Biochem. J. (1988) 249, 765–769 (Printed in Great Britain)

Regulation of lipoprotein lipase mRNA content in 3T3-L1 cells by tumour necrosis factor

Peter CORNELIUS,* Sven ENERBACK,† Gunnar BJURSELL,† Thomas OLIVECRONA‡ and Phillip H. PEKALA*§

*Department of Biochemistry, East Carolina University School of Medicine, Greenville, NC 27858, U.S.A.,
†Department of Physiological Chemistry, University of Gothenburg, S-413 46 Gothenburg, Sweden, and
‡Department of Physiological Chemistry, University of Umea, S-90187 Umea, Sweden

Tumour necrosis factor (TNF) was previously shown to suppress lipoprotein lipase (LPL) synthesis and activity in 3T3-L1 adipocytes. The present study examined the effect of TNF on amounts of mRNA for LPL in 3T3-L1 cells. Northern-blot analysis of polyadenylated RNA using a cDNA probe to guinea-pig LPL identified two predominant species of LPL message, 3.7 and 3.9 kilobases in size. The steady-state amounts of these mRNAs increased 10-fold upon expression of the adipocyte phenotype. A single dose of 1.5 nM-TNF decreased LPL mRNA by approx. 60% in 17 h with a corresponding decrease in LPL activity, an effect that was reversed 48 h after exposure to TNF. The results demonstrate that TNF reversibly down-regulates LPL mRNA in fully differentiated 3T3-L1 adipocytes. Cells induced to differentiate in the presence of 1.5 nM-TNF exhibited a delayed time course for development of the adipocyte phenotype, as judged by attenuation of the normal increase in LPL mRNA that occurs with differentiation.

INTRODUCTION

Lipoprotein lipase (LPL) catalyses the hydrolysis of lipoprotein-derived triacylglycerol, providing fatty acids for storage or catabolism in specific mammalian tissues. Its activity in adipose tissue allows the accumulation of fatty acids, which are re-esterified in adipocytes to form stored triacylglycerol. The enzyme is subject to potent regulation by both nutritional and hormonal signals, with marked loss of the adipose-tissue enzyme activity occurring upon fasting (Semb & Olivecrona, 1986). Diminished LPL activity in various disease states, such as chronic infections and malignancies, leads to derangements in lipid metabolism, resulting in severe hypertriglyceridaemia (Marclay *et al.*, 1967; Brenneman *et al.*, 1975; Kaufmann *et al.*, 1976; Rouzer & Cerami, 1980). Moreover, in many of these studies an overall catabolic state was observed. Regulation of the onset of this catabolic state, as well as the suppression of LPL activity, have been attributed to a group of proteins known collectively as monokines, which are secretory proteins from the phagocytic cells of the immune system (Bagby & Spitzer, 1980; Kawakami & Cerami, 1981; Kurzrock *et al.*, 1986; Beutler & Cerami, 1986*a*; Nathan, 1987). These proteins, interacting with specific plasma-membrane receptors on target tissues, regulate diverse metabolic pathways. In adipocytes, three monokines, i.e. tumour necrosis factor (TNF), interleukin-1 and γ-interferon, have been shown to attenuate the activity of LPL (Beutler *et al.*, 1985; Price *et al.*, 1986*a,b*; Beutler & Cerami, 1986*b*; Patton *et al.*, 1986). Price *et al.* (1986*a,b,c*) have demonstrated that at least TNF and interleukin-1 suppress LPL activity in 3T3-L1 adipocytes by inhibiting the synthesis of the enzyme. This conclusion was based on data demonstrating that, after the cells were incubated with either monokine, the incorporation of [^{35}S]-methionine into immunoprecipitable LPL was inhibited. In addition, these monokines had no effect on the half-life of LPL activity. Lipoprotein lipase is a short-lived enzyme; half-lives of approx. 30 min have been reported (Robinson, 1970; Wise & Green, 1978; Vannier *et al.*, 1985; Amri *et al.*, 1986; Semb & Olivecrona, 1986; Olivecrona *et al.*, 1987). Although there has been no direct information on the turnover of LPL mRNA, the rapid decrease of LPL synthesis in the 3T3-L1 adipocytes exposed to TNF, coupled with the short half-life for enzyme activity, suggests that the mRNA is rapidly turned over. Thus TNF regulation of LPL synthesis may occur at the level of monokine-induced alterations in LPL mRNA content, secondary to decreased gene activity or mRNA stability.

