Review Letter

LIPOLYSIS OF HEPATIC TRIACYLGLYCEROL STORES

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1. Introduction

Our knowledge concerning the mechanism of hepatic triacylglycerol synthesis and secretion has made considerable progress over the last few years and general patterns of control are beginning to emerge [1]. Yet, very little is known about the breakdown of hepatic triacylglycerols. Studies on the control and the physiological rôle of hepatic lipolysis have been hampered by the fact that it was unknown which liver lipase was responsible for the hydrolysis of hepatic triacylglycerol stores (hepatic lipolysis). Throughout the text the terms lipolytic activity and lipase activity refer to triacylglycerol lipase activity (EC 3.1.1.3). Net lipolysis is defined as the decrease in triacylglycerol stores, measured either chemically or by the disappearance of radioactivity from triacylglycerols prelabelled with radioactive fatty acids. Consequently, net lipolysis is the balance of triacylglycerol breakdown (which is termed lipolysis per se) and triacylglycerol synthesis; in the case of prelabelled triacylglycerols the latter process means reesterification of labelled fatty acids.

2. Liver triacylglycerol lipases

Lipolytic activity in rat liver homogenates was first demonstrated by Vavrinkova and Mosinger [2] and subsequently has been identified in lysosomal, microsomal, cytosolic and plasma membrane cell fractions [3–7]. The lipase of lysosomal origin has a pH optimum of 4–5 and has been partially purified from rat [8] and human liver [9]. It requires acidic phospholipids for optimal activity, and is capable of hydrolysing triacylglycerols, diacylglycerols and cholesterol esters. Rat liver lysosomes also contain phospholipase C activity producing diacylglycerols from a variety of phospholipids [10]. Monoacylglycerols produced from tri- or diacylglycerols are not processed further by the lysosomal lipase [7,10] but may be hydrolysed by a microsomal monoacylglycerol hydrolase [11].

Lipolytic activities occurring in cytosol, microsomes and plasma membranes display an alkaline pH optimum of 8–9.5 and exhibit an identical response to inhibitors such as NaCl and protamine sulfate. The highest specific activity is found in plasma membranes. Hence, the possibility has been raised that the various lipolytic activities represent the plasma membrane enzyme that is partially released during homogenization and fractionation [6,7].

Intravenous injection of heparin in the intact rat or perfusion of the isolated liver with heparin results in the immediate release of an alkaline lipase from the liver [6,12,13]. Tissue fractionation studies have shown that the heparin-releasable enzyme is associated with the plasma membrane fraction of rat liver homogenates [6,14]. In rat [15,16] and chicken [17] liver, the enzyme is synthesized and secreted by the parenchymal cells and subsequently bound to high-affinity binding sites on the non-parenchymal (endothelial)-cells [18,19]. Recent experiments have shown that there is only one alkaline lipase in rat liver [20]. Taken together, the foregoing observations suggest that the liver contains only two triacylglycerol lipases: one intracellular enzyme with acidic pH optimum and located in the lysosomes and one heparin-releasable enzyme with alkaline pH optimum and mainly associated with the exterior face of the plasma membranes of non-parenchymal cells [21]. The heparin-releasable lipase is presently thought to play a role in the uptake of cholesterol from high-density lipoproteins [21,22]. The lysosomal system is certainly involved in the degradation of lipids taken up by receptor-mediated

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endocytosis (review [23]). Evidence supporting the hypothesis that the lysosomal lipase is also responsible for hepatic lipolysis is presented below.

3. Lysosomal triacylglycerol lipase and hepatic lipolysis

The first suggestion that lysosomes and lysosomal lipase might be involved in hepatic lipolysis was raised on the basis of the following observations [24]:

(1) In isolated hepatocytes, several hypoglycemic sulfonylureas appeared to exhibit an antilipolytic effect. They inhibited ketone body production from endogenous fatty acid sources without affecting ketogenesis from exogenously added labelled oleate.

(2) In liver homogenates, these drugs inhibited the acid lipase without affecting alkaline lipase activity.

