling to 0 C and cell suspensions were rapidly washed twice with medium 199 and twice again with PBS buffer medium. Before extraction of the nucleic acids, special care was taken to completely remove the residual-free ^{14}C-thymidine by washing the pellets several times with cold 10% TCA. DNA was extracted from stromal cells and adipocytes as described previously. [2-^{14}C]Thymidine incorporation was measured in the DNA extracts.

The distribution of radioactivity was determined by paper chromatography (15,20) at different time intervals after injection and at different ages in the compounds which were present in the methanol-water layer after chloroform/methanol extraction. After evaporation of the chloroform/methanol extracts, the residue was treated by a mixture of ether/H_2O. In this procedure, the nucleotides and derivatives were recovered in the aqueous phase. This phase was concentrated by freeze-drying, and the samples were chromatographed on Whatman paper n°1 in the following solvent systems: (a) ETAA: 90% ethanol and 1M ammonium acetate containing 0.1M EDTA (70:30) and (b) EtAc form: upper phase from a mixture of ethyl acetate/water/formic acid (60:35:5). The radioactive spots were characterized by the use of unlabeled markers of thymine, thymidine, TMP, TDP, TTP and AIBA. The position of the spots was located with ultraviolet light (2537 Å), and the spots were eluted with 0.1M HCl. The amount of radioactivity present in individual spots was determined by liquid scintillation counting (15,21).

Radioactivity was measured in a liquid scintillation spectrometer (Nuclear Chicago Mark I). The degree of quenching among samples was corrected using quench curves.

The amount of proteins was determined by the method of Lowry et al. (22) in an aliquot of the KOH extract used for nucleic acid separation.

Total lipids were measured by the gravimetric method after evaporation of the chloroform/methanol extract.

Optical and electron microscopic studies were carried out with isolated adipocytes and stromal elements.

The results were analyzed by standard statistical procedures (Fischer t-test).

RESULTS

Adipose Tissue Growth and Chemical Composition (Table I)

Postnatal total weight increases varied significantly ($p<0.01$) by 56% between days 3 and 5 and by 140% between days 5 and 10.

A very rapid parallel increase in total DNA content was observed between the 3rd and 5th days (70%). After the 5th day, the total DNA content increased to a lesser extent (30%). Protein concentration was maximal at 5 days of age and then declined. The percentage of lipid rose regularly during the entire period studied. These results indicate that growth of inguinal

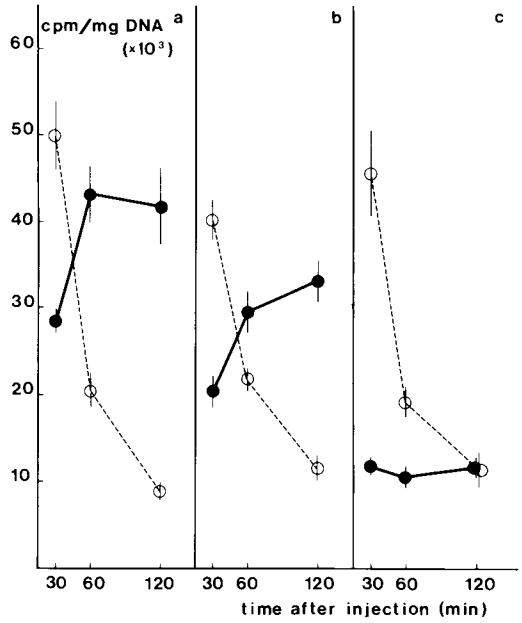

FIG. 1. Incorporation of [2-^{14}C] thymidine into the soluble fraction (○) and the DNA (●) of the inguinal tissue of (a) 3-day-old rats, (b) 5-day-old rats and (c) 10-day-old rats. Each point is the mean ± SE for 3 experiments carried out with pooled tissue from at least 3 animals.

fat tissue was for the most part correlated with cellular multiplication up to 5 days. Thereafter, the increment in adipose tissue was the result of both the increase in adipose cell number and the enlargement of cell size as shown by the fall of protein and the increase in lipid.