The availability of a cDNA probe (Enerback *et al.*, 1987) presented an opportunity to test directly whether TNF down-regulation of LPL mRNA is involved in regulation of the enzyme's activity. Using 3T3-L1 cells, we have examined the effect of TNF on LPL mRNA amounts and LPL enzyme activity, in order to define the step at which TNF inhibits LPL synthesis.

EXPERIMENTAL

Miscellaneous techniques

3T3-L1 preadipocytes obtained from Dr. Howard Green, Harvard University, Boston, MA, U.S.A., were cultured and induced to differentiate as previously described by Price *et al.* (1986*b*). Exposure of the cells to TNF was carried out by the protocol described by Price *et al.* (1986*b*) unless otherwise noted. LPL activity associated with the heparin-releasable fraction of the monolayers was determined by the method of Kawakami *et al.* (1982). Enzyme activity is reported in munits,

Abbreviations used: LPL, lipoprotein lipase; TNF, tumour necrosis factor; poly(A)$^+$ RNA, polyadenylated RNA; kb, kilobase.
§ To whom correspondence and reprint requests should be addressed.

defined as nmol of fatty acid released/min. Although we realize the limitations of determining the activity of the lipase with a heparin-releasable fraction, our previous studies have demonstrated that the activity in this fraction is the most sensitive assay for TNF and accurately reflects whole-cell lipase activity (Kawakami *et al.*, 1982). Results of these enzyme assays are summarized as means ± S.D. All assays were performed in triplicate. Protein values were determined by the method of Bradford (1976).

RNA isolation

Total RNA was isolated from cells by extraction with guanidine isothiocyanate and centrifugation through 5.7 M-CsCl as described by Chirgwin *et al.* (1979). Poly(A)$^+$ RNA was isolated from total RNA by using oligo(dT)–cellulose as per the manufacturer's instructions.

RNA analysis

For Northern analysis, 20 μg of total RNA or 4 μg of poly(A)$^+$ RNA was separated by electrophoresis in 0.8% or 1.1% agarose/2.0 M-formaldehyde gels and transferred to NitroPlus 2000, as described by Maniatis *et al.* (1982). After baking for 2 h at 80 °C in a vacuum oven, filters were prehybridized at 46 °C for 4 h in 50% (v/v) formamide containing 5 × SSPE (0.9 M-NaCl/0.05 M-NaH_2PO_4/5 mM-EDTA, pH 7.4), 0.2% SDS, 0.1% each of bovine serum albumin, polyvinylpyrrolidone and Ficoll, and 200 μg of yeast tRNA/ml. Hybridizations were carried out overnight under the same conditions in the presence of 1×10^7 c.p.m. of a nick-translated LPL cDNA probe (Enerback *et al.*, 1987)/ml and 3.0×10^6 c.p.m. of a nick-translated cDNA probe to chicken β-actin (Cleveland *et al.*, 1980)/ml. The LPL cDNA probe was a 2.2 kb fragment containing the *C*-terminal half of the coding sequence for guinea-pig LPL and 1.55 kb of the 3′-untranslated region. After hybridization, filters were washed in 0.1 × SSPE/0.1% SDS for approx. 2 h at 64 °C with constant agitation and then subjected to autoradiography by using Kodak XAR-5 film for various times at −80 °C to ensure linear film response. The relative intensity of bands on the autoradiograph was quantified with a Biomedical Instruments (Fullerton, CA, U.S.A.) laser densitometer equipped with a peak integrator. The RNA slot-blot analysis was carried out with 5 μg of total RNA which was denatured by heating for 10 min at 65 °C in 4% formaldehyde and then applied to NitroPlus 2000 with a Bethesda Research Laboratories Hybri-Slot Manifold. After baking for 2 h at 80 °C in a vacuum oven, the slot-blot was probed with nick-translated LPL-cDNA probe as described above.

Materials

Recombinant murine TNF was generously given by Biogen (Cambridge, MA, U.S.A.). The specific activity was 9.6×10^6 units/mg of protein, based on a cytotoxicity assay using L929 cells. The endotoxin contamination was 0.12 ng/mg of protein, based on a *Limulus* amaebocyte lysate assay. The chicken β-actin cDNA probe was obtained from Dr. Don W. Cleveland, Johns Hopkins University. A nick-translation kit was purchased from Bethesda Research Laboratories. [^{32}P]dCTP (800 Ci/mmol) and [^{3}H]triolein were purchased from New England Nuclear. NitroPlus 2000 (nitrocellulose) was purchased from Fisher. Oligo(dT)–cellulose was obtained from Collaborative Research.