Another important observation was that the heparin-releasable hepatic lipase was lost during preparation of hepatocytes by perfusion in the presence of collagenase [25]. Freshly prepared hepatocytes thus appeared to be a well-suited model for the study of intracellular lipases and their role in the breakdown of endogenous triacylglycerols. Furthermore, while the whole liver contains parenchymal and non-parenchymal cells, isolated hepatocytes consist of a nearly homogenous population of parenchymal cells. Although the relative volume of the non-parenchymal cells is ~8%, they may contain as much as 40% of the liver's total acid lipase activity. The lipase of non-parenchymal cells may be exclusively involved in the hydrolysis of triacylglycerols taken up as constituents of lipoproteins [26].

When isolated hepatocytes were homogenized and their lipase activity was measured, it was observed that the pH dependence of triacylglycerol lipase displayed one peak of activity with a maximum at pH 4.4. An identical pH profile was found in subcellular fractions several-fold enriched in lysosomes. The subcellular distribution of the acidic lipase activity was found to follow exactly the distribution of the lysosomal marker enzyme acid phosphatase [27]. Negligible activity was observed in the neutral to alkaline pH range. These results indicated that the only intracellular lipase of hepatocytes is the lysosomal enzyme. This has been confirmed in cultured rat [28] and chicken [17] hepatocytes and strongly suggests that the lysosomal lipase is responsible for hepatic lipolysis.

Lipolysis in intact hepatocytes cannot be measured directly since liberated fatty acids are possibly re-esterified and/or oxidized [29]. Endogenous ketogenesis, that is the production of ketone bodies from endogenous sources in the absence of added free fatty acids has been tacitly accepted as a valid index of net hepatic lipolysis. In [27] we presented evidence that, in hepatocytes from starved rats, endogenous ketones are indeed mainly derived from the breakdown of triacylglycerol. (+)-Octanoylcarnitine, an inhibitor of the carnitine palmitoyltransferase reaction, very effectively inhibited endogenous ketogenesis in hepatocytes isolated from 24 h fasted donor rats [27], indicating that the ketones originated from the oxidation of endogenously produced fatty acids. When the hapatocytes were preincubated with radioactive [^{14}C]oleate in order to label their lipids and subsequently incubated in the absence of [^{14}C]oleate, the amount of radioactivity disappearing from the hepatocyte triacylglycerols was identical with the amount of radioactivity found in CO_2 plus acid-soluble oxidation products, showing that endogenous fatty acid oxidation is a reliable index for triacylglycerol breakdown [27].

The rate of endogenous ketogenesis was found to be identical in isolated hepatocytes and in the perfused rat liver whether or not it had been pretreated with heparin [27], supporting the idea that the extracellular heparin-releasable lipase of the liver does not participate in hepatic lipolysis.

Lysosomotropic compounds [30] such as chloroquine, methylamine and ammonium chloride are known to accumulate in lysosomes and to alter several lysosomal functions, either by direct inhibition of lysosomal enzymes or more likely, through an elevation of the intralysosomal pH [31–33]. In hepatocytes from starved rats these lysosomotropic compounds inhibited endogenous ketogenesis, without affecting ketogenesis from exogenously added oleate, as well as the disappearance of label from triacylglycerols prelabelled with [^{14}C]oleate [27]. In the presence of different chloroquine concentrations the disappearance of ^{14}C activity from prelabelled triacylglycerols was proportional to the formation of labelled acid-soluble products [29]. These results fully support the idea that the lysosomal lipase is responsible for the breakdown of hepatic triacylglycerols. This also implies that prior to hydrolysis the triacylglycerols are taken up in the lysosomes, most probably by

autophagocytosis.

In the same experiments [27], there was a striking parallelism between inhibition of both endogenous ketogenesis and proteolysis. Since there is good evidence (review [34]) that endogenous proteolysis in perfused liver or isolated hepatocytes is a lysosomal process, these results again suggest that lysosomes are involved in hepatic lipolysis.