In Vivo Incorporation of Radioactive Thymidine in the Total Tissue

The data derived from these studies are shown in Figure 1 and Table II.

Thirty minutes after injection, the [2-^{14}C] thymidine incorporated into DNA of the whole tissue was high at 3 and 5 days of age (28,200 and 20,400 cpm/mg DNA, respectively, Fig. 1). At both stages of development, the time courses of incorporation were similar and attained a maximum of radioactivity at 60 min. On day 3, the difference of incorporation between 30 and 60 min was significant. Ten days after the animal's birth, the labeling of DNA was lower (ca. 11,000 cpm/mg DNA) and remained stable throughout the experiment. An inverse relationship between the disappearance of the radioactivity from the soluble compartment and the incorporation of the precursor into DNA as a function of time was observed in Figure 1 in 3- and 5-day-old but not 10-day-old rats.

TABLE II

Distribution of [methyl-^{14}C] Thymidine in the Soluble Components of Rat Inguinal Tissue during Postnatal Development[a]

Time after injection	Three days[b]			Five days[b]			Ten days[b]		
	30 Min	60 Min	120 Min	30 Min	60 Min	120 Min	30 Min	60 Min	120 Min
TMP[c] + TDP + TTP	1915 ± 323 (2.2)[e]	3460 ± 566 (7.5)[e]	1590 ± 133 (10.3)[e]	1585 ± 326 (2.7)[e]	1528 ± 93 (5.4)[e]	1012 ± 78 (8.8)[e]	1946 ± 388 (3.1)[e]	2882 ± 567 (6.6)[e]	1676 ± 187 (8.8)[e]
Thymidine	53297 ± 6463 (60.0)[e]	10765 ± 2246 (23.1)[e]	3335 ± 294 (21.7)[e]	26625 ± 484 (45.0)[e]	5482 ± 420 (13.6)[e]	2130 ± 257 (18.5)[e]	29964 ± 2770 (47.9)[e]	12173 ± 1248 (28.2)[e]	4461 ± 202 (21.0)[e]
Degradation Products[d]	26456 ± 2585 (30.6)[e]	25136 ± 2652 (55.0)[e]	7795 ± 1552 (49.8)[e]	24767 ± 386 (41.9)[e]	17221 ± 2320 (61.0)[e]	5341 ± 413 (50.5)[e]	23053 ± 1316 (37.0)[e]	22419 ± 1197 (52.1)[e]	11465 ± 1484 (53.4)[e]
Total radioactivity in soluble fraction	86271 ± 8081	46205 ± 7901	15553 ± 2277	59111 ± 845	28147 ± 3419	11486 ± 684	62430 ± 4147	43158 ± 3572	21403 ± 2151

[a]Results are expressed in cpm/mg DNA.
[b]Results are means ± SE of two experiments. Each experiment includes left and right pads from 3 animals.
[c]TMP, thymidine monophosphate; TDP, thymidine diphosphate; TTP, thymidine triphosphate.
[d]The compounds includes thymine, two unidentified products and amino-iso-butyric acid which was the main substance.
[e]Results in brackets represent percent of total soluble radioactivity in each component.

TABLE III

Incorporation of ^{14}C-Thymidine into DNA of Adipocytes and Stromal Cells Isolated from Inguinal Tissue, after Injection in vivo of the Precursor to 5-Day-Old Rats

Incorporation periods	Adipocytes[a] cpm/mg DNA	Stromal cells[a] cpm/mg DNA
30 Min	11500 ± 920 (n = 9)[b]	23100 ± 1390 (n = 9)[b]
60 Min	13000 ± 1500 (n = 7)[b]	24100 ± 960 (n = 7)[b]

[a]Results are means ± SE.
[b]n = Number of experiments carried out with pooled tissue from at least 7 animals. The experiments on adipocytes and stromal cells were always done in parallel.