RESULTS

Molecular size of LPL mRNA in the 3T3-L1 cells

The molecular size of mRNA coding for LPL was determined by using RNA isolated from both undifferentiated and fully differentiated 3T3-L1 cells as well as from mouse heart; 20 μg of total RNA or 4 μg of poly(A)$^+$RNA from each cell type or tissue was used for Northern analysis with nick-translated LPL-cDNA probe as described in the Experimental section. After autoradiography, it was possible to identify two major species of LPL mRNA in 3T3-L1 cells (Fig. 1, lanes *a*, *b* and *d*). For comparison, mRNA from mouse heart was also analysed (Fig. 1, lanes *c* and *e*). Previous work by Kirchgessner *et al.* (1987) had demonstrated the presence of two major species of mRNA for LPL in a variety of mouse tissues. Our results support the observation that two forms of LPL mRNA predominate in mouse heart, and these species appear identical with that found in the 3T3-L1 cells (Fig. 1). The molecular size of these mRNA species as determined by using calibrated RNA standards was 3.7 and 3.9 kb. The two different LPL mRNAs were most readily observed when poly(A)$^+$ RNA was analysed after separation on a 1.1%-agarose gel (Fig. 1, lanes *d* and *e*). Minor species of LPL mRNA (1.7 and 2.7 kb in the 3T3-L1 cells and 1.7 kb in the heart) were observed after prolonged autoradiography (results not shown).

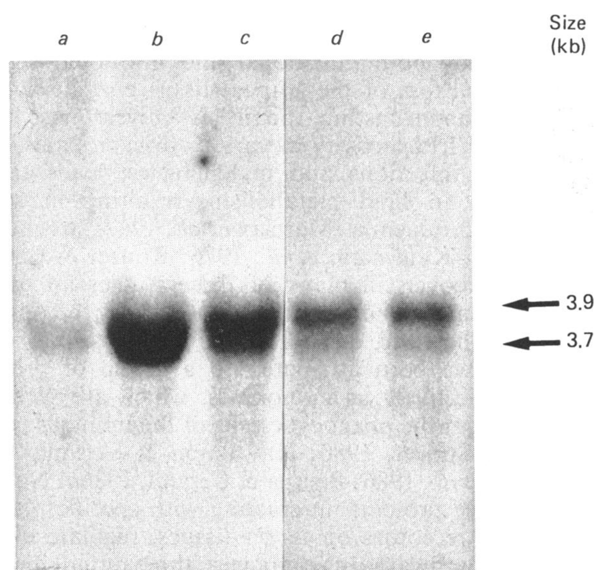

Fig. 1. Molecular size of LPL mRNA in 3T3-L1 cells

Total RNA and poly(A)$^+$ RNA were isolated from 3T3-L1 cells and from mouse heart, and examined by Northern analysis as described in the Experimental section. Samples were 20 μg of total RNA from confluent undifferentiated 3T3-L1 fibroblasts (lane *a*), fully differentiated 3T3-L1 adipocytes (lane *b*), and mouse heart (lane *c*), or 4 μg of poly(A)$^+$ RNA from fully differentiated adipocytes (lane *d*) and mouse heart (lane *e*). The results displayed are representative of an experiment performed twice with different preparations of RNA [total and poly(A)$^+$], with identical results.