In contrast to hepatocytes from starved rats, hepatocytes prepared from meal-fed donor rats, incubated for 1 h with [^{14}C]oleate [35] or $^{3}H_2O$ [36], and further incubated in a radioisotope-free buffer did not loose significant amounts of label from triacylglycerols during the second hour of incubation. This shows that net hydrolysis of prelabelled triacylglycerols does not occur in these cells. Absence of radioactivity in acid-soluble oxidation products from hepatocytes pre-incubated with [^{14}C]oleate further substantiates this observation [35]. Lund et al. [37] have shown with hepatocytes prepared from ad libitum-fed rats that only negligible amounts of [^{3}H]glycerol (pre-incorporated into triacylglycerols) disappear from these glycerolipids upon further incubation of the cells. In agreement with these studies with radioisotopes, Wirthensohn et al. [38] demonstrated that incubation of hepatocytes in the absence of added substrates led to a decrease of the content of triacylglycerols in the cell suspension, if the hepatocytes had been prepared from starved rats. In hepatocytes from fed rats this decrease was much smaller and in cells from carbohydrate-refed rats net lipolysis was absent [38]. It can be concluded that net lipolysis is minimal in the fed state. Whether this is due to a decrease in lipolytic activity per se or to an active re-esterification and/or de novo synthesis of fatty acids cannot be concluded from the above experiments.

A morphological correlate of the association between lipids and lysosomes, i.e., the lipolysosome, has been described in the liver of cholesterol-fed hamsters [39], in parenchymal cells of normal rat liver [40] and in livers of patients with various degrees of fatty infiltration [41]. Finally, deficiency of lysosomal lipase in patients suffering from Wolman's disease, is responsible for a progressive intralysosomal accumulation of triacylglycerols [42].

4. Short-term control of hepatic lipolysis by insulin and glucagon

Liver slices from starved donor rats show a glucagon- and cAMP-mediated increase in the output of fatty acids [43]. Since both glucagon and cAMP increase acyl-CoA levels in liver slices [43] and hepatocytes [44,45], and since both compounds are known to inhibit de novo hepatic fatty acid synthesis [1], the free fatty acids recovered in the incubation medium of the liver slices must have been derived from endogenous sources, most likely triacylglycerols. Bewsher and Ashmore [46] have demonstrated that rat liver homogenates prepared 30 min after the injection of glucagon into the intact animal display increased rates of fatty acid production from endogenous sources as compared to liver homogenates from rats injected with saline. Furthermore, the fall in triacylglycerol content was greater in the homogenates from glucagon-treated rats [46]. Injection of glucagon into starved rats drastically, and rapidly, lowers the proportion of triacylglycerols in total hepatic lipids [46]. This points to selective hydrolysis of triacylglycerols. It seems that glucagon activates net hepatic lipolysis. Insulin has been shown to reduce the steady-state concentration of free fatty acids in the perfusate of livers perfused without substrate [47]. However, this observation cannot be unequivocally interpreted as suggesting an inhibition of lipolysis, since insulin stimulates hepatic fatty acid incorporation into triacylglycerols [48,49].

Isolated hepatocytes are a better experimental model to study hepatic lipolysis than the intact animal or liver slices since in the former system extracellular lipolysis will not interfere [25]. Glucagon activates (within 1 h after its addition) the formation of ^{14}C-labelled acid-soluble oxidation products in hepatocytes prepared from starved rats pre-incubated with [^{14}C]oleate [35]. Insulin, on the other hand, inhibits the appearance of ^{14}C in acid-soluble products [35]. In this experimental system the radioactivity recovered in the acid-soluble products must be ultimately derived from labelled triacylglycerols since (in the presence and absence of hormones) the amount of labelled free fatty acids in the total incubation does not change [35]. The same effects of insulin and glucagon were demonstrated in a similar approach using the perfused rat liver [50]. The effect of glucagon on the production of labelled ketone bodies from prelabelled liver cells is in line with its well-known stimulatory action on the chemically measured endogenous ketogenesis [51–54].