TABLE IV

Incorporation of ^{14}C-Thymidine into DNA of Adipocytes and Stromal Cells Isolated from Inguinal Tissue of 3-Day-Old Rats: (a) Effect of Two Different Washing Procedures during Cell Fractionation and (b) Effect of Two Different DNA Extraction Methods

	30 Min		60 Min	
	Buffer + EDTA	Buffer - EDTA	Buffer - EDTA	
Incorporation periods	TCA extraction	TCA extraction	TCA extraction	DNase extraction
Adipocytes[a]	25310 ± 2700 (n = 4)[b]	23150 ± 2100 (n = 5)[b]	25700 ± 1250 (n = 7)[b]	20500 ± 2900 (n = 7)[b]
Stromal cells[a]	37630 ± 2100 (n = 4)[b]	33500 ± 1500 (n = 5)[b]	43100 ± 1700 (n = 7)[b]	40800 ± 1500 (n = 7)[b]

[a]Results are means ± SE.
[b]n = Number of experiments carried out with pooled rats from at least 12 animals.

The distribution of the radioactivity in the metabolites of the soluble compartment was measured using [methyl-^{14}C]thymidine. This precursor was used instead of [2-^{14}C]thymidine because with the latter all the degradation products were not labeled. With both precursors, no age changes occurred in the total radioactivity of the soluble fraction. A rapid decline in their radioactivity was observed between 30 and 60 min after injection. Two hours later, only 20% of the initial value was detected in the soluble fraction on days 3, 5 and 33% on day 10. The level of ^{14}C activity in the thymine nucleotides at various time periods after injection and at various ages appeared to be constant, whereas the radioactivity of free thymidine decreased. Between 30 and 120 min, this decline varied as a direct function of age. The radioactivity of the thymidine decreased 18-fold at 3 days, 12-fold at 5 days and only 5-fold at 10 days. Label present in the degradation products was constant between 30 and 60 min and then declined at all ages as a result of elimination. However, the relative proportion of the radioactivity present in these metabolites increased during the first 60 min and remained unchanged thereafter (Table II, results in brackets). Approximately 60% of the radioactivity in the degradation products was found in β-AIBA.

Thus, the decrease in the rate of labeled thymidine incorporation into DNA between days 3 and 10 was not the result of an increased rate of degradation of thymidine but a real reduction of DNA synthesis in the whole tissue.

Incorporation of ^{14}C-Thymidine into DNA of Isolated Adipocytes and Stromal Vascular Cells after Injection of the Precursor in vivo

We examined the tissue compartmentalization of radioactivity in order to provide further information about the sites of cell proliferation (Table III). The data indicate that a real incorporation of ^{14}C-thymidine into adipocytes DNA occured in 5-day-old rats even in the 30-min pulse period and remained at the same level 60 min after injection. The specific activity found in the stromal fraction was only double that of the adipocyte fraction. The similar values of incorporation found at 30 and 60 min were probably the consequence of the further 40 min incubation during cell dissociation.

In Table IV, we compared the values of

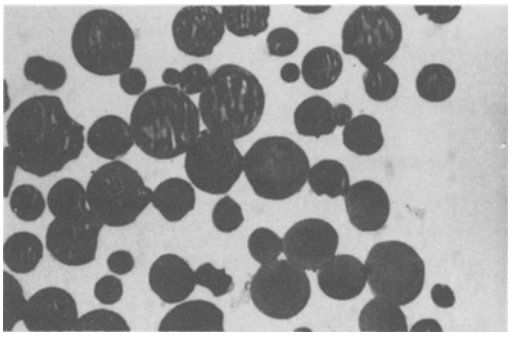

FIG. 2. Photomicrographs of free fat cells obtained from collagenase-treated rat inguinal adipose tissue. The cells were fixed in 1.7% glutaraldehyde in buffer-saline (0.1M sodium cacodylate buffer) and embedded in Epon-Araldite. Original magnification x 250.

DNA specific activity measured either after DNA digestion or after TCA extraction. In spite of slightly lower results obtained with DNase, no significant differences were found.