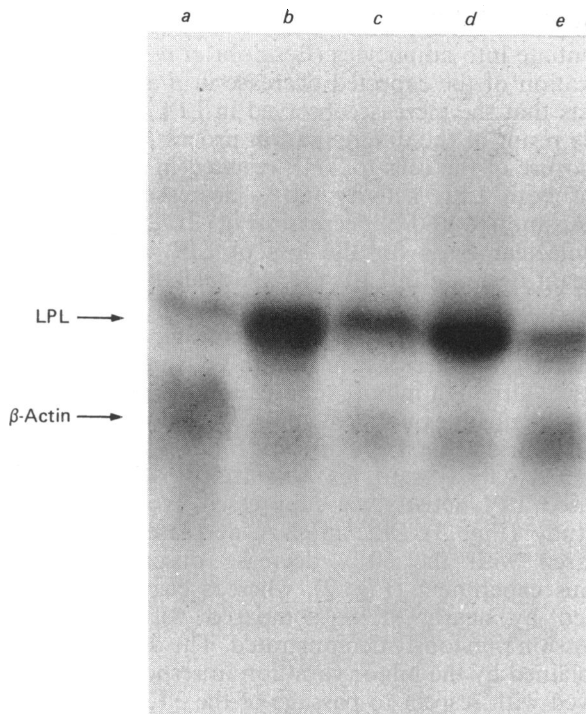

Fig. 2. Differential expression and TNF regulation of LPL mRNA in 3T3-L1 cells

Total RNA was isolated from 3T3-L1 cells at various times and 20 μg samples were examined by Northern analysis as described in the Experimental section. Lane (*a*), confluent undifferentiated fibroblasts; lane (*b*), fully differentiated adipocytes; lane (*c*), adipocytes treated for 17 h with 1.5 nM-TNF (250 units/ml); lane (*d*), adipocytes 48 h after TNF addition; lane (*e*), cells induced to differentiate in the presence of 1.5 nM-TNF, with RNA isolated 6 days later. The autoradiogram displayed is representative of an experiment performed twice with different preparations of RNA, with identical results.

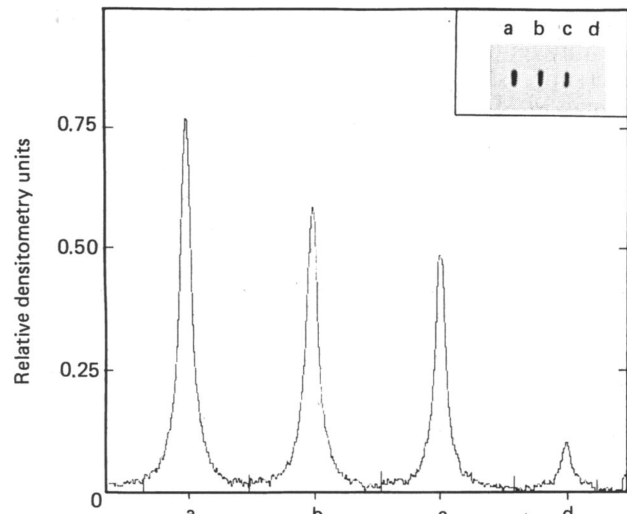

Fig. 3. Suppression of LPL mRNA by TNF

Total RNA was extracted from fully differentiated 3T3-L1 adipocytes at various times after exposure to 1.5 nM-TNF (250 units/ml), and 5 μg samples were subjected to slot-blot analysis as described in the Experimental section. Inset shows (a) control adipocytes, and adipocytes (b) 3 h, (c) 6 h and (d) 17 h after TNF. The main Figure shows densitometric analysis of the autoradiogram in the inset. The data displayed are representative of an experiment performed twice with identical results.

As demonstrated in Fig. 1 (lane *a*), confluent undifferentiated 3T3-L1 cells contain a measurable quantity of LPL mRNA, although in these same cells enzyme activity is barely detectable ($\leqslant$ 0.1 munit/mg of protein). The amount of LPL mRNA increased 10-fold, as determined by densitometric analysis, upon adipocyte conversion (Fig. 1, lane *b*). This corresponded to an increase in enzyme activity to 48 ± 4 munits/mg of protein.