5. Mechanisms underlying hormonal control of hepatic lipolysis

Several possible mechanisms may underly hormonal control of hepatic lipolysis. Net lipolysis may be indirectly controlled by fatty acid oxidation which is under hormonal control. In isolated hepatocytes glucagon stimulates and insulin inhibits the oxidation of fatty acids, both hormones being effective within 1 h after their addition [35]. An increased rate of fatty acid oxidation might lower the intracellular acyl-CoA and fatty acid levels resulting in an increased rate of lipolysis. Inhibition of fatty acid oxidation might depress lipolysis by increasing the fatty acid and acyl-CoA levels. No experimental evidence for such feedback regulation of lipolysis has been obtained. Since changes in the rates of fatty acid oxidation and esterification are reciprocally related, it is likely that fatty acid oxidation at least partly controls net hepatic lipolysis by altering the rate of fatty acid esterification or re-esterification. A possible direct inhibition (glucagon) [37,45,49] or stimulation (insulin) of esterification [49] and re-esterification will also influence net hepatic lipolysis.

Yet another possibility is that lipolysis per se is under direct hormonal control. This would imply that hepatic triacylglycerol lipase is under endocrine control, a view that has often been put forward. In analogy to the hormone-sensitive lipase in adipose tissue, it has been suggested on the basis of indirect evidence, that the liver contains a cAMP-sensitive triacylglycerol lipase [43,46,48,54–56]. The adipose tissue lipase exists in two interconvertible forms, one is phosphorylated and catalytically active, the other one is dephosphorylated and inactive [57]. In several laboratories evidence has been obtained that glucagon rapidly activates hepatic lipase activity. Bewsher and Ashmore [46] observed an increased production of free fatty acid from endogenous sources in bicarbonate-buffered (pH 7.4) liver homogenates prepared from rats injected with glucagon 30 min prior to sacrifice. Likewise, Vavrinkova and Mosinger [58] demonstrated that administration of glucagon to intact rats increased free fatty acid formation by liver homogenates from either endogenous substrate or added triacylglycerols at pH 5.0 and 4.8, respectively. Similar results were obtained when liver slices were incubated with glucagon for 10 min [58].

Although several authors [43,46,48,54–56] have proposed that hepatic lipase activity is regulated through phosphorylation–dephosphorylation cycles as is the adipose tissue lipase, no direct evidence for this proposal has been demonstrated. Debeer et al. [27] have shown that incubation of hepatocyte homogenates with cAMP, ATP and Mg^{2+} did not affect lipolytic activity. The activity of adipose tissue lipase is increased under this condition [57]. Furthermore, inclusion of NaF in the homogenization and/or the lipase assay mixture in order to inhibit possible phosphoprotein phosphatase activities, did not affect lipolytic activity [27]. These results suggest that hepatic lipase is not regulated by a phosphorylation–dephosphorylation mechanism.

On the other hand, autophagocytosis has been proposed as the mechanism underlying hepatic lipolysis [27]. During autophagocytosis, an organelle or portions of cytoplasm become surrounded by a membrane and the resulting autophagosome fuses with a lysosome. Glucagon induces the formation of hepatic autophagic vacuoles in vivo [59] or in the perfused liver [60]. During this process, lysosomes become more labile and have an increased mechanical and osmotic fragility [59]. Insulin and amino acids seem to inhibit autophagocytosis as well as the coupled process of lysosomal proteolysis [61].

In agreement with an autophagocytotic process of hepatic lipolysis, Debeer et al. [27] could not find any indication for an activation by glucagon of triacylglycerol lipase assayed in homogenized hepatocytes in the presence of detergent and with an emulsion of radioactive triolein as substrate. Likewise, Guder et al. [53] did not find an increase in lysosomal lipase activity after perfusion of isolated livers with glucagon when the enzyme was assayed under hypotonic conditions (total lipase activity). However, when assayed under isotonic conditions, there was an increase in the activities (free activities) of both the acid lipase and *N*-acetyl-β-D-glucosaminidase, another lysosomal enzyme. This increase in free activity was interpreted as the expression of the increased fragility of the lysosomes during active phagocytosis. As mentioned before, glucagon pretreatment in vivo increased the release of free fatty acids from endogenous sources in liver homogenates prepared under isotonic conditions [46]. In these experiments lipolysis may have taken place within intact lysosomes explaining the apparent discrepancy between these results and those obtained by Guder et al. [53] and Debeer et al. [27].