In order to check whether some contamination of the adipocyte fraction with stromal cells occurred, additional experiments were performed using PBS deprived of Ca^{++} and Mg^{++} and supplemented with EDTA in the washing procedures to avoid cellular aggregation (Table IV). Both cell pools incorporated ^{14}C-thymidine at the same level with or without EDTA.

Furthermore, stromal vascular cells were absent from fat cell preparations when examined by optical microscopy (Fig. 2).

In vitro ^{14}C-Thymidine Incorporation into DNA of Isolated Cell Fractions

In order to verify that the presence of the labeled DNA in the adipocytes was not due to a rapid differentiation occurring during the course of the experiment, we separated the cells in vitro before the incorporation of radioactive thymidine.

When stromal cells and adipocytes were incubated separately for 60 min with labeled thymidine in a culture medium, an appreciable amount of radioactivity was recovered in the DNA of both cell types (Table V). In the stromal cell types, the incorporation rate of thymidine was again double that of the adipocytes.

A parallel experiment using epididymal adipose tissue from 6-month-old adult rats was performed in order to determine the basal activity of a tissue which has ceased to multiply (1). Here, very low activity was observed in the DNA of the 2 cell pools, about 5 to 10 times lower than in the inguinal tissue of young rats (Table V).

DISCUSSION

The results presented here show that a high rate of cell multiplication and DNA synthesis occured between birth and 5 days after birth in the inguinal fat tissue of the infant rat. We observed that neither the penetration of the precursor into the cell nor its rate of degradation was modified during this period. In the inguinal tissue, 60% of the radioactivity was recovered in thymidine 30 min after the beginning of the experiment. In contrast, Chang and Looney (15) in an adult regenerating rat liver showed that, 2 min after precursor injection, 85% of the total radioactivity was found in the degradation products. The high level of radioactivity found in the DNA of the adipocytes after cellular separation supports the hypothesis of a real participation of these cells in the proliferative process of the young rat's inguinal tissue. The incorporation of thymidine into the DNA of the adipocytes has been a matter of discussion for a long time. It was assumed by many authors that it was a conse-

TABLE V

In vitro Incubation during 60 Min of Adipocytes and Stromal Cells with 1 μCl/1 ml ^{14}C Thymidine, after Separation of the Cells from Inguinal Tissue of Five-Day-Old Rats. Comparison with the Incorporation into DNA of Cells Isolated from the Epididymal Tissue of Adult Rats

Age	Adipocytes[a] cpm/mg DNA	Stromal cells[a] cpm/mg DNA
5 days	12800 ± 1200[b] (n = 9)[b]	29400 ± 2560[b] (n = 9)[b]
adults 6 months	2400 ± 300 (n = 3)[c]	3310 ± 820 (n = 3)[c]

[a]Results are means ± SE.
[b]Significantly different from the adult rats. $P<0.01$.
[c]n = Number of experiments carried out with pooled tissue from at least 10 rats aged three days and two adults.

quence of contamination of the adipose cells with stromal elements. Most of these experiments were conducted with adult animals, and the preparations probably contained connective and supportive tissue cells (8,9). However, 60 min after ^{3}H-thymidine injection, Greenwood and Hirsch (10) observed a real incorporation of the precursor into the DNA of adipocytes from epididymal tissue of 9-day-old rats. We could corroborate these results using inguinal tissue in the early stages of development and short labeling periods. The assumption of contamination by cellular aggregation could be discarded. Using a medium deprived of Ca^{++} and supplemented with EDTA during the washing procedures, we did not improve appreciably our results. Furthermore, taking as a basis the relative levels of radioactivity observed in the adipocytes and stromal cells, one would have to assume stromal contamination of at least 50%. Electron and optical microscopic studies with isolated adipocytes showed an homogeneous cell type presenting a characteristic spherical shape with a large central droplet and a flattened eccentric nucleus. These observations ruled out extensive contamination. In the adipocytes isolated from 5-day-old rats and incubated in vitro in a culture medium with ^{14}C-thymidine, we found label in the DNA. Hollenberg and Vost (8) did not find any radioactive thymidine in the adipocytes from epididymal tissue incubated in vitro. In these experiments, adult rats were used, and the authors reported that the cells were probably damaged during separation. The results presented here show that at an early stage of development, when the tissue is increasing especially by hyperplasia, adipocytes, like stromal cells, incorporated ^{14}C-thymidine into the DNA. This ability decreased as the animals grew older. The hypothesis, according to which the radioactivity accumulated in the adipocytes could originate in the very rapidly differentiating stromal cells, appeared unlikely. Indeed, the very short incorporation times used in our experiments do not warrant this interpretation if we take durations of various known cell cycles into consideration (23). Can one say, though, that these differentiated cells which actively incorporated thymidine into the DNA would be able to divide? One might suppose, with present knowledge, that the adipocytes having incorporated thymidine remained blocked in G 2 of the cell cycle. This phenomenon has been described for other cells (24). We can imagine that, under the influence of certain hormonal or nutritional stimuli, particularly in the young animal, these cells can become free of lipids and divide.