Effects of TNF on amounts of LPL mRNA

In order to characterize the effect of TNF on amounts of mRNA coding for LPL, total RNA was prepared from 3T3-L1 cells of the same passage, plated at the same time. Undifferentiated and fully differentiated cells, and cells exposed to 1.5 nM-TNF, were included in the following experiment. From each preparation 20 μg of RNA was used for Northern analysis as described in the Experimental section. In this experiment the 10-fold increase of LPL mRNA upon differentiation was again observed (Fig. 2, lanes *a* and *b*), with an increase in LPL activity to 33 ± 1.3 munits/mg of protein. After these fully differentiated cells were exposed to 1.5 nM-TNF for 17 h (Fig. 2, lane *c*), the amount of LPL mRNA decreased by nearly 60 %, and activity was depressed to approx. 8 ± 0.4 munits/mg of protein, a decrease of 76 %. However, after 48 h of continuous exposure to TNF (Fig. 2, lane *d*), LPL mRNA content and enzyme activity approached the values seen in control fully differentiated cells (mRNA, 85 % of control; enzyme activity, 25 ± 0.6 munits/mg of protein, 77 % of control). Thus the effects were reversed in the continued presence of TNF. As a control for these determinations, the amounts of β-actin mRNA were also measured. Amounts of β-actin mRNA have previously been shown to decrease by 50 % upon differentiation (Bernlohr *et al.*, 1985), and lanes (*a*) and (*b*) of Fig. 2 confirm those findings. A 12 % decrease in β-actin mRNA is observed after 17 h of exposure to 1.5 nM-TNF (lane *c*), with amounts returning to normal at 48 h (lane *d*). Lane (*e*) is an analysis of mRNA isolated from 3T3-L1 cells induced to differentiate in the presence of TNF. That is, at 2 days post confluence, the cells were treated with 0.5 mM-isobutylmethylxanthine, 1 μM-dexamethasone and 10 μg of insulin/ml to induce differentiation, while at the same time the medium was made 1.5 nM in TNF. The medium was then replaced every 48 h with fresh medium supplemented only with 2.5 μg of insulin/ml for the first two feedings, with insulin omitted for the final feeding. At 6 days after removal of isobutylmethylxanthine and dexamethasone (the same age and treatment as with cells used for analysis in lanes *b* and *c*), RNA was isolated and analysed as described above. The results (lane *e*) indicate that this single exposure to TNF was sufficient to prevent full expression of LPL mRNA (only 35 % of fully differentiated; lane *b*) and to prevent the full attenuation of the β-actin mRNA. LPL activity also remained low, at 15 ± 0.6 munits/mg of protein (45 % that of fully differentiated cells).

Our previous work demonstrated that exposure of the fully differentiated 3T3-L1 cells to TNF resulted in a rapid suppression of both activity and synthesis of LPL (Price *et al.*, 1986*b*,*c*), with maximal suppression occurring between 12 and 17 h after addition of the monokine. To determine if the observed decreases in activity and synthesis mirrored a decrease in LPL mRNA content, total RNA was isolated from fully differentiated 3T3-L1 cells throughout a 17 h time course of exposure to TNF. By using a slot-blot technique, 5 μg of total RNA from each time point was applied to nitrocellulose, hybridized to nick-translated LPL cDNA probe, and the degree of hybridization was quantified by densitometry as described in the Experimental section. The results demonstrate that, relative to LPL mRNA isolated from fully differentiated untreated control cells (Fig. 3, inset, lane *a*), LPL mRNA decreases after TNF addition, by 20% after 3 h (lane *b*), 40% after 6 h (lane *c*), and 85% after 17 h (lane *d*). The activity of LPL, measured throughout the time course of exposure to TNF, was also suppressed (54.9 ± 2.9, 16.3 ± 0.4, 6.6 ± 1.2, 2.7 ± 0.9 munits/mg of protein for control and at 3, 6 and 17 h respectively). In additional experiments, the amount of LPL mRNA had begun to increase 24 h after addition of TNF. Moreover, no selectivity was observed with respect to either species of mRNA (3.7 or 3.9 kb) that were suppressed after TNF treatment (P. Cornelius & P. H. Pekala, unpublished work).