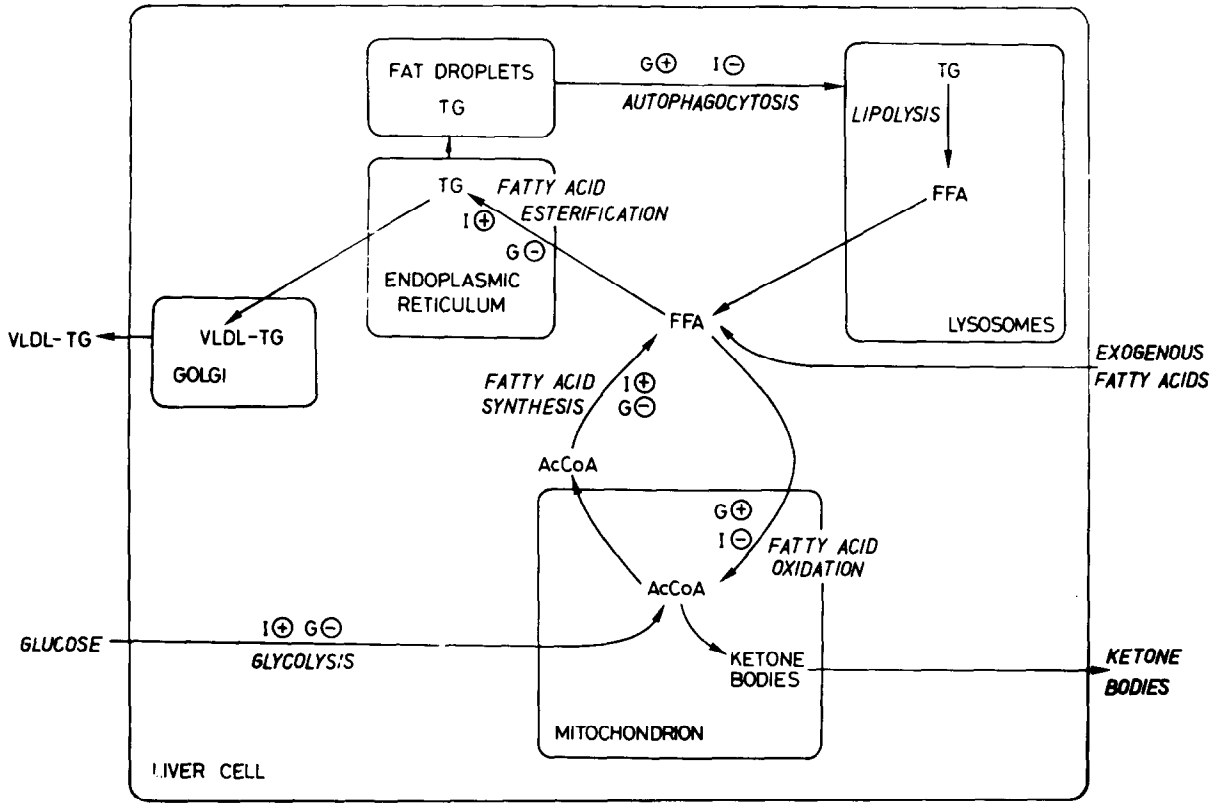

Fig.1. Nutritional and hormonal control of intrahepatic lipolysis: G, glucagon; I, insulin; ⊕, a positive effector; ⊖, a negative effector; TG, triacylglycerols; FFA, free fatty acids; AcCoA, acetyl coenzyme A; VLDL, very low density lipoproteins.

6. Conclusion

Our present knowledge of the nutritional and hormonal (insulin and glucagon) control of intrahepatic lipolysis is summarized in fig.1.

In the fed state, the predominance of insulin over glucagon in the circulation represses hepatic autophagocytosis and hence hepatic lysosomal lipolysis. In addition, insulin stimulates re-esterification of fatty acids liberated from hepatic triacylglycerols so that net lipolysis is low and the appearance of labelled acid-soluble oxidation products from prelabelled triacylglycerols is barely detectable.

In the fasted state, the hormonal balance shifts in favor of glucagon. This results in a more active autophagocytosis and lipolysis. In addition the stimulatory effect of glucagon on fatty acid oxidation counteracts active re-esterification so that net lipolysis is high.