ACKNOWLEDGMENT

We are grateful to Dr. M. Lesourd from the Department of Electron Microscopy (Institut Pasteur) for verifying the cell preparations with optical and electron microscopy. We thank Dr. J.M. Roux for helpful criticism during the course of this work. We are indebted to Prof. P. Hahn (Departments of Pediatrics, Obstetrics and Gynecology, University of British Colombia, Vancouver) for his interest in this work and valuable comments in the preparation of this manuscript.

REFERENCES

1. Hirsch, J., and P.W. Han, J. Lipid Res. 10:77 (1969).
2. Knittle, J.L., and J. Hirsch, J. Clin. Invest. 47:2091 (1968).
3. Lemonnier, D., J. Clin. Invest. 51:2907 (1972).
4. Hirsch, J., and J.L. Knittle, Fed. Proc. 29:1516 (1970).
5. Adebonojo, F.D., Yale J. Biol. Med. 48:9 (1975).
6. Hirsch, J., and E. Gallian, J. Lipid Res. 9:110 (1968).
7. Stern, J.S., and M.C.R. Greenwood, Fed. Proc. 33:1952 (1974).
8. Hollenberg, C.H., and A. Vost, J. Clin. Invest. 47:2485 (1968).
9. Kazdova, L., P. Fabry, and A. Vrana, Diabetologia 10:77 (1974).
10. Greenwood, M.C.R., and J. Hirsch, J. Lipid Res. 15:474 (1975).
11. Johnson, P.R., L.M. Zucker, J.A.F. Cruce, and J. Hirsch, J. Lipid Res. 12:706 (1971).
12. Johnson, P.R., and J. Hirsch, J. Lipid Res. 13:2 (1972).
13. Pequignot-Planche, E., P. de Gasquet, A. Boulangé, and Nhu Thuy Tonnu, Biochem. J. 162:461 (1974).
14. Schneider, N.C., and A.E. Greco, Biochim. Biophys. Acta 228:610 (1971).
15. Chang, L.O., and W.B. Looney, Cancer Res. 25:1817 (1965).
16. Folch, J., M. Lees, and G.H. Sloane-Stanley, J. Biol. Chem. 226:497 (1957).
17. Schmidt, G., and S.J. Thannhauser, J. Biol. Chem. 161:83 (1945).
18. Burton, K., Biochem. J. 62:315 (1956).
19. Steudel, H., and E. Peiser, Z. Physiol. Chem. 120:292 (1922).
20. Rodbell, M., J. Biol. Chem. 239:375 (1964).
21. Fink, K., R.E. Cline, and R.M. Fink, Anal. Chem. 35:369 (1963).
22. Lowry, O.H., J.J. Rosebrough, A.L. Farr, and R.J. Randall, J. Biol. Chem. 193:265 (1951).
23. Graham, C.F., in "The Cell Cycle in Development and Differentiation," edited by M. Balls and F.S. Billett, Cambridge, Great Britain, 1973, p. 293.
24. Prescott, D.M., in "Reproduction of Eukaryotic Cells," Academic Press, New York, 1976, p. 81.

[Revision received February 21, 1979]