DISCUSSION

Our results demonstrate that 3T3-L1 cells express multiple species of mRNA coding for LPL. Similar results were obtained by Enerback *et al.* (1987) working with guinea-pig adipocytes, where three major (3.8, 3.3 and 2.1 kb) and one minor (4.5 kb) species of LPL mRNA were detected. Wion *et al.* (1987) have also detected two LPL mRNA species, of 3.3 and 3.8 kb, in human adiposse tissue, and Kirchgessner *et al.* (1987) observed mRNA species of 3.4 and 3.6 kb in both mouse adipose and heart tissue, with an additional 1.7 kb species in the heart. Furthermore, Senda *et al.* (1987), on analysis of bovine mammary-gland LPL mRNA, observed species of 3.2, 2.7 and 1.7 kb. These differences have been attributed to the presence of multiple polyadenylation signals (Kirchgessner *et al.*, 1987). In the present study, the distribution patterns of both mouse heart and 3T3-L1 LPL mRNA were identical with those obtained by Kirchgessner *et al.* (1987). However, minor differences in molecular size were observed; the sources of these variations are unknown. We note that estimations of molecular size were derived from calibrated RNA standards that exhibited linearity [mobility versus log(molecular size)] over a range of 4.5–1.4 kb. Our data further indicate that LPL mRNA is available for hybridization in confluent undifferentiated cells, which synthesize barely detectable amounts of LPL protein (Olivecrona *et al.*, 1987). Densitometric measurements from Northern analysis (Figs. 1 and 2) indicate that LPL mRNA increases approx. 10-fold as the cells differentiate and mature into adipocytes, whereas enzyme activity increases at least 100-fold (Wise & Green, 1978; Olivecrona *et al.*, 1987). As a control for this measurement, the amounts of β-actin mRNA were also measured. This mRNA has previously been shown to decrease by approx. 50% as the 3T3-L1 cells differentiate into adipocytes (Bernlohr *et al.*, 1985). The observation of the expected decrease in β-actin mRNA suggests that the increase observed in LPL mRNA is a specific result of the differentiation process.

Exposure of the cells to TNF resulted in a significant loss of both LPL activity (80% decrease) and LPL mRNA amounts (60% decrease, Fig. 2), evidence that the molecular basis for the loss of LPL activity is a significant decrease in LPL mRNA available for translation. A minor decrease (12%) was also observed in amounts of mRNA for β-actin, suggesting that a small percentage of the decrease in LPL mRNA may be due to a general, although minor, suppression of transcription. In the subsequent experiment (Fig. 3), the time course for loss of hybridizable LPL mRNA followed the loss of enzyme activity. Again, as amounts of LPL mRNA decreased, LPL activity was suppressed. We note that in this study (Fig. 3) LPL mRNA decreased by 85%, compared with the 60% decrease observed in the previous experiment (Fig. 2), whereas enzyme activity declined by nearly 95%, compared with the 80% suppression previously demonstrated. The difference can be explained by the minor variation in response to TNF observed with respect to passage of the 3T3-L1 cells. A 15% variation has been observed and documented previously (Beutler *et al.*, 1985). The observation that amounts of LPL mRNA returned to 85% of control after a single, continuous, exposure to TNF (Fig. 2) provides further support that the monokine is a potent, but reversible, regulator of adipose-tissue metabolism.

Studies of the effects of TNF *in vivo* on LPL in guinea pigs have demonstrated a 20% decrease in adipose-tissue LPL mRNA coupled with a 46% decrease in LPL activity 16 h after TNF treatment (30 μg, intraperitoneally) (S. Enerback, unpublished results). This lends further support to the hypothesis that TNF regulates LPL activity by suppressing amounts of mRNA available for translation. Dobson *et al.* (1987) have demonstrated that TNF down-regulates the activity of glycerophosphate dehydrogenase by suppressing the mRNA for this enzyme in F442-A cells (an adipogenic clone of 3T3 cells related to the 3T3-L1 cells), indicating that control of mRNA amounts may be a generalized mechanism of TNF action.

It has been suggested that both crude and recombinant preparations of TNF block differentiation of preadipocytes to adipocytes (Torti *et al.*, 1985). In the present study, however, evidence is provided that a single dose of TNF added at the induction of differentiation of 3T3-L1 cells did not stop the differentiation process in its entirety (Fig. 2, lane *e*). LPL mRNA had nearly doubled, whereas β-actin mRNA decreased significantly. Moreover, the cells had begun to accumulate lipid ($\sim$ 20% relative to control; P. Cornelius & P. H. Pekala, unpublished work), indicating that these cells began the transition, although somewhat delayed, to adiposity. The results may differ upon exposure of the cells to multiple doses of the monokine; this requires investigation, together with the effects of this monokine on the transcriptional process.

We gratefully acknowledge the expert technical assistance of Melissa Marlowe and Diane Eades in the performance of this work, as well as the excellent secretarial assistance of Laura Miner. This work was supported by N.I.H. grant GM 32892,

and by grants from the Swedish Medical Research Council (grant nos. B-131-7894 and B-03X-00727), the Swedish National Board for Technical Development (3/86), Hassle AB, Sweden, the Department of Trade and Industry, City of Gothenburg, and the Lundbergs Forskningsstiftelse, and from the Lions in Umea Research Fund (426/86).