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References

[1] Geelen, M. J. H., Harris, R. A., Beynen, A. C. and McCune, S. A. (1980) Diabetes 29, 1006–1022.
[2] Vavřínková, H. and Mosinger, B. (1965) Physiol. Bohemoslov. 14, 439–445.
[3] Mahadevan, S. and Tappel, A. L. (1968) J. Biol. Chem. 243, 2849–2854.

[4] Guder, W., Weiss, L. and Wieland, O. (1969) Biochim. Biophys. Acta 187, 173–185.
[5] Hayase, K. and Tappel, A. L. (1970) J. Biol. Chem. 245, 169–175.
[6] Assmann, G., Krauss, R. M., Frederickson, D. S. and Levy, R. I. (1973) J. Biol. Chem. 248, 1992–1999.
[7] Colbeau, A., Cuault, F. and Vignais, P. M. (1974) Biochemie 56, 275–288.
[8] Teng, M.-H. and Kaplan, A. (1974) J. Biol. Chem. 249, 1064–1070.
[9] Warner, T. B., Dambach, L. M., Shin, J. H. and O'Brien, J. S. (1981) J. Biol. Chem. 256, 2952–2957.
[10] Matsuzawa, Y. and Hostetler, K. Y. (1980) J. Biol. Chem. 255, 646–652.
[11] Ikeda, Y., Okamura, K., Arima, T. and Fujii, S. (1977) Biochim. Biophys. Acta 487, 189–203.
[12] La Rosa, J. C., Levy, R. I., Windmueller, H. G. and Frederickson, D. S. (1972) J. Lipid Res. 13, 356–363.
[13] Krauss, R. M., Windmueller, H. G., Levy, R. I. and Frederickson, D. S. (1973) J. Lipid Res. 14, 286–295.
[14] Waite, M., Sisson, P. and El-Maghrabi, R. (1978) Biochim. Biophys. Acta 530, 292–298.
[15] Jansen, H., Kalkman, C., Zonneveld, A. J. and Hülsmann, W. C. (1979) FEBS Lett. 98, 299–302.
[16] Sundaram, G. S., Shakir, K. M. M., Barnes, G. and Margolis, S. (1978) J. Biol. Chem. 253, 7703–7710.
[17] Jensen, G. J., Baly, D. L., Brannon, P. M. and Bensadoun, A. (1980) J. Biol. Chem. 255, 11141–11148.
[18] Jansen, H., Van Berkel, T. J. C. and Hülsmann, W. C. (1978) Biochem. Biophys. Res. Commun. 85, 148–152.
[19] Kuusi, T., Nikkila, E. A., Virtanen, I. and Kinnunen, P. K. J. (1979) Biochem. J. 181, 245–246.
[20] Groener, J. E. M. and Knauer, T. E. (1981) Biochim. Biophys. Acta 665, 306–316.
[21] Van Tol, A., Van Gent, T. and Jansen, H. (1980) Biochem. Biophys. Res. Commun. 94, 101–108.
[22] Jansen, H. and Hülsmann, W. C. (1980) Trends Biochem. Sci. 5, 265–268.
[23] Brown, M. S., Kovanen, P. T. and Goldstein, J. L. (1981) Science 212, 628–635.
[24] Debeer, L. J., Thomas, J., Mannaerts, G. P. and De Schepper, P. J. (1977) J. Clin. Invest. 59, 185–192.
[25] Thomas, J., Debeer, L. J. and Mannaerts, G. P. (1978) Biochem. J. 172, 177–179.
[26] Van Berkel, T. J. C. (1979) Trends Biochem. Sci. 4, 202–205.
[27] Debeer, L. J., Thomas, J., De Schepper, P. J. and Mannaerts, G. P. (1979) J. Biol. Chem. 254, 8841–8846.
[28] Davis, R. A., Engelhorn, S. C., Pangburn, S. H., Weinstein, D. B. and Steinberg, D. (1979) J. Biol. Chem. 254, 2010–2016.