REFERENCES

Amri, E., Vannier, C., Etienne, J. & Ailhaud, G. (1986) Biochim. Biophys. Acta **875**, 334–343
Bagby, G. J. & Spitzer, J. A. (1980) Am. J. Physiol. **238**, H325–H331
Bernlohr, D. A., Bolanowski, M. A., Kelly, T. J. & Lane, M. D. (1985) J. Biol. Chem. **260**, 5563–5567
Beutler, B. & Cerami, A. (1986*a*) Nature (London) **370**, 584–588
Beutler, B. & Cerami, A. (1986*b*) J. Immunol. **135**, 3969–3971
Beutler, B., Mahoney, J., LeTrang, N., Pekala, P. H. & Cerami, A. (1985) J. Exp. Med. **161**, 984–995
Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254
Brenneman, D. E., Mathur, S. N. & Spector, A. A. (1975) Eur. J. Cancer **11**, 225–232
Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) Biochemistry **18**, 5294–5299
Cleveland, D. W., Lopata, M. A., MacDonald, R. J., Cowan, N. J., Rutter, W. J. & Kirschner, M. W. (1980) Cell **20**, 95–105
Dobson, D. E., Groves, D. L. & Spiegelman, B. M. (1987) J. Biol. Chem. **262**, 1804–1809
Enerback, S., Semb, H., Bengtsson-Olivecrona, G., Carlsson, P., Hermansson, M., Olivecrona, T. & Bjursell, G. (1987) Gene, in the press
Kaufmann, R. L., Matson, C. F., Rowberg, A. H. & Beisel, W. R. (1976) Metab. Clin. Exp. **25**, 615–619
Kawakami, M. & Cerami, A. (1981) J. Exp. Med. **154**, 631–639
Kawakami, M., Pekala, P. H., Lane, M. D. & Cerami, A. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 912–916
Kirchgessner, T. G., Svenson, K. L., Lusis, A. J. & Schotz, M. C. (1987) J. Biol. Chem. **262**, 8463–8466
Kurzrock, R., Rohde, M. F., Quesada, J. R., Gianturco, S. H., Bradley, W. A., Sherwin, S. A. & Gutterman, J. U. (1986) J. Exp. Med. **164**, 1093–1101
Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor
Marclay, M., Skipski, V. P., Terebus-Kekish, T., Merker, P. L. & Cappucino, J. G. (1967) Cancer Res. **27**, 1158–1165
Nathan, C. F. (1987) J. Clin. Invest. **79**, 319–326
Olivecrona, T., Chernick, S. S., Bengtsson-Olivecrona, G., Garrison, M. & Scow, R. O. (1987) J. Biol. Chem. **262**, 10748–10759
Patton, J. S., Shepard, H. M., Wilking, H., Lewis, G., Aggarwal, B. B., Eessalu, E. T., Gavin, L. A. & Grunfeld, C. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 8313–8317
Price, S. R., Mizel, S. B. & Pekala, P. H. (1986*a*) Biochim. Biophys. Acta **889**, 374–381
Price, S. R., Olivecrona, T. & Pekala, P. H. (1986*b*) Arch. Biochem. Biophys. **251**, 738–746
Price, S. R., Olivecrona, T. & Pekala, P. H. (1986*c*) Biochem. J. **240**, 601–604
Robinson, D. S. (1970) Compr. Biochem. **18**, 51–116
Rouzer, C. A. & Cerami, A. (1980) Mol. Biochem. Parasitol. **2**, 31–38
Semb, H. & Olivecrona, T. (1986) Biochim. Biophys. Acta **876**, 249–255
Senda, M., Oka, K., Brown, W. V., Qasba, P. K. & Furuichi, Y. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 4369–4373
Torti, F. M., Dieckmann, B., Beutler, B., Cerami, A. & Ringold, G. M. (1985) Science **229**, 867–869
Vannier, C., Amri, E., Etienne, J., Negrel, R. & Ailhaud, G. (1985) J. Biol. Chem. **260**, 4424–4431
Wion, K. L., Kirchgessner, T. G., Lusis, A. J., Schotz, M. C. & Lawn, R. M. (1987) Science **235**, 1638–1641
Wise, L. S. & Green, H. (1978) Cell **13**, 233–242

Received 18 August 1987/26 October 1987; accepted 3 November 1987