[29] Beynen, A. C., Van der Molen, A. J. and Geelen, M. J. H. (1981) Pharm. Weekbl. Sci. Ed. 3, 118–121.
[30] De Duve, C., De Barsy, T., Poole, B., Trouet, A., Tulkens, P. and Van Hoof, T. (1974) Biochem. Pharmacol. 23, 2495–2531.
[31] Reijngoud, D. J. and Tager, J. M. (1976) FEBS Lett. 64, 231–235.
[32] Reijngoud, D. J., Oud, P. S., Káš, J. and Tager, J. M. (1976) Biochim. Biophys. Acta 448, 290–302.
[33] Ohkuma, S. and Poole, B. (1978) Proc. Natl. Acad. Sci. USA 75, 3327–3331.
[34] Amenta, J. S. and Brocher, S. C. (1981) Life Sci. 28, 1195–1208.
[35] Beynen, A. C., Van der Molen, A. J. and Geelen, M. J. H. (1981) Horm. Metabol. Res. 13, 183–184.
[36] Beynen, A. C., Haagsman, H. P., Van Golde, L. M. G. and Geelen, M. J. H. (1981) Biochim. Biophys. Acta 665, 1–7.
[37] Lund, H., Borrebaek, B. and Bremer, J. (1980) Biochim. Biophys. Acta 620, 364–371.
[38] Wirtensohn, G., Brocks, D. G. and Guder, W. G. (1980) Hoppe-Seyler's Z. Physiol. Chem. 361, 985–993.
[39] Nehemiah, J. L. and Novikoff, A. B. (1974) Exp. Mol. Pathol. 21, 398–423.
[40] Hayashi, H. and Winship, D. H. (1976) Clin. Res. 24, 286A.
[41] Hayashi, H., Winship, D. H. and Sternlieb, I. (1977) Gastroenterology 73, 651–654.
[42] Patrick, A. D. and Lake, B. D. (1969) Nature 222, 1067–1068.
[43] Claycomb, W. C. and Kilsheimer, G. S. (1969) Endocrinology 84, 1179–1183.
[44] Goodridge, A. G. (1973) J. Biol. Chem. 248, 4318–4326.
[45] Christiansen, R. Z. (1977) Biochim. Biophys. Acta 488, 249–262.
[46] Bewsher, P. D. and Ashmore, J. (1966) Biochem. Biophys. Res. Commun. 24, 431–436.
[47] Bates, E. J., Topping, D. L., Sooranna, S. P., Saggerson, D. E. and Mayes, P. A. (1977) FEBS Lett. 84, 225–228.
[48] Topping, D. L. and Mayes, P. A. (1972) Biochem. J. 126, 295–311.
[49] Beynen, A. C., Vaartjes, W. J. and Geelen, M. J. H. (1980) Horm. Metab. Res. 12, 425–430.
[50] Poledne, R. and Mayes, P. A. (1970) Biochem. J. 119, 47 P.
[51] McGarry, J. D., Takabayashi, Y. and Foster, D. W. (1978) J. Biol. Chem. 253, 8294–8300.
[52] Menahan, L. A. and Wieland, O. (1969) Eur. J. Biochem. 9, 182–188.
[53] Guder, W. G., Fröhlich, C., Patzelt, C. and Wieland, O. (1970) FEBS Lett. 10, 215–218.
[54] Müller, P., Singh, A., Orci, L. and Jeanrenaud, B. (1976) Biochim. Biophys. Acta 428, 480–494.
[55] Hems, D. A. (1977) FEBS Lett. 80, 237–245.
[56] Mayes, P. A. (1977) in: Review of Physiological Chemistry (Harper, H. A. et al. eds) p. 303, Lange Medical, Los Altos CA.
[57] Steinberg, D. (1976) Adv. Cyclic. Nucl. Res. 8, 157–198.
[58] Vavřínková, H. and Mosinger, B. (1971) Biochim. Biophys. Acta 231, 320–326.
[59] Deter, R. L. and De Duve, C. (1967) J. Cell Biol. 33, 437–449.
[60] Ashford, T. P. and Porter, K. R. (1962) J. Cell Biol. 12, 198–202.
[61] Aronson, N. N. (1980) Life Sci. 27, 95–104.